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Epigenetic and Transcriptional Dysregulation in T cells of Patients with Atopic Dermatitis

Amy A. Eapen
Henry Ford Health, aeapen1@hfhs.org

Leah Kottyan

Sreeja Parameswaran

Carmy Forney

Lee Edsall

See next page for additional authors

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Authors

Amy A. Eapen, Leah Kottyan, Sreeja Parameswaran, Carmy Forney, Lee Edsall, Daniel Miller, Omer Donmez, Matthew Weirauch, Katelyn Dunn, Xiaoming Lu, Marissa Granitto, Hope Rowden, Adam Magier, Mario Pujato, Xiaoting Chen, David Bernstein, Ashley Devonshire, and Marc Rothenberg

013 Local IL-4Ra Blockade at Sites of Allergic Skin Inflammation Dampens Inflammation and Promotes S. aureus Clearance in Mice

Juan-Manuel Leyva-Castillo, PhD[1], Alex McGurk[1], Raif Geha, MD FAAAAI[1]; [1]Boston Children's Hospital.

RATIONALE: Atopic dermatitis (AD) is characterized by Th2-dominated skin inflammation and colonization with *S. aureus*. Systemic IL-4Ra blockade is beneficial in AD and results in decreased skin colonization by *S. aureus* via mechanisms that are not well understood.

METHODS: The skin of Balb/c mice was sensitized epicutaneously with ovalbumin (OVA) for 8 days and then injected intradermally with a single dose of anti-IL-4Ra blocking antibody or isotype control followed by topical application of *S. aureus* or saline. Two days later the sensitized skin was examined for cell infiltrates by flow cytometry, cytokine mRNA expression by quantitative PCR, global gene expression by transcriptome analysis, and bacterial burden by *in vivo* imaging and counting colony-forming units in skin homogenates.

RESULTS: IL-4Ra blockade in OVA sensitized skin significantly decreased epidermal thickening, diminished eosinophil and mast cell infiltration by ~30% ($p<0.05$) and increased cutaneous *Il17a* mRNA levels (1.5 folds, $p<0.05$) compared to control, without affecting cutaneous *Il4* and *Il13* mRNA expression or the systemic response to OVA. Transcriptome analysis revealed that IL-4Ra blockade increased the expression of genes regulated by IL-1b and IL-17A. IL-4Ra blockade in OVA sensitized and *S. aureus* infected skin had a similar effect and Importantly, it decreased *S. aureus* load ($20\pm3.8x10^3$ versus $3\pm1.6x10^3$ CFUs, $p<0.005$).

CONCLUSIONS: Local IL-4Ra blockade in an antigen-driven mouse model of AD improves allergic skin inflammation and enhances *S. aureus* clearance, possibly by enhancing the local responses known to restrain cutaneous *S. aureus* infection.

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

Melina Butuci, PhD[1], Zachary Benet[1], Alan Wong[1], Julia Schanin[1], Alan Xu[1], Amol Kamboj, MD[1], Brad Youngblood, PhD[1]; [1]Allakos, Inc.

RATIONALE: 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. 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. While MCs and eosinophils have been shown to be elevated in AD, their roles in pathogenesis remain poorly understood. We hypothesized that MRGPRX2-mediated MC activation contributes to local inflammation and sensory neuron excitation in AD.

METHODS: Single-cell suspensions were prepared by enzymatic digestion of fresh biopsies from AD patients and non-diseased skin tissue. Fresh biopsies were cultured ex vivo overnight followed by collection of supernatants to quantify mediators. Flow cytometry was used to assess MRGPRX2 activity.

RESULTS: MCs in AD biopsies displayed increased expression of activation and degranulation markers. In addition, supernatants from ex vivo cultured AD biopsies had elevated levels of MC- and eosinophil-derived mediators and cytokines, indicative of local functional activation and inflammation. Biopsy supernatants also showed elevated levels of endogenous MRGPRX2 ligands and ex vivo stimulation of AD skin biopsies with MRGPRX2 ligands induced significant MC activation. Lastly, MRGPRX2-mediated MC activation significantly activated human sensory neurons.

CONCLUSIONS: MCs are locally activated and respond to MRGPRX2 ligands, implicating MCs as potential pathogenic drivers of chronic skin inflammation and itch. Targeting both MRGPRX2-mediated MC activation and eosinophils may represent a potential therapeutic strategy for AD patients.

015 Epigenetic and Transcriptional Dysregulation in T cells of Patients with Atopic Dermatitis

Amy Eapen, MD, MS[1], Leah Kottyan, PhD[2], Sreeja Parameswaran[3], Carmy Forney[4], Lee Edsall, PhD[4], Daniel Miller[5], Omer Donmez[4], Matthew Weirauch, PhD[3], Katelyn Dunn[4], Xiaoming Lu[5], Marissa Granitto[4], Hope Rowden[4], Adam Magier, MS[3], Mario Pujato[4], Xiaoting Chen[4], David Bernstein, MD FAAAAI[6], Ashley Devonshire, MD MPH[7], Marc Rothenberg, MD PhD FAAAAI[3]; [1]Henry Ford Health System, [2]Cincinnati Children's Hospital Medical C, [3]Cincinnati Children, [4]Cincinnati Children's Hospital Medical Center, [5]CCHMC, [6]Bernstein Allergy Group, Inc, [7]University of Cincinnati, Cincinnati Chi.

RATIONALE: Atopic dermatitis (AD) is linked to genetic and environmental risk factors. The effect of these factors on molecular and transcriptional events is not well understood. Immunologically, AD involves skin barrier defects and CD4+ T cells that produce inflammatory cytokines and amplify epidermal dysfunction Our objective was to investigate epigenetic mechanisms that may account for genetic susceptibility in CD4+ T cells.

METHODS: We measured chromatin accessibility (ATAC-seq), NFKB1 binding (ChIP-seq), and gene expression (RNA-seq) in anti-CD3/CD28 stimulated CD4+ T cells from 6 subjects with active moderate-to-severe AD and 6 age-matched non-allergic controls.

RESULTS: AD genetic risk loci were enriched for open chromatin regions in stimulated CD4+ T cells. The majority of ATAC-seq peaks were shared between matched AD-control pairs, consistent with those sections of chromatin being equally available. In contrast, NFKB DNA binding motifs were enriched in AD-dependent open chromatin. NFKB1 ChIP-seq identified genomic regions that were more strongly bound in AD cases, more strongly bound in controls, or shared between cases and controls. Chromatin that was strongly accessible and bound by NFKB1 in AD was enriched for AD genetic risk variants. Using whole genome sequencing data, we identified genotype-dependent accessible chromatin at AD risk loci corresponding to 32 genes with genotype-dependent expression in stimulated CD4+ T cells.

CONCLUSIONS: The response of CD4+ T cells to stimulation is AD-specific and results in differential chromatin accessibility and transcription factor binding. These differences in transcriptional regulation result in epigenetic and transcriptional dysregulation in CD4+ T cells of patients with AD.