**Innate Immunity, Microbiology, and Microbiome | ABSTRACTS**

**205**

**Evaluation of SARS-CoV-2 spike protein response on PI3K agonist-mediated IL-8 release**

C. Birocota, A. Thyagarajan, C. Rapp, J.R. Travess and R. Sah Pharmacy & Toxicology, Wright State University, Dayton, Ohio, United States

A novel coronavirus related to a condition known as a severe acute respiratory syndrome (SARS) is currently ravaging the world. In the current study, we examined the immune response of PI3K agonist-mediated IL-8 release. We observed that treatments with only PI3K but not COVID-19 were able to induce dose-dependent IL-8 release from both K562 and A549 cell lines. Our next study determined the effects of COVID-19 pretreatment with PI3K and vice versa to evaluate if any of these combinations would exert synergistic effect on IL-8 release. We observed no significant differences in IL-8 release with either of these combinations when compared with PI3K alone group. However, significantly increased IL-8 release was noticed by PI3K + COVID-19 combination a when compared with COVID-19 alone group. Overall, these studies indicate that PI3K signaling does not directly mediate COVID-19-induced IL-8 release in these cellular models.

**206**

**The distinct skin microbiota of congenital ichthyoses**

A. Paler, K. Tham, R. Lefler, D. Duan, S. Lim, E. Biler, M. Chima, H. Kim, B. Wu, A. Angler, T. Beil and C. Galen, North-Western University Feinberg School of Medicine, Chicago, Illinois, United States, 2 Skin Research Institute of Singapore, Agency for Science Technology and Research, Singapore, Singapore, 4 Dermatology, Kain School of Medicine at Mount Sinai, New York, New York, United States

The ichthyoses are genetic keratinization disorders with an impaired epidermal barrier and increased risk of microbial infections. Congenital forms have recently been found to have a TH17 immune signature with increased antimicrobial peptides, but the skin microbiota is largely unexplored. We analyzed the metagenome profile of the skin microbiome for major congenital ichthyosis subtypes. Body site matched skin surface samples were collected from the scalp, upper arm, and lower back of 23 adult patients with ichthyosis and 16 healthy controls for whole metagenomics sequencing analysis. Taxonomic profiling showed changes in bacteria, fungi, and virus abundance across the subtypes. Cutibacterium acnes and Malassezia were significantly reduced in ichthyotic skin. Analysis of lipidome revealed that a lipid signature as a possible candidate biomarker in ichthyosis.

**207**

**Targeting of HDAC8 and HDAC9 in keratinocytes to enhance skin immune defense**

Y. Sawada, T. Dokoshi, N. Kulkarni, M. Legg, T. Nakatsuji, G. Sen and R. Gallo, Dermatology, University of California, San Diego, La Jolla, California, United States

We recently reported that short-chain fatty acids (SCFA) promote an inflammatory response in keratinocytes by suppression of HDAC8 or HDAC9, specific histone deacetylases whose activity increases tolerance of the skin to inflammatory signals. Upon silencing of HDAC8 or 9 in keratinocytes, subsequent exposure to LPS, TNF or L727 ligands affects inflammatory cytokine production in keratinocytes, but this effect does not occur in bone marrow derived cells, thus demonstrating epidermal specificity of this mechanism. Chip-Seq and signal pathway analysis by RNA-Seq identified MAP2K3 as a key intermediate in this process, with increased acetylation at H3K9 and H3K27 in the MAP2K3 promoter after silencing HDAC8 and HDAC9 or inhibition of HDAC activity by SCFA butyrate. Antibody pull-down and mass spec analysis showed that HDAC8 and HDAC9 bind the FACT complex and signal pathway analysis by RNA-Seq identified MAP2K3 as a key intermediate in this process. Immortalized keratinocytes were transfected with siRNA targeting HDAC8 and HDAC9 and the effect on cell viability monitored over time. These responses were ablated with MAP2K3 knock down. Further, HDAC8 and HDAC9 silencing in keratinocytes lead to IFN-β-dependent activation of antigen presentation ability in cultured dendritic cells and enhanced T cell proliferation in culture. Increased immune responses of keratinocytes was also seen in K14Cre; HDAC8/9floxed mice in response to UV radiation or imiquimod application, thus validating the critical role of this epigenetic mechanism in the skin. To exploit a potential benefit of HDAC8 and HDAC9 inhibition, we evaluated the impact of HDAC inhibition by topical application of SCFAs on survival of S. aureus. Topical treatment of mice with butyrate upregulated anti-microbial peptide production (Cath and mBD4) and subsequently inhibited S. aureus in mice despite increased Th2 cytokines generated in an MC903-induced AD mouse model. These observations show a novel approach to enhance host defense against pathogens on human skin.

**208**

**Sarecycline demonstrates reduced activity against representative fungal and bacterial species commonly found in the human gastrointestinal tract**

M. Chemin, L. Long, S. Joussef, T. McCormick and A. Grada, 1 Dermatology, University of Michigan, Ann Arbor, Michigan, United States and 3 Dermatology, Case Western Reserve University, Cleveland, Ohio, United States

Use of broad-spectrum antibiotics (e.g., doxycycline, minocycline) significantly alter the gut flora and skin microbiome leading to dysbiosis, resulting in microbial imbalance and has been associated with exacerbation of infection. Sarecycline was developed as the first narrow-spectrum tetracycline-class antibiotic to treat acne. Narrow-spectrum antibiotics are hypothesized to cause minimal interference with endogenous gastrointestinal (GI) tract microbiota, thereby maintaining innate microbial diversity. To examine the breadth of this effect, a panel of microorganisms that reflect the diversity of the gut microbiome were evaluated with sarecycline compared to the broad-spectrum antibiotic doxycycline using in vitro susceptibility testing and time-kill assays. Sarecycline had a lower minimum inhibitory concentration (MIC) against 3 out of 4 isolates from Actinobacteria phylum, 10 out of 12 isolates from Bacteroidetes, and 5 out of 7 isolates from the Firmicutes. Furthermore, sarecycline was less active against E. coli, and significantly less active against P. aeruginosa when compared to minocycline. Against fungi, sarecycline showed less activity against 4 representative Candida species. Time-kill curves for E. coli and C. tropicalis showed significantly less activity against E. coli for sarecycline compared to minocycline at all time-points (p-values <0.05). Similarly, sarecycline was significantly less effective in inhibiting C. tropicalis compared to minocycline at 20 and 22 hours exposure. Overall, sarecycline showed reduced antimicrobial activity against 79% of gut microflora tested compared to minocycline, suggesting that it has less potential to cause dysbiosis. Further in vivo testing is warranted.

**209**

**Epidermal interferon production is positively regulated by Staphylococcus aureus in SLE and involves the STING pathway**

S. Subrahmanyan, M.K. Sarkar, H. Stickey, J. Banfield, J. Gudjonsson and J.M. Kahlenberg, University of Michigan, Ann Arbor, Michigan, United States

Cutaneous inflammation is exhibited by many systemic lupus erythematosus (SLE) patients. Keratinocytes are an important source of type I interferons. In the current study, we examined the emergence of interferon production in SLE. We recently demonstrated that SLE lesional skin is highly colonized by Staphylococcus aureus (50%) secondary to effects of elevated type I IFN signaling. In this work we examined whether S. aureus can also induce keratinocyte IFN production, and we identified the specific intracellular signaling pathway utilized by S. aureus in this process. Immortalized keratinocytes (NHK) were exposed to live and heat killed S. aureus followed by gene expression analysis via quantitative real time PCR (qRT-PCR). IFNβ expression occurred rapidly (1 hour) in NHK-TERTs while other IFNs such as IFNα were produced with longer exposure. A panel of keratinocytes with a functional reporter to test IFNβ cytokine activity were infected with S. aureus. Notably, S. aureus induced IFNβ and epidermides, the ubiquitous skin colonizer, did not induce IFN production in keratinocytes. Also, co-culturing S. aureus with S. epidermidis decreased IFN production in keratinocytes in a dose-dependent manner suggesting that S. epidermidis can potentially regulate immunomodulation induced by S. aureus. In order to identify signaling pathways leading to IFN upregulation by S. aureus, MyD88 and TME4173 (STING) knockout keratinocyte cell lines, generated by CRISPR-Cas9, were treated with live S. aureus. Loss of TME4173, but not MyD88, was required for keratinocyte IFN production in response to S. aureus colonization. In summary, S. aureus, but not S. epidermidis, generates an epidermal IFN response in a TME4173-dependent manner. Further study of the impact of microbial dysbiosis will be important for understanding the pathobiology of IFN-driven skin diseases.

**210**

**Eosinophil-derived IL-17 protects against epicutaneous Staphylococcus aureus infections**

N. Orlando, C. Yuan, S. Nolan, M. Alphonse, D. Dikeman, Y. Wang, G. Patrick, I. Miller and N.K. Arch Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States

Staphylococcus aureus is the predominant cause of skin and soft tissue infections in humans and skin microflora which has prompted the search for alternatives to antibiotics through a better understanding of host immune responses. IL-17-mediated immunity is crucial for host defense against S. aureus skin infections, but the immune cells involved in these responses are not entirely defined. Recently, eosinophils were identified to produce IL-17 during fungal infections. However, the role of eosinophils in IL-17-mediated immunity against S. aureus skin infections is unexplored. To investigate the role of eosinophils against S. aureus skin infections, wildtype (wt) and eosinophil-deficient mice were epicutaneously exposed to a low bacterial strain of S. aureus and bacterial burden monitored over time. Eosinophil-deficient mice had increased bacterial burden compared to wt mice, suggesting that eosinophils contributed to host defense against S. aureus. Next, we used an IL-17A-deficient KO strain of S. aureus to test whether eosinophils produced IL-17A or IL-17F during S. aureus infection. Interestingly, ~50% of IL-17A-producing cells in the S. aureus-exposed skin were eosinophils, whereas eosinophils did not produce IL-17A. To determine whether eosinophils produce IL-17F during S. aureus infections, eosinophils were adaptively transferred into IL-17A/F-deficient mice treated with neutralizing mAbs against IL-17A/F or an isotype control. Indeed, eosinophils restored the host defense defect in IL-17A/F-deficient mice, but not in the presence of IL-17A/F mAbs. Overall, these findings implicate eosinophils in skin defense against S. aureus infections, and may have implications in the development of immune-based therapies against S. aureus and potentially other skin infections.