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Trichodysplasia spinulosa-associated polyomavirus small T antigen activates MAPK pathway

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Trichodysplasia Spinulosa (TS) is a disfiguring skin condition characterized by abnormal maturation of hair follicles, dysplasia of inner root sheath cells, keratin spine formation on the central face, and alopecia. TS occurs exclusively in immunocompromised patients and results from infection with Trichodysplasia Spinulosa-Associated Polyomavirus (TSPyV). Although the association between TSPyV and TS has been recognized, the mechanism by which TSPyV mediates the pathogenesis of TS is poorly understood. Similar to other polyomaviruses, the TSPyV genome encodes the small T (sT) antigen. While several polyomavirus sT antigens bind and inhibit protein phosphatases, the specific function of protein phosphatase 2A (PP2A) in small T antigen pathogenesis remains to be further defined. Moreover, it remains unknown whether and which phosphatase might be implicated in TSPyV sT antigen actions. The purpose of the current study is to understand the proliferative role of TSPyV sT antigen by investigating its binding capacity to protein phosphatase 2A (PP2A) and to determine its effect on the activation of MAPK and AKT, two characteristic PP2A targets that influence cell proliferation. We performed co-immunoprecipitation and His-tag pull-down assays to evaluate the interaction between TSPyV sT antigen and PP2A. Under both assay systems, we observed that TSPyV sT antigen physically associated with PP2A. We subsequently examined the phosphorylation of MEK-1/2, ERK-1/2, and AKT in cultured cells overexpressing TSPyV sT antigen. Our data showed that overexpression of TSPyV sT antigen enhanced the phosphorylation of MEK-1/2 and ERK-1/2, but not AKT. These findings provide novel evidence for a distinct role of PP2A in mediating the actions of TSPyV small T antigen. Our results demonstrate that TSPyV sT antigen, by binding and inhibiting PP2A, can activate the MAPK pathway and thus contribute to the proliferative nature of TS.

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Recognition of melanoma by an innate immune receptor Dectin-1 orchestrates innate immune cells for anti-tumor responses

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The eradication of cancer cells requires communication between cells which constitute the complex immune system. Natural killer (NK) cells are essential tumor-killing effector cells of the innate arm of the immune system; however, little is known about whether or how other innate immune cells recognize tumor cells to assist NK cells. In addition, although signal-transducing pattern recognition receptors are known to play important roles in innate immune responses against invading pathogens through recognition of pathogen-associated molecular patterns (PAMPs), it is still enigmatic whether and how these receptors contribute to anti-tumor immune responses. In this study, we show that the innate immune pattern recognition receptor Dectin-1 expressed on dendritic cells (DCs) and macrophages is critical to NK cell-mediated killing activity against melanoma. We also show that tumor cell-triggered Dectin-1 signaling in DCs and macrophages is instigated by the receptor recognition of N-glycan structures on tumor cells, which we term tumor-associated molecular patterns (TAMPs). The TAMP-Dectin-1 signaling causes activation of the Interferon Regulatory Factor 5 (IRF5) transcription factor and subsequent induction of genes required for the full-blown killing activity of NK cells. The importance of these events is underscored by the observation of massive melanoma metastasis in mice genetically deficient in either Dectin-1 or IRF5. Thus, these results reveal a hitherto unrecognized facet of pattern recognition receptors in the orchestration of anti-tumor innate immune responses; recognition of TAMPs. This study is the first to demonstrate that an innate immune pattern recognition receptor potentiates anti-tumor immune responses, offering new insight into anti-tumor activity of the innate immune system with implications for anti-tumor immunotherapy.

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Use of a translational human *ex-vivo* skin model to predict clinical effective dose of a topical drug candidate

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We postulated that topical target engagement could be demonstrated in human skin prior to clinical POC trials. Indeed, a therapeutic compound formulated for topical delivery can be shown to affect specific disease-relevant biomarkers using a novel ex-vivo human skin culture system. The custom design of Franz Cells allows topical delivery of drug compounds through the skin barrier to be interrogated with repeated applications of drug to imitate clinical trial applications. Treatment using Franz cells ensured localized, contained, and reproducible application. Systemic application, in the basolateral compartment, of a Th17 "polarization" cocktail was studied in the presence of a potent and selective control antagonist applied apically. Polarization of skin resident T cells into Th17 cells and compound efficacy was monitored by qPCR, and the integrity of the skin barrier was assessed by TEWL. The dose range finding implications allow for better prediction of efficacious concentrations prior to first time in humans. This experimental design is the first to reliably limit the exposure of the compound to the surface of human skin to detect dermal and epidermal target engagement. This unique design enabled us to perform multiple applications of the compound to assess PK/PD relationships in a human tissue maintained in an air-liquid interface mimicking physiological conditions. Thus, we can not only analyze disease-state biomarker regulation, but also compare different concentrations and formulations proposed as candidates for human testing. This approach employs a novel chamber system designed specifically for culture of human skin, more directly testing compound interactions without the need for animal subjects. This methodology provides a platform to interrogate novel topical therapeutics we are advancing for the treatment of skin disease.

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GSK compound A Inhibits keratinocyte and T lymphocyte proliferation and cytokine production by blocking cell cycle progression at the G1-S transition phase

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In psoriasis, an excessive accumulation of cells including keratinocytes and lymphocytes are observed in disease pathology. Dysregulated keratinocytes proliferate rapidly and epidermal differentiation is altered resulting in raised, psoriasiform skin placques. In addition, accumulation of inflammatory T cells secreting IL-17a(Th17) in perivascular clusters of the dermis and epidermis have been implicated in contributing to disease pathology. Phenotypic screening showed that compound A inhibited IL-17 cytokine production by T cells as well as keratinocyte proliferation. Therefore, we sought to determine if a) the biological effect of Compound A is due to a direct effect on cytokine production or to an effect on the proliferation of inflammatory Th17 cells and b) whether the inhibition of keratinocyte proliferation is mediated by alterations in the cell cycle. Compound A treatment during Th17 polarization of CD4 T cells inhibited cell division in a concentration dependent manner. As a result of this block, Th17 differentiation was inhibited resulting in a reduction of IL-17a+ cells. Cell cycle studies showed that Compound A treatment of keratinocytes altered the frequency of cells within the G1, S, G2/M cell cycle phases. Chemically induced cell synchronization experiments in keratinocytes further implicate a cell cycle block at the G1/S transition. We propose that prolonged inhibition of the cell cycle by compound A preceded and ultimately resulted in the induction of apoptosis. Overall, compound A mediates inhibition of proliferation and cytokine production in two major cell types of the skin, keratinocytes and T cells, implicated in chronic inflammation in psoriasis.

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AN2728, a new boron-based topical anti-inflammatory agent, inhibits phosphodiesterase 4 (PDE4)

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These studies were conducted to further characterize the mechanism of action of AN2728, a novel boron-based topical anti-inflammatory agent being investigated in phase 3 clinical trials for treatment of mild-to-moderate atopic dermatitis. The mechanism of action of AN2728 was characterized via enzyme kinetic assays, PDE4 subtype inhibition studies, protein crystallography, and an analysis of cytokine inhibition in peripheral blood mononuclear cells. To investigate reactivity against other targets, AN2728 was tested against 50 receptors and ligand-gated ion channels for inhibition at 10 μM. AN2728 competes with cAMP to inhibit the PDE4B1-catalytic domain with a Ki of 173±26 nM; thus AN2728 interacts at the enzyme-active site. The X-ray structure of PDE4B-catalytic domain with AN2728, and its structural relative, AN2898, reveals that the boron atom interacts with the bimetal center and occupies a position in the catalytic site similar to that of the cAMP phosphate. AN2728 has good affinity across the PDE4 gene products A-D. Its selective affinity for PDE4 is 4- to 10-fold greater than its affinity for PDE1, 2, 3A, 6, or 7B. It is inactive on PDE3B, 5, 7A1, and 8-11. The activity of AN2728 results in an increase in intracellular cAMP and activation of PKA, followed by phosphorylation and negative regulation of transcription factors of various cytokines. In the analysis of AN2728 binding to 50 receptors and ligand-gated ion channels, inhibition was less than 25% for all of the receptors tested; thus, AN2728 is specific for PDE4. AN2728 exerts its anti-inflammatory effect by inhibition of PDE4, one of 11 subtypes of the enzymes that catalyze the breakdown of cyclic nucleotides to their inactive monophosphates (in the case of PDE4, cAMP to AMP). Through inhibition of cAMP-dependent PDE4 activity, AN2728 inhibits the production of specific cytokines with a pattern that is notably similar to that of other established PDE4 inhibitors and distinctly different from those of a glucocorticoid and a calcineurin inhibitor.

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Dysbiotic microbiota drives atopic inflammation in *Adam17*$^{fl/fl}$*Sox9*-Cre mice

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Staphylococcus aureus universally colonizes skin of patients with atopic dermatitis (AD), while *Corynebacterium* species can also be detected in genetic diseases that manifest eczematous dermatitis. The causal relationship between dysbiosis and AD has yet to be determined. Recently, ADAM17-deficiency was identified as a new genetic disease that manifests eczematous dermatitis. Here we show in *Sox9*-Cre; *Adam17*$^{fl/fl}$ (*Adam17*$^{\Delta Sox9}$) mice, that the atopic phenotype they exhibited, dry, eczematous and pruritic skin, high serum IgE and CCL17 and a mixture of enhanced T cell responses, critically depended on dysbiotic microbiota. Longitudinal microbiome analysis revealed sequential changes in skin bacterial communities. While microbiota in WT and *Adam17*$^{\Delta Sox9}$ mice were diverse and indistinguishable at week 2 after birth, *Corynebacterium mastitidis* emerged in *Adam17*$^{\Delta Sox9}$ mice at week 4, followed by *S. aureus* from week 6. *C. mastitidis* was abruptly replaced by *C. bovis* at week 8. Antibiotic treatments reversed dysbiosis and protected mice from developing eczematous inflammation. On the contrary, withdrawal of antibiotics resulted in rapid emergence of dysbiosis and eczematous inflammation. Inoculation of *S. aureus* onto antibiotic-pre-treated *Adam17*$^{\Delta Sox9}$ mice markedly enhanced eczematous dermatitis whereas *C. bovis* inoculation induced prominent Th2 responses, formally demonstrating that specific components of the skin microflora can modulate eczematous inflammation. Impairment of EGFR signaling, a major downstream pathway of Adam17, was sufficient to initiate dysbiosis. Taken together, this study reveals crucial contributions of commensal bacteria during eczematous inflammation in mice and provides impetus for exploring novel therapies that target the microbiota-host immunity axis in AD.

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IL-1β-independent neutrophil recruitment induces long-term protection against a *Staphylococcus aureus* skin reinfection

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S. aureus skin reinfections in patients are common, especially those caused by community-acquired methicillin-resistant *S. aureus* (CAMRSA). We previously reported that IL-1β induced neutrophil (PMN) recruitment and bacterial clearance during a primary *S. aureus* skin infection in mice. However, the immune responses that promote protection against *S. aureus* skin reinfections are not clear. We developed a CA-MRSA (USA300 LAC::*lux*; $3x10^7$ CFU, intradermally) skin reinfection model in mice involving a primary infection (1°) in the lower back (which cleared by 14 days) followed by a secondary infection (2°) in the upper back on day 28. Wt mice developed little protection as 2° wt mice had smaller lesions than 1° wt but the bacterial burden was identical. In contrast, 2° IL1β-/- mice were rescued from the larger lesions and higher bacterial burden in 1° IL-1β-/- mice. This protection was long-lasting as it was still present if we waited 8 weeks between 1° and 2° infections. By histology on day 1, the impaired PMN abscess formation in 1° IL1β-/- mice was restored in 2° IL-1β-/- mice to levels seen in wt mice. In the blood at 6 hours, the PMN trafficking defect in 1° IL-1β-/- mice (indicated by higher numbers of $CD11b^+/Ly6G^+$ PMNs) was also rescued in 2° IL-1β-/- mice, which had lower numbers of PMNs, similar to wt mice. In the skin at 6 hours, a 23-plex protein array for inflammatory cytokines/chemokines revealed a marked defect in IL-6 levels in 1° IL-1β-/- mice, which was restored in 2° IL-1β-/- mice to levels seen in wt mice, suggesting a role for IL-6 in the immune protection. Taken together, in the absence of IL-1β, an alternative and potentially novel mechanism for PMN recruitment developed that mediated long-term protection against a CA-MRSA skin reinfection.

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Role of filaggrin-deficiency, skin injury and *Staphylococcus aureus* in atopic dermatitis-like skin inflammation in mice

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Atopic dermatitis (AD) is associated with filaggrin loss-of-function mutations, which contribute to the skin barrier defect in this disease. In addition, *Staphylococcus aureus* colonizes and exacerbates AD, however, it is unclear whether *S. aureus* contributes to the pathogenesis of AD in the context of filaggrin mutations. To model AD-like skin inflammation, we performed scalpel cuts on the backs of filaggrin-deficient (ft/ft) and wt (Balb/c) mice (which are Th2 biased) to mimic skin injury from scratching in AD patients. Three weeks following skin injury, ft/ft mice but not wt mice developed AD-like skin inflammation at the site of injury (clinically: erythema, scale; histologically: acanthosis and dermal inflammation with influx of eosinophils, neutrophils and mast cells). Of note, histologic analysis of lesional and non-lesional skin revealed constitutive IL1β expression in the epidermis of ft/ft but not wt mice, suggesting a potential role for IL-1β in mediating AD-like skin inflammation. To determine the role of *S. aureus* in contributing to the AD-like skin inflammation, *S. aureus* (SF8300, $3x10^6$ CFU) was inoculated onto the injured skin. Interestingly, the combination of skin injury and *S. aureus* resulted in AD-like inflammation in wt mice but did not alter the AD-like skin inflammation induced by skin injury alone in ft/ft mice. Taken together, skin injury alone was sufficient to induce AD-like inflammation in the setting of filaggrin deficiency whereas the combination skin injury and *S. aureus* inoculation resulted in the development of AD-like inflammation in wt mice. These results suggest that skin injury may be a more important determinant than *S. aureus* colonization for the development of AD in patients with filaggrin mutations.

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Integration of IL17 and TNF responses in human keratinocytes

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Psoriasis is a chronic inflammatory disorder characterized by hyperproliferation of keratinocytes and consequent development of red scaly skin plaques. The pro-inflammatory cytokines IL17 and TNF are strongly implicated in psoriasis, most directly by the clinical efficacy of anti-TNF and anti-IL17 biologics. TNF and IL17 are proposed to act synergistically to induce a gene expression profile in keratinocytes that shares significant overlap with the transcriptome of psoriatic lesions. However, there are several potential points of cross-talk between TNF and IL17 signaling pathways and the mechanism of TNF and IL17 synergy remains unclear. Here, we demonstrate directionality within this synergy, wherein IL17 augments TNF-induced gene expression in human keratinocytes. The effects of TNF are shaped by the chronicity of stimulation: the duration of keratinocyte exposure to TNF can drastically alter the magnitude and kinetics of pro-inflammatory gene expression. Consequently, a single brief exposure to TNF results in a short-lived transcriptional response in cultured keratinocytes. Simultaneous exposure to IL17 drastically alters the response of keratinocytes to TNF stimulation. As a result, production of pro-inflammatory factors by keratinocytes exposed to brief pulses of TNF in the presence of IL17 mimic the response to sustained, high-level TNF stimulation. This enhanced response is independent of IL-17 effects on NF-kB activation. Instead, we find that the IL17 increases stability of several TNF-induced transcripts that are known to be upregulated in psoriasis. These findings provide a basis for understanding why TNF and IL17 are necessary but not sufficient for psoriasis pathogenesis and highlight the potential of developing anti-psoriatics that spare normal, protective, biological functions of cytokine by targeting mechanisms of cytokine synergy

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Expression of toll like receptors 3, 7, 8, and 9 in peripheral blood mononuclear cells from patients with psoriasis

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This study was to evaluate gene expression level of intracellular TLR in peripheral blood mononuclear cells (PBMC) from psoriatic patients. Changes in TLR3, TLR7, TLR8 and TLR9 expression were evaluated using quantitative real-time reverse transcription polymerase chain reaction and flow cytometry in PBMC from 22 patients with psoriasis and 5 healthy controls, along with changes in inflammatory cytokine levels. The gene expression of all TLRs evaluated were significantly augmented in psoriatic patients compared with controls, and patients with more recent aggravation showed more up-regulation of TLR. Cytokine levels, including TNF-α, IFN-γ, IL-10, IL-12, IL-17A, IL-22 and IL-23, were significantly increased compared with healthy controls, while increased IL-6, IL-8, and IL-21 level was not statistically significant. Increased expression of intracellular TLRs, TLR3, TLR7, TLR8 and TLR9 on PBMC in psoriatic patients and clinical correlations together with inflammatory cytokine profiles suggest that the innate immune response involving TLRs could play a role in the pathomechanism of psoriasis.

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Bacteria in the human skin microbiome mediate glycerol fermentation against *Malassezia furfur*

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Malassezia furfur (M. furfur), a fungus in the human skin microbiome, is found growing on the skin of most adults. By fingerprint analysis, we have identified two skin bacteria that can fermentatively metabolize glycerol and create inhibition zones against *M. fufur*. Co-culture of skin bacteria with *M. furfur* in the presence of glycerol *in vitro* hinders the growth of *M. furfur*. By nuclear magnetic resonance (NMR) analysis, we found that acetic and butyric acids are two major short-chain fatty acids (SCFAs) in the fermentation media of skin bacteria. Butyric acid, but not acetic acid, increases the acetylation levels of Histone 3 at Lysine 9 (*AcH3K9*) in primary human keratinocytes. Treatment of human keratinocytes with butyric acid markedly reduces the level of *interleukin* (IL)-1β. Results from the minimal bactericidal concentration (MBC) assays demonstrate that both acetic and butyric acid display excellent anti-fungal activities against *M. fufur. By performing the 16S rRNA and 28S rDNA sequencing, we have identified four bacteria and five fungi including M. furfur* coexisting in human dandruff. Incubation of butyric or acetic acid with dandruff significantly decreases the growth of fungi in dandruff. Our study emphasizes the probiotic activity of bacteria in the human skin microbiome against *M. fufur*, which has been linked to several skin diseases, including seborrheic dermatitis, folliculitis, confluent and reticulated papillomatosis, and pityriasis versicolor. SCFAs and their analogs as novel anti-fungal agents with high potential for treatment of *M. fufur*-associated skin disorders.

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Unprocessed IL-36α regulates psoriasis-like skin inflammation in co-operation with IL-1α

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Psoriasis has been linked to overzealous IL-1 and IL-36 signaling; however, whether these cytokines have redundant, interrelated or independent functions have not been examined. It is also unknown if the IL-36 cytokines undergo processing *in vivo*. Using the imiquimod-induced skin inflammation model, we previously showed that IL-1α, via the receptor IL-1R1, plays an important role in initiating psoriasiform disease. Here, we examined IL-36 protein expression, functional involvement and interaction with IL-1α/IL-1R1 signaling. Normal skin did not express detectable levels of IL-36α, IL-36β, or IL-36γ. Following imiquimod treatment all 3 cytokine mRNAs were up-regulated; however, only IL-36α secretion could be detected in explant skin cultures. Significantly less (25%, $p<0.01$) was released from IL-1R1 deficient skin. Surprisingly, the released IL-36α migrated in SDS-PAGE similar to a full-length recombinant form suggesting that IL-36α does not undergo proteolytical processing. Using knockout (KO) mice for each individual IL-36 cytokine, we found that IL-36α, but not IL-36β and IL-36γ, significantly contributed to pathology. Specifically, we observed thicker epidermis (approximately 2-fold, $p<0.01$) and increased dermal edema (1.5 fold, $p<0.05$) and inflammation in wild type compared to IL-36α KO mice. Neutrophil recruitment to the epidermis was impaired in the IL-36α KO mice, but could be rescued with application of the chemokine CXCL1. IL-36α also regulated IL-1α expression through a feedback mechanism. To examine interactions between IL-1 and IL-36α signaling, we generated IL-1R1/IL-36α double KO mice. After imiquimod challenge epidermal and dermal thickness was significantly greater ($p<0.05$) in single than double KO mice. This demonstrates that IL-1α and IL-36α augment the psoriasis promoting activity of the other cytokine. In summary, we conclude that IL-1α and IL-36α form a self-amplifying inflammatory loop that in patients with insufficient counter regulatory mechanisms may become hyper-engaged and/or chronic.