8-1-2022

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Hidradenitis suppurativa is characterized by suppression of antimicrobial effector perforin-2

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Hidradenitis suppurativa (HS) is characterized by dysfunctional immune response and robust bacterial colonization, however little is known about specific molecular events involved in disease development and progression. We previously demonstrated essential role of antimicrobial effector Perforin-2 (P-2) in the cutaneous barrier repair and control of wound infection. Here we evaluated ability of HS tissue to respond to wounding and infection through *ex vivo* wound model and cell-specific evaluation of P-2. HS tissue samples (n=14) were collected during standard of care procedures. Location-matched healthy skin specimens were used as controls (n=3). H&E staining was performed to evaluate histopathology. We aimed to establish *ex vivo* HS model and compare wound healing rates between lesional and location-matched control skin. Wounds were created through the epidermis with 3mm punch, maintained at air liquid interphase and rate of re-epithelialization was evaluated by histomorphometry. Moreover, cellular composition of the immune cell infiltrates and the expression of P-2 was determined by FISH-Flow and flow cytometry. Histopathology of HS tissue confirmed epidermal hyperplasia, elongated rete ridges, dermal inflammation, and fibrosis. Inflammation was confirmed by flow cytometry; HS lesions had higher frequency of multiple cell subsets including CD8+ T cells, GD T, B cells, macrophages, and neutrophils. However, we found suppression of P-2 in all cell subsets. High level of inflammation was also accompanied by lack of re-epithelialization in *ex vivo* wound healing model. Our data show inhibition of re-epithelialization, increased inflammation and suppression of P-2 in the tissue from HS patients. Newly established *ex vivo* HS model can enable pre-clinical testing of novel treatments, including targeting P-2. A thorough understanding of the P-2 regulation in HS could be invaluable in the development of targeted treatments or the re-purposing of existing treatments for HS by inducing P-2.

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Using swabs and scanning electron microscopy (SEM) to detect biofilms in chronic epidermolysis bullosa (EB) wounds

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EB is a group of genetic disorders that cause skin and mucous membranes fragility resulting in blisters after minor mechanical trauma, which collapse/rupture, leaving open wounds. Currently, no cure is available and management focusses on wound care and managing complications. Whether skin microbiome exist in biofilms in EB remains to be explored, which we investigate in this study. Biofilms are microbial aggregates embedded in a self- or host-produced matrix along with inflammatory cells attached to a surface. Biofilms are resistant to antimicrobial therapies and may contribute to wound chronicity and impaired healing. Acquisition of skin biopsies, the gold standard method for biofilm diagnosis, from EB patients is challenging. We report the use of cotton swabs in an attempt to visualize biofilms by SEM. Swabs were obtained from wounds, post-irrigation with saline to remove planktonic microorganisms, from patients with different EB subtypes, whose wounds failed to heal for months/years but demonstrated no obvious signs of infection. Results demonstrated clear aggregated coccoid objects measuring 0.2-0.5µm in diameter suspended in a matrix along with inflammatory cells. Besides, yeast-like structures were found forming aggregations within the previously mentioned matrix. The images imply for the first time the presence of intricate biofilms in chronic EB wounds, which may contribute to wound chronicity. Moreover, wound swabs offer a promising alternative to more invasive tissue biopsies. Further studies are needed to characterize the visualized biofilms in EB chronic skin wounds as this offers new promising therapeutic approaches in treating such recalcitrant wounds.

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Characterization of myeloid cell subsets in the tumor microenvironment of merkel cell carcinoma

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PD-1 pathway blockade has changed the landscape for advanced Merkel cell carcinoma (MCC) as over 50% of patients initially respond to therapy. However, for patients who do not respond (or later recur), there is a need for additional therapies. In the US, ~80% of MCC tumors are caused by the Merkel cell polyomavirus, while ~20% are caused only by UV-induced mutations. MCC tumors are thus highly immunogenic because they express either non-self viral antigens or numerous UV-induced neoantigens. Historically, studies have been focused on adaptive immunity, and little is known about innate immunity in MCC which may play an important role in immune evasion. Myeloid cells are heterogeneous with diverse lineages and roles in the tumor microenvironment (TME). It has been challenging to accurately identify specific myeloid cell subsets. We have employed recently available technologies to accurately identify and quantitate myeloid cells in the MCC TME, to assess whether they are associated with unresponsiveness to PD-1 pathway blockade. We performed single-cell transcriptional and cell surface protein (CITE-seq) analyses on PBMC and tumor samples from 8 MCC patients. We identified two major myeloid subsets in MCC tumors: tumor associated macrophages (TAMs) that expressed genes consistent with an "M2-like" signature and plasmacytoid dendritic cells (pDCs). Both of these myeloid subsets are associated with poor prognosis across other tumor types. To explore a possible association of these myeloid subtypes to response to PD-1 pathway blockade, we integrated relevant antibodies into multiplex immunohistochemistry panels and determined their expression in tumor samples obtained prior to PD-1 pathway blockade treatment. Studies are ongoing to determine if the presence of pDCs is associated with failure to respond to treatment. Presently, our findings suggest that TAMs are abundantly expressed in MCC tumors and may thus play a role in establishing an immunosuppressive TME that promotes immune evasion.

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Murine epidermis harbors functionally distinct langerhans cell subsets

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Epidermal Langerhans cells (LCs) derive from embryonic myeloid progenitors at the steady-state and monocyte progenitors under inflammatory conditions. LCs have the capacity to induce both immunity and tolerance in the skin, but how a single population of LCs mediates both these functions has perplexed researchers for decades. We hypothesized that LCs in murine epidermis have functionally heterogenous subpopulations. We employed single-cell RNA sequencing (scRNAseq) and scATACseq to identify transcriptional and epigenetic heterogeneity in LCs during late embryonic development, adult steady-state and inflamed-state. We found three transcriptionally distinct clusters in adult at steady-state: $ATF3^{hi}CD207^{lo}$ (cLC1), $ATF3^{lo}CD207^{hi}$ (cLC2), and $CD207^{+}$ cells expressing keratinocyte (KC) genes (kLCs). Ingenuity pathway analysis showed LC1 had downregulated immunostimulatory pathways and LC2 had upregulated immunostimulatory pathways. LCs from ATF3 knockout mice promoted Th1/2/17 immunity in co-culture experiments, confirming the immunotolerant function of cLC1s. cLC1 and cLC2 clusters had corresponding scATACseq clusters but kLCs did not, suggesting that kLCs may acquire their KC-"fingerprint" through interactions with KCs. scRNAseq analyses of E18.5 pre-LCs and 3 weeks post UVC-treatment also identified $ATF3^{hi}$ and $ATF3^{lo}$ clusters, but kLCs were neither present at E18.5 nor after UVC treatment. Overall, our single cell analyses uncover murine epidermal LC subsets with distinct functions during late embryonic development, steady-state and inflamed-state.

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Chronic wound environment shapes virulence of human commensal bacteria

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Staphylococcus epidermidis is one of the most abundant skin-commensal known to modulate cutaneous immune response. Emerging evidence suggests S. epidermidis isolates from healthy skin improve barrier integrity and response to wounding. However, S. epidermidis could also carry a reservoir of antimicrobial resistance genes(ARGs), adding this microbe to the list of "accidental" pathogens. Hence, we aimed to characterize S. epidermidis isolates from healthy skin and chronic wounds (CW) to evaluate their virulence potential and effect on wound healing. Shotgun metagenomic sequencing was performed to analyze presence of ARG and virulence genes in isolates from both environments. Furthermore, antimicrobial susceptibility was tested using the microdilution method. To assess virulence traits of selected isolates, biofilm formation and adhesion to components of the extracellular matrix (ECM) were performed. Human *ex vivo* wound model was used to assess the effect of S. epidermidis isolates on healing. Results pointed to the prevalence of ARG in S. epidermidis isolates from CW associated with gentamicin, ampicillin, erythromycin, norfloxacin, tetracycline, and trimethoprim resistance. This was functionally confirmed, chronic wound isolates showed higher minimal inhibitory concentration (MIC) values for these antibiotics and for benzalkonium chloride, a widely used disinfectant. All CW strains exhibited a higher ability to bind to ECM components compared to healthy skin strains. This feature of CW isolates correlates with their high biofilm formation potential in both *in vitro* and *ex vivo* assays. Infection of human *ex vivo* wounds showed increased accumulation of CW isolates in the wound bed suggesting the strong ability of *in vivo* biofilm formation. Our study suggests that CW microenvironment influenced selection of S. epidermidis strains with prevalence of ARG and capacity to bind to ECM and form biofilm. Our data reflects the dangers of antibiotic overuse due to the frequency of antibiotic resistance and virulent potential of S. epidermidis strains found in CW.

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Commensal induced accumulation of monocyte-derived cells in neonatal skin regulates long-term cutaneous type 17 inflammation

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Early life immune interactions help shape longer-term skin health and homeostasis. Prior work has shown that commensal microbes facilitate neonatal skin accumulation of innate and adaptive T cells, thereby promoting fundamental needs such as immune tolerance to commensals and wound healing. Comparatively little is known about commensal-myeloid cell crosstalk in neonatal skin and the functional consequences of these interactions. Using mass cytometry, we surveyed the longitudinal composition of the myeloid cell compartment in murine skin from D6 to D30 of life, in SPF, gnotobiotic and conventionalized animals. This revealed classical monocytes to be a population uniquely enriched in the skin of microbially replete versus germ-free neonates. Corroborative studies revealed that skin monocytes rapidly accumulate between D1 and D3 of life, after which their numbers gradually decline. This early monocyte wave was prevented in antibiotic-treated SPF pups as well as in $Myd88^{-/-}$ but not $IL1R1^{-/-}$ mice, suggesting a key role for tonic toll-like receptor signaling in their accumulation. To dissect the functional relevance of these cells in cutaneous biology, we developed an antibody-based regimen to temporarily deplete monocytes in the first two weeks of life (NeoDmono). scRNA sequencing revealed a heightened type 17 signature in skin T cells from D15 NeoDmono mice. Flow cytometry assays confirmed sustained elevation of IL-17A production by NeoDmono skin T cells through adulthood. This reflected a heightened response to commensal microbes as IL-17 production was significantly reduced in antibiotic-treated NeoDmono mice. While there was no visible skin pathology in NeoDmono mice under homeostatic conditions, imiquimod treatment of the ears in adulthood led to significantly increased ear swelling and neutrophils. Taken together, our data demonstrate a previously unappreciated, commensal-dependent regulatory imprinting function of cutaneous classical monocytes in the early life window.