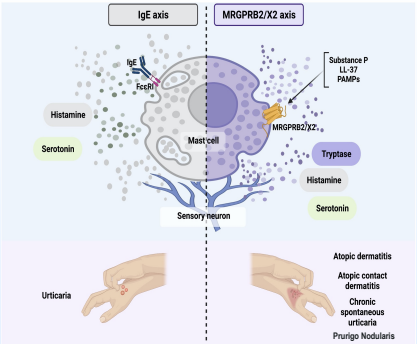
Poster 191

MRGPRX2-Mediated Mast Cell Activation is a Shared Pathogenic Mechanism in Atopic Dermatitis and Prurigo Nodularis Patients that can be Inhibited by Siglec-6

Zachary Benet[1], Thuy Luu[1], Emily C. Brock[1], Robert Sanchez[1], Julia Schanin[1], and Bradford A. Youngblood[1]
[1]Allakos Inc., San Carlos, CA

BACKGROUND

- Prurigo nodularis (PN), atopic dermatitis (AD), and chronic spontaneous urticaria (CSU) are inflammatory diseases of the skin characterized by chronic pruritus
- IgE-dependent mast cell activation is a well-established driver of histamine-dependent itch that's associated with CSU
- Mas-related G-protein receptor X2 (MRGPRX2)-mediated activation is a newly discovered pathogenic mechanism of mast cell (MC) activation independent of IgE
- Crosstalk between MCs and sensory neurons contribute to itch and inflammation via MRGPRX2 which has been implicated in AD and PN disease pathogenesis
- Inhibiting MC activation via agonist sialic acid-binding Ig-like lectin (Siglec) antibodies represents a therapeutic option for inflammatory diseases
- Here, we characterized MCs in skin biopsies from patients with PN and AD and evaluated the activity of agonist Siglec-6 and -8 antibodies in models of MRGPRX2-driven inflammation

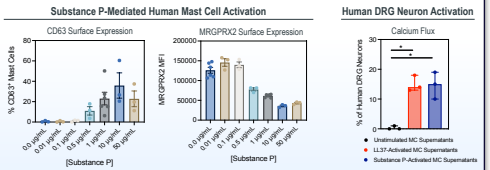


METHODS

- Fresh skin biopsies from AD and PN patients were immunologically profiled
- The function of MRGPRX2-activated MCs was assessed ex vivo and in vivo
- Antibody dependent cellular phagocytosis (ADCP) activity of AK006 was assessed using unstimulated or IFNγ-stimulated human macrophages
- Siglec-6 and Siglec-8 transgenic (Tg) mice were used to evaluate the activity of AK006 (Siglec-6 mAb) or AK002 (Siglec-8 mAb) in a model of MRGPRX2-induced skin inflammation

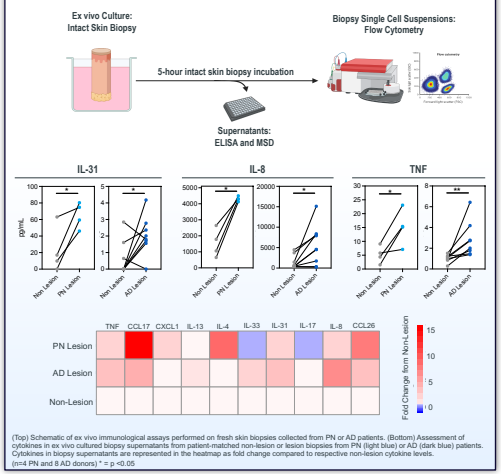
RESULTS

Figure 1. Substance P-Mediated Mast Cell Activation Induces MRGPRX2 Internalization and Excites Human Sensory Neurons



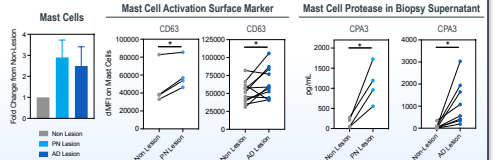
MRGPRX2-expressing CD34+ derived human mast cells were stimulated with Substance P at indicated concentrations and flow cytometry was used to assess activation (left) and MRGPRX2 surface expression (center). Supernatants from MRGPRX2-ligand pulsed MCs were used to assess human DRG neuron responses as measured by calcium flux. (n=3 human DRG donors) * = p <0.05

Figure 2: PN & AD Skin Lesions Display Mixed Inflammatory Profile



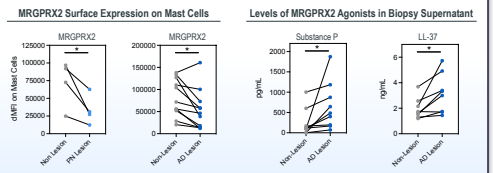
(Top) Schematic of ex vivo immunological assays performed on fresh skin biopsies collected from PN or AD patients. (Bottom) Assessment of cytokines in ex vivo cultured biopsy supernatants from patient-matched non-lesion or lesion biopsies from PN (light blue) or AD (dark blue) patients. Cytokines in biopsy supernatants are represented in the heatmap as fold change compared to respective non-lesion cytokine levels. (n=4 PN and 8 AD donors) * = p <0.05

Figure 3: Mast Cells are Elevated and Activated in PN & AD Skin



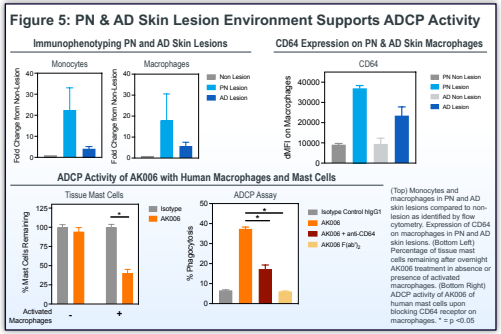
Analysis of mast cells from PN and AD skin biopsies. Mast cells were measured via flow cytometry as defined by CD117+FcεRIa+ events (left). Expression of the activation marker CD63 on mast cells taken from PN (left center) or AD (right center) patients. Supernatant levels of CPA3 from ex vivo biopsy cultures from PN or AD patients (right). (n=4 PN and 8 AD donors) * = p <0.05

Figure 4: Mast Cells in PN & AD Skin Display MRGPRX2 Activation



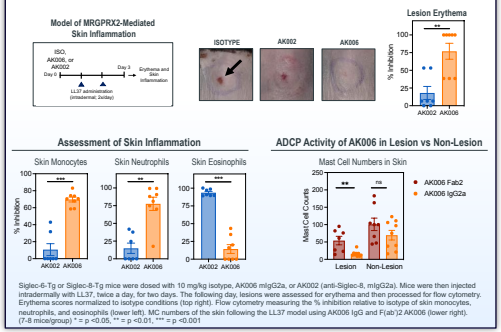
MRGPRX2 expression was analyzed on mast cells from PN and AD biopsies by flow cytometry (left). Supernatant levels of MRGPRX2-ligands Substance P and LL37 were measured after 5-hour incubation of ex vivo cultured biopsies from AD patients (right). (n=4 PN,8 AD donors) * = p <0.05

Figure 5: PN & AD Skin Lesion Environment Supports ADCP Activity



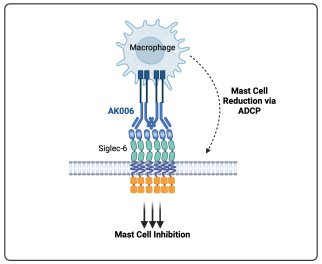
(Top) Monocytes and macrophages in PN and AD skin lesions compared to non-lesion as identified by flow cytometry. Expression of CD64 on macrophages in PN and AD skin lesions. (Bottom Left) Percentage of tissue mast cells remaining after overnight AK006 treatment in absence or presence of activated macrophages. (Bottom Right) ADCP activity of AK006 of human mast cells upon blocking CD64 receptor on macrophages. * = p <0.05

Figure 6: AK006, but not AK002 Reduces MRGPRX2-Induced Skin Inflammation



Siglec-6-Tg or Siglec-8-Tg mice were dosed with 10 mg/kg isotype, AK006 mIgG2a, or AK002 (anti-Siglec-8, mIgG2a). Mice were then injected intradermally with LL37, twice a day, for two days. The following day, lesions were assessed for erythema and then processed for flow cytometry. Erythema scores normalized to isotype conditions (top right). Flow cytometry measuring the % inhibition relative to isotype of skin monocytes, neutrophils, and eosinophils (lower left). MC numbers of the skin following the LL37 model using AK006 IgG and F(ab')2 AK006 (lower right). (7-8 mice/group) * = p <0.05, ** = p <0.01, *** = p <0.001

CONCLUSIONS



- MRGPRX2-mediated MC activation may contribute to AD and PN pathogenesis through the release of pruritic and inflammatory mediators
- The inflammatory environment in PN and AD skin lesions supports AK006 induced ADCP activity
- Therapeutic strategies that broadly silence and deplete MCs, such as AK006, may present potential therapeutic approaches in pruritic skin diseases

References: 1. Roy, S et al. J Allergy Clin Immunol. 2021.

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