223 Murine cutaneous microbiota composition is largely mouse strain dependent with microbiota changes during acute wound healing showing mouse strain specific responses.


To date little research has investigated the genetic determinants of cutaneous microbiota composition and how microbiota composition can effect acute wound healing. Here we used 114 mice totalling 30 different mouse strains from an advanced cross-breeding program. The Collaborative Cross, and performed large, 1.5x1.5cm, full excisional wounds of mouse dorsal skin. 16S RNA sequencing immediately before wounding and at days 3 and 10 post-wounding show microbiota compositional changes are largely strain dependent with different mouse strains showing different roles in microbiota composition as a result of wound healing. Principal component regression of centred-log ratio abundances and wound healing speed, time-to-wound closure, show an adjusted R-squared <5%. PERMANOVA analysis suggests <4% of variance in microbiota compositional changes during healing are explained by wounding alone with >40% variance explained by mouse strain and no specific response to wounding. Murine microbiota composition is largely mouse strain dependent with microbiota changes during wound healing largely determined by mouse strain specific responses.

224 Rho Kinase deficiency protects mice from UVB-induced skin inflammation by inhibition of neutrophil NETosis

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Background: Ultraviolet B (UVB) is an important risk factor for lupus. UVB induces skin inflammation with recruitment of a network of cells that include neutrophils and exhibit NET-associated proinflammatory cytokines. We have recently identified that ROCK2-mediated nuclear lamin B phosphorylation is responsible for nuclear envelope rupture, nuclear DNA release and NET formation. Interestingly, inhibition of Rho Kinase (ROCK2) with its inhibitor HA1077 attenuates cell assembly, PKCε nuclear translocation, and NETosis in vitro. Intrapерitoneal application of HA1077 alleviates NETosis in vivo and NET-associated proinflammatory cytokines in the skin of UVB-irradiated C57BL6 wildtype (WT) mice. Here, we sought to further study the causal role of ROCK2 in NET formation and UVB-induced skin inflammation.

Method: We have generated CD45.1 mice with hematopoietic specific ROCK2 deficiency by bone marrow transplantation (BMT) of hematopoietic stem cells (HSCs) from ROCK2 deficient mice, followed by UVB exposure (150 mJ/cm², 5 consecutive days). We examined neutrophil NET formations in vitro and in vivo, as well as NET-associated IFNγ, TNFα, IL-17A expression and exhibition in skin of the UVB-irradiated mice. Results: We found that ROCK2 deficiency decreases NET formation in vitro from neutrophils from ROCK2 deficient mice as compared to those from WT mice. Very importantly, NET formation and NET-associated IFNγ, TNFα, IL-17A were significantly reduced in skin of UVB-irradiated BMT mice with ROCK2 deficiency as compared to those transplanted with WT HSCs. In an ex vivo experiment, exhibition of neutrophil NET-associated IFNγ, TNFα, IL-17A were significantly reduced in PAF-treated neutrophils from ROCK2 deficient mice as compared to those from WT mice. Conclusion: Hematopoietic specific ROCK2 deficiency attenuates neutrophil NET release and NET-associated proinflammatory cytokine display in UVB-induced skin inflammation.

225 Induction of protective antimicrobial responses mediated by NOD2 as a treatment for wounds infected with multidrug-resistant bacteria

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Of critical concern is the increasing antimicrobial resistance of bacteria and the decreasing availability of effective treatments for these multi-drug resistant (MDR) strains. The antimicrobial activity of the pattern recognition receptor NOD2 is increased by the pyrinium synthesized into N-Methylphosphonic acid (PA) in vitro. We investigated the efficacy of a topical PALA formulation to enhance NOD2-mediated protective immune responses as novel therapeutic approach for wounds infected with MDR bacteria. In a mouse model of MRSA infected biopsy wounds, topical application of PALA increased bacterial clearance from wound tissue as early as day 4 post-infection without impairing wound healing kinetics. PALA enhanced bacterial clearance corresponded with increased epithelial and dermal prodollum formation (APD), AMP expression, and Th17 cytokine expression. Molecular analyses in vitro demonstrate that PALA treatment increased NOD2 activation of the transcription factor IRF1 and upregulation of IL-17C, IL-22, and NOD2 expression. PALA-enhanced IRF1 activation required expression of NOD2 and the protein kinase RIP2, but not the adaptor protein MAVS, as demonstrated by siRNA-mediated knockdown analysis. However, PALA enhanced clearance of MRSA required expression of all three proteins, suggesting crosstalk between canonical and non-canonical NOD2 signaling pathways enhances protective antimicrobial responses. These results indicate that induction of protective antimicrobial immune responses mediated by NOD2 is achievable and well tolerated, suggesting this could be an effective therapeutic approach to MDR bacterial infections.

226 A positive feedback loop between mTORC1 and cathelicidin promotes skin inflammation in rosacea

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Rosacea is a chronic inflammatory skin disorder whose pathogenesis is unclear. Here, we focus on addressing the role of mTOR signaling in the pathogenesis of rosacea. We report that mTORC1 signaling is hyperactivated in both rosacea patients and mouse models. Functionally, both mTORC1 ablation and pharmacological inhibition by its specific inhibitors restrained the development of rosacea in an LL37 induced rosacea-like mouse model. On the contrary, hyperactivation of mTORC1 signaling in TSC2+/- mice exacerbated rosacea development. Furthermore, we revealed a positive feedback circuit between mTORC1 signaling and cathelicidin, in which LL37 activates mTORC1 signaling by binding to TLR2, which in turn enhances the expression of cathelicidin. Subsequently, cathelicidin LL37 derived from skin metabolizes NF-kB signaling, cytokines and chemokines production which are key factors associated with rosacea development through mTORC1 signaling. Finally, our pilot clinical study showed that topical application of rapamycin had a significant curative effect on rosacea patients. Collectively, these findings suggest a pivotal role for mTORC1 signaling in the pathogenesis of rosacea and reveal a potential therapeutic target for rosacea treatment.

227 Effect of triacsinclone & manuka honey on Staphyloccocus aureus aureus growth & hemolytic activity

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Atopic dermatitis (AD) is an inflammatory skin disease commonly treated with topical corticosteroids. NSAIDs (e.g. MIF) is an alternative therapy to AD. The role of TSC and MH has not been studied in skin. M. F. Molysins. To address the effect TCS & MH have on skin microbial shifts among different age groups are known from several culture- and sequencing-based studies, the influence of sexual maturation, skin microbiome, and skin physiology.

228 Shifts in the skin bacteria and fungi in healthy children transitioning through puberty

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Sexual maturation, skin microbial shifts among different age groups are known from several culture- and sequencing-based studies, the influence of sexual maturation, skin microbiome, and skin physiology.

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