Targeting the Inhibitory Receptor Siglec-8 on Mast Cells Represents an Attractive Approach to Reduce MRGPRX2-Mediated Mast Cell Activation

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BACKGROUND

- Atopic dermatitis (AD) is a debilitating inflammatory disease of the skin characterized by pruritus[1,2]
- Mast cells (MCs) and eosinophils are elevated in AD tissue and poised both physically and biochemically to be key drivers of itch[3-5]
- Crosstalk between skin MCs and sensory neurons contribute to non-histaminergic itch via activation of Mas-related G-protein coupled receptor X2 (MRGPRX2)
- MRGPRX2-mediated MC activation has been implicated in chronic urticaria and allergic contact dermatitis[6]
- However, the role of MRGPRX2 in contributing to AD pathogenesis has not been well studied
- We hypothesize that activation of MCs through MRGPRX2 contributes to chronic inflammation and itch in patients with AD

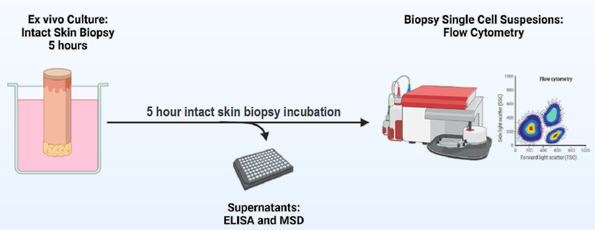
OBJECTIVE

- To study MC biology in AD by measuring both soluble mediators and examining various aspects of the inflammatory cell profiles and status of MC activation in matched non-lesional and lesional skin punch biopsies from otherwise healthy donors not undergoing any form of biologic treatment

METHODS

- Supernatants were collected from intact *ex vivo* cultured lesional and non-lesional skin biopsies from donor-matched patients with AD or healthy volunteers
- Following *ex vivo* culture, secreted mediators were quantified using ELISA and meso scale discovery (MSD)
- Biopsies were enzymatically digested and MRGPRX2 expression and function were measured using flow cytometry and MSD
- Acute MRGPRX2 activation *in vivo* and the effects of a Siglec-8 monoclonal antibody (mAb), compared with an isotype control, were studied in Siglec-8 transgenic mice

Figure 1. Schematic of Experimental Protocol

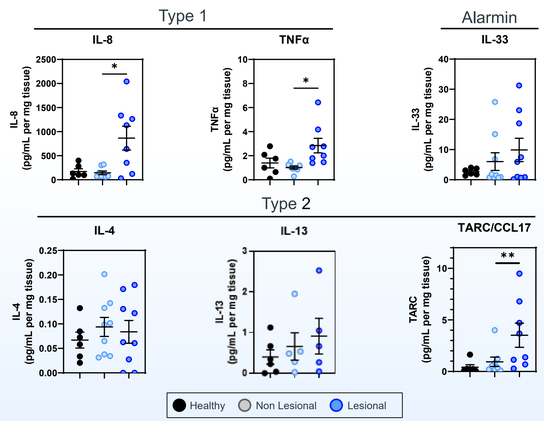


Evaluation of local inflammation in fresh skin biopsies via *Ex Vivo* immunological assays

RESULTS

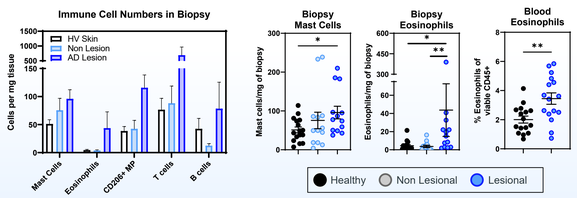
- MC and eosinophil numbers were increased in lesional biopsies from patients with AD compared with patient-matched non-lesional and healthy volunteer control biopsies
- MCs from AD lesional biopsies showed significant evidence of activation and degranulation compared to MCs in non-lesional biopsies
- Levels of MC-specific proteases, inflammatory, and pruritic mediators were significantly elevated in supernatants from ex vivo cultured lesional biopsies, including CPA3, CCL17 (TARC), IL-8, IL-13 and IL-31
- Furthermore, we found significantly elevated levels of MRPGRX2 ligands in supernatants from AD lesional biopsies
- *Ex vivo* challenge of healthy skin tissue with MRGPRX2 ligands partially replicated the AD lesional microenvironment. Lastly, intradermal injection of MRGPRX2 ligands in Siglec-8 transgenic mice induced inflammation and itch, which were inhibited with a Siglec-8 mAb

Figure 2. AD Skin Lesions Display Mixed Inflammatory Profile



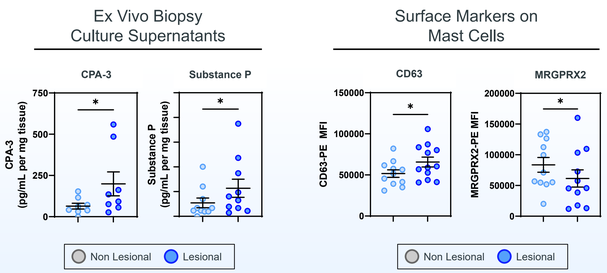
AD skin biopsies show evidence of both Th1 and Th2 inflammation

Figure 3. Mast Cells and Eosinophils are Elevated in AD Lesions



MCs and eosinophils may play a role in AD pathogenesis

Figure 4. MRGPRX2-Substance P Axis is Active in AD Lesional Skin



MRGPRX2-mediated MC activation is found in AD skin

Figure 5. Substance P-mediated MC Activation Induces MRGPRX2 Internalization

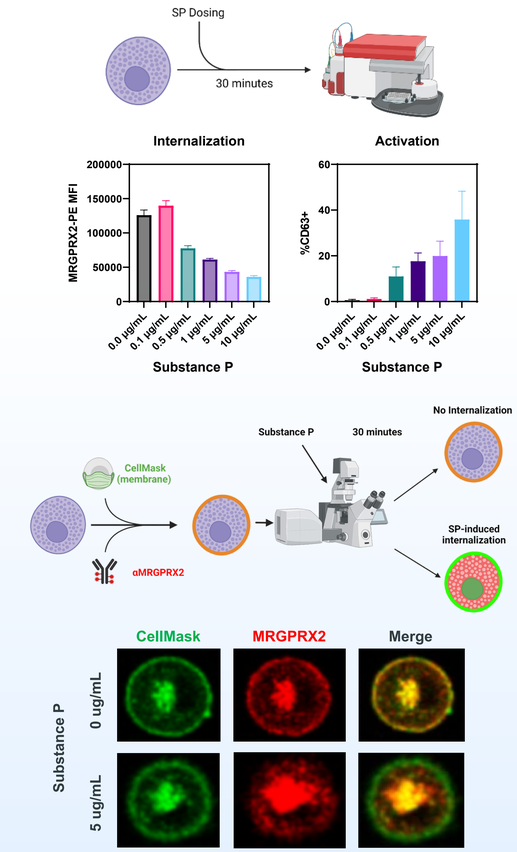
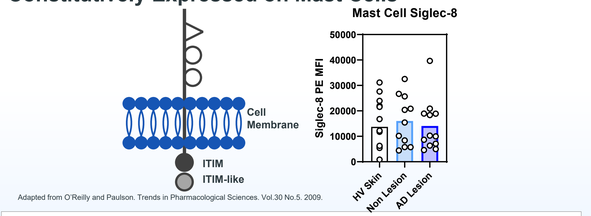
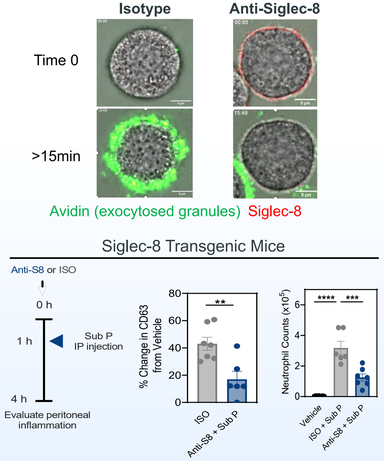


Figure 6. Siglec-8 is an Inhibitory Cell Surface Receptor Constitutively Expressed on Mast Cells



- Sialic Acid-binding Immunoglobulin-like Lectin (Siglec)-8 is a cell surface receptor of the CD33-related subfamily of Siglecs only found in humans
- Selectively expressed on eosinophils and mast cells
- Upon antibody engagement, Siglec-8 induces eosinophil depletion and mast cell inhibition

Figure 7. Siglec-8 mAb Inhibits Substance P-mediated Activation of MCs



Siglec-8 represents a novel target to inhibit MRGPRX2-mediated MC activation

CONCLUSIONS/DISCUSSION

- MRGPRX2-mediated mast-cell (MC) activation contributes to inflammation in patients with atopic dermatitis through the release of chemotactic, proteolytic, pruritic, and inflammatory factors
- Targeting the inhibitory receptor Siglec-8 on MCs represents an attractive approach to reduce MRGPRX2-mediated MC activation
- These findings support the rationale of the ongoing phase 2 proof-of-concept, randomized, double-blind, placebo-controlled study of lirentelimab, an anti-Siglec-8 antibody, in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical treatments (NCT05155085, "ATLAS")

Reference: (1) Weidinger S, et al. Nat Rev Dis Primers 2018; (2) Weidinger S, Novak N. The Lancet 2016; (3) Voss M, et al. Int J Mol Sci 2021; (4) Stander S. NEJM 2021; (5) Oetjen LK, et al. Cell 2017; (6) Gebremeskel S, et al. Allergy (EAACI 2020)

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