**ABSTRACT: Adaptive and Auto-Immunity**

**031** Single cell transcriptomic analysis of cutaneous T cells in psoriasis  
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Psoriasis is a chronic inflammatory skin disease characterized by the dysregulated expression of cytokines and chemokines that affects 125 million people worldwide. However, the underlying pathways contributing to psoriasis pathogenesis have not been fully elucidated. This project utilizes single-cell transcriptomics of T cells from healthy and psoriatic skin in an effort to identify key biomarkers and pathways of psoriasis. T cells were clustered into subsets and differential gene expression analysis was performed between lesional and healthy skin to identify psoriatic marker genes in each T cell subset. Regulatory CD4+ T cells in psoriatic lesional skin were found to be enriched for transcriptional programs, such as IL-7R, that are associated with tissue repair and regeneration. The identification of these specific transcriptional programs will provide insights into the mechanisms underlying psoriasis pathogenesis and potential targets for therapy.

**032** A multiplexed skin-targeted COVID-19 vaccine elicits robust humoral and cellular immune responses  
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Considerable progress has been made toward development of COVID-19 vaccines in the past year. However, there is still a need to understand the immune response to SARS-CoV-2 vaccines to enable sustainable immunization programs against COVID-19. To address this need, we hypothesized that harnessing the immune-responsive cutaneous microenvironment using microarray patches (MAPs) to deliver integrated SARS-CoV-2 vaccine components would bring together biologic advantages of targeting the endogenous immune circuitry of the skin with a more user-friendly cutaneous vaccine delivery platform. We show that immunologically rich cutaneous microenvironments in both murine and human skin evoke potent adaptive immune responses when exposed to MAPs delivering a recombinant SARS-CoV-2 protein antigen, with or without an innate immune agonist. Immune response to vaccine-loaded MAPs generates robust antibody and cellular immune responses, and multiplexed (antigen plus adjuvant) MAP vaccination obviates the need for additional vaccine components, such as adjuvants, and IgG2c responses, which are vital for control of SARS-CoV-2 viral infection. Notably, multiplexed MAP vaccination results in increased immune responses compared to immunization via traditional intramuscular injection, and MAP immunization obviates adverse effects of intramuscular delivery of adjuvants, suggesting improved safety and efficacy compared to conventional vaccination routes. These results are supported by our translational studies utilizing freshly excised human skin, suggesting that multiplexed MAPs induce greater expression of co-stimulatory molecules by human skin-migratory DCs, which may contribute to enhanced immune responses. Ultimately, the simplicity, cost-effectiveness, immuno-modularity, and versatility of MAPs may enable novel vaccination strategies and incorporate more patient data.

**033** IL-15 is an unexpected guardian of hair follicle immune privilege and promotes human hair growth ex vivo  
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Successful, long-lasting alopecia areata (AA) therapy is required for re-initiating hair growth and for preventing relapse of AA. We show that IL-15+ cell number is increased while IL-15 receptor alpha protein expression is decreased in AA-affected human scalp HFs compared to healthy human scalp skin. When organ-cultured, healthy human anagen scalp HFs were treated with recombinant human IL-15 (15-15), anagen was significantly prolonged and hair matrix keratinoctyes apoptosis inhibited. Moreover, expression of MICA and MHC class I was reduced while hair bulb expression of the potent IP guardian, a-MSH, was increased by 50% and 100 mg/ml IL-15 ex vivo. Importantly, if IL-15 was administered before the HF IP collapse-inducing IFN-g, the increased expression of the NK2G2-activating “danger” signal, MICA, and MHC class I as well as the decreased expression of a-AH induction by IFN-g were all prevented. Taken together, despite its involvement in autoimmune diseases, IL-15 operates as an IP guardian and hair growth promoter in human HFs, while IL-13RAI signaling is detectable in AA. Therefore, selective stimulation, rather than inhibition, of IL-15RA-mediated signaling is likely to be beneficial in the future management of AA and possibly other inflammatory hair loss disorders.

**034** Dysregulation of VISTA expression and functionality in psoriatic monocytes and Mo-MDSCs  
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V-domain Immunoglobulin Suppressor of T cell activation (VISTA) is an inhibitory B7 family immune checkpoint molecule. VISTA is highly expressed on myeloid, hematopoietic and cancer cells and participates in T cell-mediated autoimmunity and antitumor immunity, playing a broad role in regulation of myeloid- and T cell-mediated immunity. VISTA is upregulated on myeloid-derived suppressor cells (MDSCs) from AML patients. We previously reported MDSCs are increased but functionally impaired in psoriasis (Pso), VISTA knock-out (KO) mice exhibit Pso-like inflammation. Whether VISTA signaling is related to Pso MDSC dysfunction is unknown. We analyzed delivery and healthy control (HC) monocytes (Mo) using flow cytometry. Mo-MDSC (CD14+HLA-DRneg) were elevated in Pso patients, and, as hypothesized, VISTA surface expression was elevated (1.6 ± 0.9 vs 13.2 ± 4.0 % of Mo in HC vs Pso, n = 4, p < 0.01). Immune signaling for human Mo activation via LPS attenuated VISTA gene expression in HC and Pso patients, suggesting VISTA expression is sensitive to inflammatory status. A novel VISTA ligand in V-Set and Immunoglobulin domain containing 3 (VSG-3) activity, is a functional role for VISTA in human Mo, we found that VSG-1 stimulation of CD14+ Mo decreases IL-6 in Pso patients. VSG-3 was less effective in reducing IL-6 in Pso-Mo compared to HC average IL-6 after VSG-3 relative to LPS alone of 66±7.7 % in HC versus a minimal effect on IL-6 of 89±7.0 % in Pso, n = 3, p > 0.05). Thus, in addition to T cell signals, VISTA expression/signaling is implicated in human Mo activation. In Pso, VISTA pathway targeting may represent a novel immune rebalancing approach in Psoriasis and related inflammatory diseases whose engagement inhibits T cell proliferation as well as cytokine production, demonstrated previously by VSG-3 inhibition of anti-CD3-induced IL-17 secretion in PBMCs.

**035** Expansion of bacterial phosphatidyglycerol reactive CD4+ T cells in atopic dermatitis  
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CD1a, a lipid antigen-presenting molecule structurally related to MHC class I, is constitutively expressed on Langerhans cells in human epidermis. Studies in recent years have suggested a pathogenic role for CD1a in inflammatory and allergic skin disease. We have recently detected a subset of CD1a-restricted CD4+ T cells that specifically responds to bacterial phosphatidyglycerol. In particular, lysophosphatidyglycerol (LPG), an aminoacylated membrane lipid present in many gram-positive bacteria, including S. aureus, binds to CD1a and is recognized by these T cells. Using CD1a tetramers loaded with LPG, we detected CD1a LPG staining T cells in the peripheral blood of multiple donors, and were able to isolate and expand these T cells in vitro. The majority of tetramer+ T cells were CD4+ T cells, and expanded in a dose-dependent manner to LPG. CD1a LPG reactive T cell lines showed a predominantly Th2 cytokine profile, with abundant IL-4 and IL-13 release. Beyond the recognition of purified lipid antigen, CD1a-LPG reactive T cells also responded to whole bacteria, as CD1a-expressing dendritic cells pre-incubated with S. aureus induced IL-13 release from the T cell lines. The increased bacterial skin colonization in atopic dermatitis (AD), specifically with S. aureus, prompted us to investigate the presence of CD1a-LPG reactive T cells in AD skin. A pilot study in AD patients showed a significantly increased frequency of CD4+ CD1a-LPG tetramer+ T cells in the blood of AD patients. Ongoing work aims to understand the contribution of CD1a-LPG reactive T cells to Th2 mediated pathology in AD.