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Neutrophil extracellular traps and type 1 IFN contribute to autoimmunity in hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a neutrophilic inflammatory skin disorder with an unknown etiology primarily affecting intertriginous areas.Considering the predominant cellular infiltrate, we sought to understand the role of neutrophil extracellular traps (NETs) in HS.In peripheral blood samples from HS patients, neutrophils had enhanced NETosis and WB analysis revealed that these NETs possessed proteins recognized by autoantibodies (AAbs) present in HS serum, namely antibodies against IL17B.Furthermore, serum from HS patients had significant titers of total IgG and contained AAbs against citrullinated proteins, including filaggrin, vimentin and enolase corresponding to levels detected in sera from patients with rheumatoid arthritis ($p<0.05$).Moreover, NETs were confirmed in HS tissue via immunofluorescent detection of citrullinated histone 4 (cit-H4). With ELISA, HS tissue homogenates revealed a positive correlation of detected cit-H3/double-stranded DNA complexes with disease stage ($r^2=0.7107$, $p=0.0043$).Finally, HS tissue displayed a significant upregulation of type I interferon (IFN) genes.Taken together, these results suggest unreported roles of autoimmunity and neutrophils in the pathogenesis of HS, identifying NETs as a source of AAbs and the type I IFN signature in HS tissue, which could impact alterations in therapeutic approaches.

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Lack of skin mast cell activation and sphingosine-1-phosphate elevation in male mice may explain gender disparity observed in pre-symptomatic atopic dermatitis

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We recently reported the essential roles of mast cells (MC) and sphingolipid metabolite sphingosine-1-phosphate (S1P) in pre-lesional skin remodeling observed in female mice, using a human atopic dermatitis (AD)-like preclinical model. In human adults, females have a greater propensity to develop AD than males. Accordingly, most AD mouse models only utilize females as males do not exhibit AD-like changes. We previously showed epidermal and dermal thickening with cellular infiltration that occurred in the hypodermis of female mice after a single exposure to antigen ovalbumin (OVA), compared to controls and prior to IgE elevation. Using male mice in a similar preclinical model, we observed hypodermal cell infiltration after OVA exposure although to a lesser extent than in female mice ($p \leq 0.0001$), but no skin layer thickening or increased skin S1P levels, compared to female mice. Moreover, the number of activated skin MC was not increased in male mice, as opposed to female mice. The current work supports our previously reported results establishing MC as major effectors of remodeling in pre-lesional AD. In sum, we identified that the absence of local mast cell activation and elevated S1P levels observed in male mice may explain gender differences at the onset of AD.

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Assessment of the anti-inflammatory effects of cannabidiol and its fluorinated derivative in *in vitro* and *in vivo* models of atopic dermatitis

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Fluorination can significantly increase the efficacy of the active components in pharmaceuticals The aim of the study was to assess the potential anti-inflammatory effects of cannabidiol (CBD), the major non-psychoactive component of the plant *Cannabis sativa*, and its fluorinated derivative (HUF-101) in various experimental systems modeling atopic dermatitis (AD). For the *in vitro* AD model, keratinocytes were challenged with the combination of *Staphylococcus aureus* enterotoxin B and thymic stromal lymphopoietin and expressions of certain marker molecules were assessed by RT-qPCR and ELISA. For the *in vivo* model, mice were sensitized with 2% oxazolone (OXA) before elicitation. Test compounds were applied topically (1 and 10 µM) after inducing skin inflammation and edema formation (in the ears) was measured with a micrometer. In the *in vitro* model, expressions of certain pro-inflammatory cytokines (e.g. interleukin [IL]-1α, IL-1β, IL-6 and IL-8) were significantly down-regulated upon the administration of CBD and HUF-101. However, HUF-101 exhibited significantly higher potency in comparison to CBD. In the *in vivo* model, topical application of 1 µM CBD significantly reduced the OXA-induced ear edema; however, 10 µM CBD exerted insignificant effect. In contrast, HUF-101 attenuated OXA-induced edema formation at both concentrations. Intriguingly, the anti-inflammatory potency of HUF-101 was significantly greater than that of CBD. Our study provides the first evidence that CBD and its fluorinated derivative exert significant anti-inflammatory actions in models of AD. Further pre-clinical/clinical studies are needed to exploit the therapeutic potential of certain CBD derivatives in inflammatory skin conditions.

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Origin of type 2 innate lymphoid cells in the skin

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Type 2 innate lymphoid cells (ILC2s) are a recently discovered subset of immune cells that have been found to be an important source of the cytokines that characterize parasitic infections and allergic immune responses. Accordingly, ILC2s have been shown to contribute to the immune response in mouse models of allergy and atopic dermatitis, but the details of where and when ILC2s originate are poorly understood. To determine the proportion of skin ILC2s that are locally derived, we surgically joined pairs of congenically distinct mice so as to allow sharing of blood circulation. Results from these parabiosis experiments were consistent with tissue residency and limited circulation of skin ILC2s. To determine the origin of adult skin ILC2s, we used a lineage-tracing strategy using two independent strains of tamoxifen-inducible Cre mice to mark the progeny of cells based on the timing of tamoxifen administration. Results from prenatal administration of tamoxifen showed that while a small fraction of adult skin ILC2s are embryonically derived, the majority of skin ILC2s are derived postnatally. Results from postnatal administration of tamoxifen showed a significantly higher fraction of lineage-traced ILC2s, but in contrast to other tissues in which the ILC2 pool is relatively stable throughout adulthood, skin ILC2s were replaced by *de novo* ILC2s at a faster rate, perhaps reflecting responses to inflammatory signals from from microbial or environmental stimuli. The origin of ILC2s has implications for the timing of therapies targeting these cells, and further work will be necessary to determine factors contributing to the turnover of these potent mediators of type 2 immune responses.

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Identification of mycobacteria species in skin tissue using amplification and melt curve analysis of the *hsp65*-gene

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Species identification of mycobacteria by molecular methods in formalin fixed, paraffin embedded skin tissue has been suboptimal. Conventional microbiological culture is sensitive but requires weeks, and traditional species-level identification is challenging. Patients that received culture for mycobacteria and histopathology of skin biopsy were studied (2011-2017). Culture was performed on automated BACTEC-MGIT broth incubation system. RT-PCR melt curve assay of the *hsp65*-gene was performed on broth following growth. Consensus primers amplified the *hsp65*-gene, and fluorescence resonance energy transfer probes were used. Conventional identification from solid media was also done. We identified mycobacterial growth in 27 of 944 (2.86%) unique patients. The number of tissue specimens per-patient ranged from 1-21. The mean number of samples from positive patients was 2.13 (95% CI:1.133.12). Mean age of patients with positive culture was 62.3±18.5 years and no gender differences where observed. The cutaneous location of infection were fingers 18.5%, feet 14.8%, arms 14.8%, legs 11.1%, neck 11.1%, forearms 7.4%, abdomen 7.41%, hands 3.7%, and back 3.7%. Histopathology revealed supurative granuloma 48.1%, non-supurative granuloma 18.5%, and mixed inflammation 66.6%. Paucibacillary disease was seen in 85.1%. The rapidly growing species identified were *M. chelonae* 29.6%, *M. abscessus* 14.8%, *M. fortuitum* 11.1%, and *M. goodii* 3.7%. The slowly growing species were *M. avium-intracellulare* complex 14.8%, *M. marinum* 14.8%, *M. tuberculosis* complex 7.4%, and *M. kansaii* 3.7%. PCR melt curve analysis from MGIT broths provided rapid identification 8-24 h, following positive culture. For comparison, growth and species level identification can require weeks using conventional methods. Thus, the use of this assay provides rapid and accurate species identification allowing for more timely initiation of therapy.

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Distinct *Cutibacterium acnes* strains isolated from lesional and non-lesional regions of acne promote differential immune responses

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Cutibacterium acnes (AKA *Propionibacterium acnes*), (PA), is a ubiquitous skin commensal that is tolerated by the immune system of healthy human skin. There is evidence to suggest a crucial role for strain-specific actions of PA during acne pathogenesis. We investigated the population structure and functional diversity of PA strains by swabbing surface skin of healthy volunteers as well as lesional and non-lesional sites of acne patients with moderate to severe acne. Inflammatory potential was assessed by measuring IL-8 secretion from SEB-1 sebocytes and human primary keratinocytes exposed to sterile supernatant of PA isolates grown anaerobically with a lipid substrate. Strain-level identity of PA was determined by single locus sequence typing (SLST). A significantly higher frequency of phylotype IA-1 strains, namely C1 and C2 sequence types (STs) were found from lesional sites of acne subjects compared to non-lesional sites. C1/C2 STs were undetectable from healthy skin. In contrast, sequence types K1 and K2 were found exclusively on healthy and non-lesional skin, but absent from acne lesional sites. Whereas K1/K2 dominated on healthy skin, they promoted greater IL-8 production in sebocytes and keratinocytes compared to lesional C1/C2 STs. Although glycerol utilization differed greatly between strains, GC-mass spec analysis of bacterial supernatants revealed that all PA strains produced similar amounts of propionate, acetate and isobutyrate, and all PA strains tested similarly enhanced H3K9 acetylation in SEB-1 cells. Since clonal isolates within the same sequence type strain had distinct functions, higher resolution whole genome sequencing is being performed to resolve the PA genetic associations with this functional difference. We conclude that the genetic basis for differential immune responses to PA cannot be resolved by sequence typing, and regional variation of PA strains within an individual is an important and previously unrecognized element to understand the physiological relevance of the acne-associated microbiome.