Mast Cells Are Locally Activated and Respond to MRGPRX2 Stimulation in Atopic Dermatitis Ex Vivo Skin Biopsies

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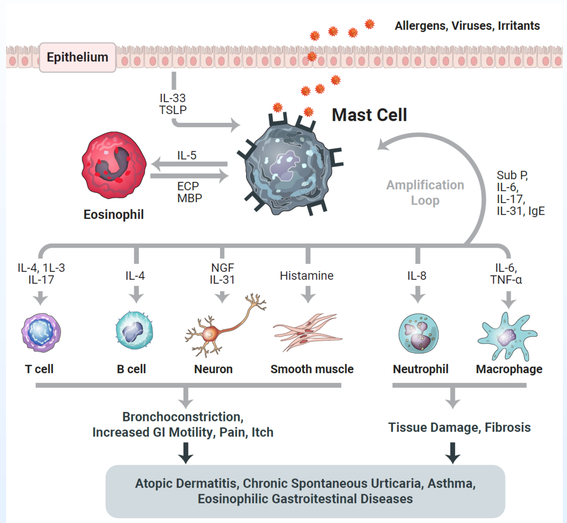
BACKGROUND

- Atopic dermatitis (AD) is characterized largely by type 2 inflammation of the skin and often debilitating symptoms such as pruritis. Mast cells (MCs) and eosinophils are considered drivers of itch in skin diseases via crosstalk with sensory neurons[1]
- The identification of MRGPRX2 as a MC-specific receptor for a broad range of neuropeptides further implicates crosstalk between MCs and nerves in neurogenic inflammation[2]
- While MCs and eosinophils have been shown to be elevated in AD, their potential role in disease pathogenesis remains poorly understood
- In order to investigate local inflammation in ongoing chronic AD, we developed an intact biopsy culture system that enables assaying active release of soluble mediators while simultaneously assessing immune cell numbers and MC activation status
- Here, we demonstrate MRGPRX2-mediated MC activation is a relevant, disease-driving pathway in patients with AD

Figure 1. Mast Cells and Eosinophils Are Key Drivers of Acute and Chronic Inflammation



METHODS

- Fresh, intact biopsies and donor-matched blood were obtained from healthy volunteers (n= 16) and atopic dermatitis patients (n = 16)
- Intact whole biopsies were cultured in media for 5 hours followed by collection of supernatants
- Following ex vivo culture, whole biopsies were enzymatically and mechanically digested, and flow cytometry was performed to quantify immune cells and evaluate the activation state of mast cells
- Cell-free supernatants were quantified for secreted mediators using ELISA and meso scale discovery (MSD)
- The following mediators were analyzed: IL-4, IL-5, IL-8, IL-13, IL-31, IL-33, CCL2 (MCP-1), CCL11 (Eotaxin-1), CCL17 (TARC), CCL26 (Eotaxin-3), TNFα, TSLP, CPA-3, and Substance P
- Human dorsal root ganglia (DRG) neurons were imaged in response to supernatants from Substance P and LL37 activated MCs

Figure 2. Study Design

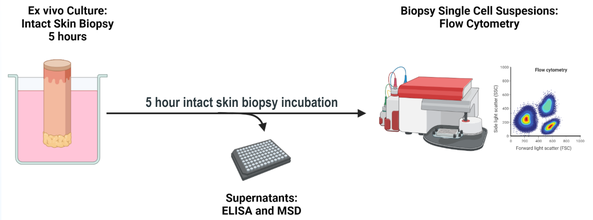
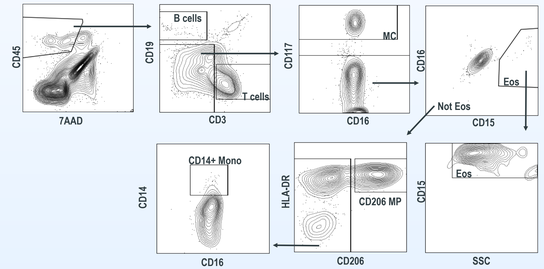


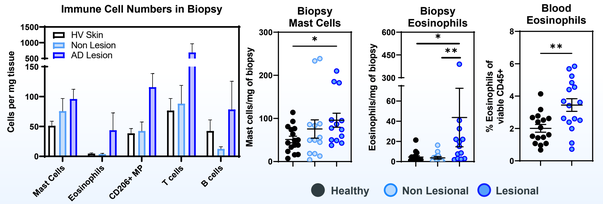
Figure 3. Strategy to Identify and Phenotype Eos and MCs in Biopsies



Gating strategy used to identify cell subsets in skin biopsies from healthy and AD patients. Live immune cells were gated using CD45-BV785 and 7AAD. B cells and T cells were identified using CD19-BV421 and CD3-BV650, respectively. MCs were then identified using CD117-APC. Eosinophils were identified using CD15-BV510 and CD16-APC-Cy7 and SSC. Finally, CD206 Macrophages were identified using CD206-PE-Cy7 and HLA-DR-HV605. Gating was performed in FlowJo.

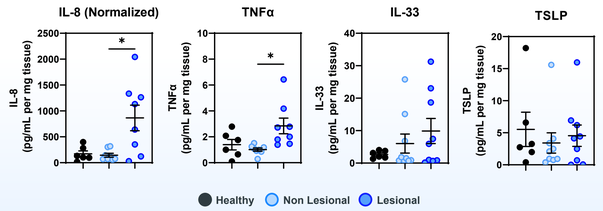
RESULTS

Figure 4. Mast Cells and Eosinophils Were Elevated in AD Lesions



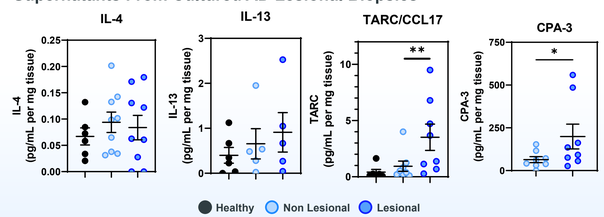
Cell counts from healthy (HV), Normal, and AD lesions normalized to the total mass of biopsy (far left). Biopsy mass normalized MC counts (center left). Biopsy mass normalized eosinophil counts (center right). Frequency of Eosinophils in the blood of HV and AD patients. Data are shown as mean +/- SEM for n=16 HV and n=13-16 AD. *, p<0.05. **, p<0.005

Figure 5. Th1-cytokines, but Not Alarmins Were Elevated in Supernatants From Cultured AD Lesional Biopsies



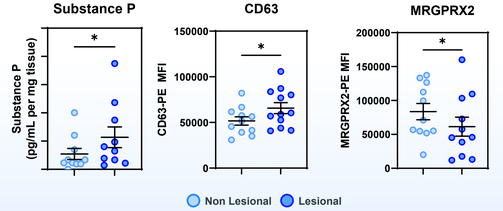
Levels of IL-8 (far left), TNFα (center left), IL-33 (center right), and TSLP (far right) in the supernatants after 5 hours of intact whole biopsy culture as measured by Meso Scale Discovery (MSD). Data are shown as mean +/- SEM for n=6 HV and n=8 Normal/AD. *, p<0.05.

Figure 6. Th2-cytokines and MC-derived Mediators Were Elevated in Supernatants From Cultured AD Lesional Biopsies



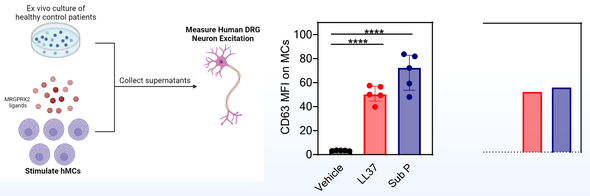
Levels of IL-4 (top left), IL-13 (top middle), IL-31 (top right), TARC/CCL17 (bottom left), and CPA-3 in the supernatants after 5 hours of intact whole biopsy culture as measured by Meso Scale Discovery (MSD). Data are shown as mean +/- SEM for n=5-6 HV and n=5-8 Normal/AD. *, p<0.05. **, p<0.005.

Figure 7. Substance P-induced MRGPRX2-mediated MC Activation in AD lesions



Levels of Substance P (left) in the supernatant after 5 hours of intact whole biopsy culture as measured by ELISA. Expression of CD63 (center) and MRGPRX2 (right) on MCs as measured by FACS from single cell suspensions. Data are shown as mean +/- SEM for n=5-6 HV and n=5-8 Normal/AD. *, p<0.05.

Figure 8. MRPGRX2 Stimulated MCs Activate Human DRG Neurons



Schematic of supernatants collected for human DRG neuron stimulation assays (left). LL37 (10 μM) and Substance P (10 μg/mL)-mediated MC activation supernatant-responsive human DRG neurons as a percentage of the total capsaicin-responsive neurons (right). *, p<0.05.

CONCLUSIONS

- Mast cells, eosinophils and T cells were specifically elevated in lesional skin biopsies from patients with AD
- Inflammatory mediators were significantly elevated in supernatants from cultured AD lesional skin biopsies compared to non-lesional skin, including CCL17, TNF, IL-8 and the MC protease CPA3
- Lesional skin biopsies from AD patients showed evidence of MRGPRX2-mediated MC activation via Substance P
- Substance P-stimulated MCs induced activation of human sensory neurons
- Activation of MCs via MRGPRX2 may contribute to neurogenic inflammation seen in AD lesions, highlighting a previously unrecognized role for MCs in AD pathogenesis

References: (1) Voss, M, et al. *Int. J. Mol. Sci.* 2021.; (2) Roy, S, et al. *J Allergy Clin Immunol.* 2021.

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