Chronic Inflammation

• Here, we demonstrate MRGPRX2-mediated MC activation is a relevant, disease-driving pathway in patients with AD.

• In order to investigate local inflammation in ongoing chronic AD, we developed an intact biopsy culture system that enables assaying active immune cell crosstalk with sensory neurons.

• While MCs and eosinophils have been shown to be elevated in AD, their numbers and MC activation status have not been extensively evaluated in vivo.

• Following ex vivo culture, whole biopsies were enzymatically and mechanically digested, and flow cytometry was performed to quantify immune cell numbers and MC activation status.

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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.

• Cell-free supernatants were quantified for secreted mediators using ELISA and meso scale discovery (MSD).

• Following ex vivo culture, whole biopsies were enzymatically and mechanically digested, and flow cytometry was performed to quantify immune cell numbers and MC activation status.

• Human dorsal root ganglia (DRG) neurons were imaged in response to supernatants from Substance P and LL37 activated MCs.

• The following mediators were analyzed: IL-4, IL-5, IL-8, IL-13, IL-31, IL-33, CCL2 (MCP-1), CCL17 (TARC), CCL26 (Eotaxin-3), TNFα, TSLP, CPA-3, and Substance P.

• Intact whole biopsies were cultured in media for 5 hours followed by collection of supernatants.

• Supernatants from Substance P and LL37 activated MCs were shown to significantly elevate levels of IL-8 (p<0.05), TNFα (p<0.005), IL-33 (p<0.005), TARC (p<0.05), and CPA-3 (p<0.05). Levels of IL-33 were shown to be significantly higher in patients with AD compared to healthy volunteers (p<0.005).

RESULTS

• The following mediators were analyzed: IL-4, IL-5, IL-8, IL-13, IL-31, IL-33, CCL2 (MCP-1), CCL17 (TARC), CCL26 (Eotaxin-3), TNFα, TSLP, CPA-3, and Substance P.

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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, TNFα, 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.

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[References and funding information would be included here if applicable.]