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IL-15/IL-15Rα signaling is a guardian of human hair follicle immune privilege and promotes hair growth

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Though these drugs tend to be effective alopecia areata (AA) therapeutics, AA quickly relapses after discontinuing treatment with JAK1/3 inhibitors (JAKi). One reason could be that JAKi also block the signaling of AA-protective cytokines that signal via the common γ-chain, which is also used by interleukin-15 (IL-15). IL-15 is known to suppress mouse HF apoptosis, promote murine HF growth *in vivo*, and is required to maintain (AA-protective!) iNKT cells. Moreover, it is unclear how IL-15 affects human hair follicle (HF)-immune privilege (IP) and catagen development, namely via signaling through its private receptor, IL-15Rα. To clarify this, organ-cultured, healthy human anagen scalp HFs were treated with rhIL-15 before or after IFNγ-induced HF-IP collapse. This showed that IL-15 protects from HF-IP collapse, restores HF-IP (assessed by MHC class Ia, MICA, and α-MSH quantitative immunohistomorphometry), and retards catagen development. Instead, IL-15Rα silencing *ex vivo* induces premature catagen, inhibits hair matrix proliferation and weakens the HF-IP (=increased expression of MHCIa/β2-mg; decreased production of the HF IP guardians, α-MSH and TGFβ2). Moreover, lesional skin of AA patients showed significantly reduced protein expression of both, IL-15Rα and the common γ-chain, not only in lesional but already in non-lesional AA HFs, indicating reduced sensitivity to IL-15 signaling in AA patients. Thus, contrary to often reverberated misconceptions, IL-15 also promotes human hair growth and acts as a human HF IP guardian (but might simultaneously promote selected AA-related immune responses). Thus, selectively stimulating *intrafollicular* IL-15Rα signaling could effectively promote hair (re-)growth and prevent rapid AA relapse after discontinuation of JAKi therapy.

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Tyrosine Kinase 2 Inhibition Ameliorates the Phenotype of Lesional Alopecia Areata Scalp Skin Ex Vivo, and Reverses the Induction of Human Alopecia Areata in a Humanized Mouse Model

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Alopecia areata (AA) is an immune-mediated hair follicle (HF) disorder induced by elevated IFNγ levels and a Tc1-driven inflammatory response. We have previously shown that treatment with IL-12 and IL-18 induces IFNγ-dependent, immune privilege collapse of the hair bulb, which can be inhibited by blocking IL-12 receptor signaling with the TYK2 inhibitor, BMS-986202 (BMS), in human microdissected HFs. Here, we further investigate the role of TYK2 and its inhibition in AA pathogenesis. We cultured human full thickness non-lesional and lesional scalp skin biopsies from acute or chronic AA patients *ex vivo* with BMS. In addition, oral treatment with BMS or tofacitinib was performed in the well-established humanized mouse model for AA. Samples were then processed for quantitative (immuno-)histomorphometry. BMS treatment prolonged anagen in non-lesional and lesional HFs of acute AA scalp biopsies, and reduced expression of MHC I and number of MHC II^+ cells in the bulb of acute lesional samples. Moreover, it diminished the number of intra- and peri-follicular $CD3^+$ T cells in unstimulated acute, and unstimulated/stimulated chronic, lesional AA scalp biopsies. IFNg release was decreased in the presence of BMS in the culture medium of stimulated acute and chronic lesional biopsies. In the humanized mouse model, treatment with BMS or tofacitinib significantly increased hair shaft numbers and microscopically detected anagen I-VI HFs, reduced HM keratinocyte apoptosis and HF dystrophy, decreased MHC class I and II expression, and diminished numbers of MHC class II^+ cells and infiltrating $CD3^+$ T cells. Taken together, our results suggest TYK2 inhibition is a novel, pharmacological strategy for AA management, deserving clinical exploration.

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Clonal sharing of skin T cells within different skin compartments and blood

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The human skin harbors a heterogenous pool of cytotoxic tissue-resident memory T (TRM) cells seeded during infections and inflammation. TRM cells are retained at the site of viral infections and provide local immunity. These cells persist for years in the skin after bone marrow transplantation but circulating ex-TRM cells indicate several potential fates. Here, we explored the clonal relationships between human T cells in different skin compartments and circulation. 10x Genomics 5 prime immune profiling coupled with feature barcoding technology was employed to investigate the clonal relationship, transcriptomic and proteomic profiles of single T cells sorted from blood, subcutis, dermis, and epidermis. A few CD8+ TRM cell-clones dominate epidermis and cytotoxic CD8+CD103+ CD49a+ showed the least diverse T cell receptor (TCR) repertoire. Clonal overlap was found between all three compartments: circulating blood T cells, dermal and epidermal TRM cells. The highest degree of overlap was detected between epidermis and dermis. Clones overlapping was scarce between blood and highly abundant epidermal clones. Our results suggest that lowly abundant skin clonotypes have a circulatory counterpart, which can react to reinfection at secondary sides, whereas highly abundant epidermal clones might be skin specific.

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PC111, a novel, First In Class, human anti-FasL antibody for the treatment of Pemphigus: a translational study

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Pemphigus is a rare and debilitating autoimmune blistering disease due to keratinocyte cell-cell detachment (acantholysis). Treatments focus on immune suppression but are often associated with severe side effects, slow onset of action and frequent clinical relapses; therefore, innovative and non-immunosuppressive therapies are needed to provide rapid, safer and long-lasting responses. Patients' autoantibodies (PVIgG) are fundamental for initiating the pathologic mechanisms of pemphigus. We have recently demonstrated that soluble Fas Ligand (sFasL), that is massively up-regulated and released from keratinocytes upon PVIgG binding, is essential for blister formation. In fact, FasL/Fas binging activates caspases, which degrade desmogleins thus inducing acantholysis. Furthermore, animals that specifically lack sFasL are protected from PVIgG-induced acantholysis. Against this background, we have developed PC111, an anti-FasL human IgG4 monoclonal antibody with high affinity to human FasL (KD<500 pM) and potent inhibitory activity in human keratinocyte acantholysis assays (IC50 < 0.05 μg/ml). While the use of a corresponding mouse antibody against murine FasL blocked blister formation in passive transfer and active pemphigus mouse models, we now confirm protective effects of PC111 in two different ex-vivo pemphigus models. Firstly, a subcutaneous injection of PC111 reduced blister formation up to 50% in a dose dependent manner in an anti-desmoglein 1 and 3 scFv-induced model. Secondly, we demonstrated a very potent inhibitory effect of PC111 in blocking blister formation in a PVIgG-induced model. These initial results indicate that PC111 is a promising clinical candidate for the treatment of pemphigus. To further test the efficacy of PC111, we have developed a new murine model, by swapping the FasL murine gene with the human one, to be used as a platform for further in-vivo PoC studies.

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Single-cell T cell receptor (TCR) repertoire of skin and blood reveals skin-specific characteristics in health and HIV infection

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Human skin harbours a large proportion of the resident memory T (Trm) cells of the body. Trm cells promote immune homeostasis and exert anti-microbial and anti-cancer function in tissue. We recently demonstrated that skin Trm cells are permanently depleted in people living with HIV (PLWH) with a low nadir and possibly replaced by blood-derived T cells. We linked the loss of Trm cells in PLWH with the increased risk of developing cutaneous and mucosal cancer. The T-cell receptor (TCR) diversity analysis with next generation sequencing might give new insights into the biology of skin Trm cells at homeostasis and in HIV. We investigated the TCR profile of skin- compared to blood-derived T cells in healthy controls (HC) and PLWH (n=5). We first performed bulk RNA-sequencing to study diversity, richness and clonality distribution among the study groups. We then continued with single-cell RNA-Sequencing (scRNA-Seq) coupled with single-cell TCR-Sequencing (scTCR-Seq) to get more insights into the distribution of TCR clones in the different skin and T cell clusters. We observed that only few clones are shared between skin and blood in healthy controls (8.39%), suggesting that Trm cells are quite self-sustained with scarce immune interactions with other compartments. On the contrary, the percentage of TCR clones shared between skin and blood circulating T cells in PLWH was 24.5% and mostly represented by effector memory CD8+ T cells. Further analysis suggested an oligoclonal expansion of the TCR repertoire in CD8+ T cells of PLWH. Considering the increased risk in PLWH for psoriasis and cancer, we believe that the ongoing analysis will bring new insights into the pathogenesis of inflammatory skin diseases and cancer susceptibility of barrier organs.

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Imiquimod perturbs amino acid metabolism in human $CD8^+$ T cells

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Imiquimod (IMQ), a TLR7 agonist, is a standard local treatment for non-melanoma skin cancers (NMSC). IMQ triggers inflammation, ultimately resulting in immunological tumor destruction. Albeit IMQ promotes the recruitment of effector T (T_E) cells, IMQ triggers anergy in $CD4^+$ T cells. Meanwhile, how IMQ affects human $CD8^+$ T_E cells, pivotal to immunological cancer control, remains unclear. To address this, we studied $CD8^+$ T_E cell lines from NMSC and circulating, skin-homing CLA^+CD8^+ T_E cells. In both models, IMQ-treated T_E cells showed significantly reduced proliferation and IFN-γ production. Because metabolism fundamentally underlies the function of T cells, we hypothesized that metabolic changes underlie the suppressive effects of IMQ on T_E cells. Along this idea, we found that IMQ-treated T_E cells have reduced mTOR activity. To gain broader insight into metabolic adaptations of IMQ-treated T cells, we performed proteomics, revealing dysregulation of amino acid (AA) transporters, especially the SLC1A5 transporter in IMQ-treated $CD8^+$ T_E cells. Meanwhile, IMQ reduced intracellular levels of AA transported by SLC1A5, including glutamine, asparagine, aspartic acid. Consistent with a known supportive role of several AA, including glutamine and asparagine, in T_E responses, adding these AA to IMQ-treated T_E cells restored effector functions. Our finding that SLC1A5-dependent AA rescue IMQ-induced hypo-responsiveness of $CD8^+$ T_E cells provides a rational for studying if exogenous AA can improve effectiveness of IMQ-based destructive NMSC therapies.