Targeting keratinocytes to potentiate non-viral DNA skin immunization

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Skin is a uniquely accessible and responsive target for vaccine delivery. Emerging evidence suggests that keratinocytes can modulate skin immunity in response to diverse stimuli, producing either proinflammatory or immune suppressive mediators depending on the nature of the exogenous stress. To improve the immunogenicity of skin targeted vaccines, we engineered keratinocytes to support a proinflammatory local environment. Keratinocytes were genetically engineered to express the stress response transcription factor x-box binding protein 1 (XBP1). In a mouse model, keratinocyte-specific overexpression of XBP1 was transient and induced a proinflammatory skin microenvironment characterized by increased expression of proinflammatory cytokines, localized inflammatory infiltrates, including localized increases of dermal CD103+ DCs, XCR1+DCs, plasmacytoid DCs, γδ T cells, and group 1 innate lymphoid cells. Simultaneous non-viral delivery of plasmids driving expression of XBP1 and antigen OVA resulted in increased antigen expression and increased the induction of antigen-specific cellular and humoral responses, including durable antigen-specific skin-resident memory CD8 T cells and efficacious protective immunity, compared to delivery of antigen by traditional routes. This translated to improved survival in a ZIKV infection model, and improved therapeutic immunity in a clinically reflective endogenous melanoma model. Further, overexpression of XBP1 in keratinocytes in human skin resulted in a proinflammatory skin microenvironment. These findings support the feasibility of keratinocyte-targeted DNA vaccines to induce a proinflammatory skin microenvironment for effective immunization.

Highly Multiplexed Immunophenotyping of Dermatomyositis Skin Lesions

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Dermatomyositis (DM) is a systemic inflammatory muscle disease. The pathogenesis of cellular skin inflammation has yet to be investigated. Previous work revealed a type 1 interferon gene signature characterized predominantly by interferon-beta (β). To investigate the type 1 interferon signature, we identified pathways and cellular phenotypes in a subset of DM patients. Healthy controls (HC) and DM formalin-fixed, paraffin-embedded (FFPE) samples obtained from trunk, arm, or leg were stained with a panel of 35 metal conjugated antibodies. Regions of interest (ROIs) of 500x800μm were ablated at a frequency of 200Hz on the Hyperion Imaging System (Fluidigm). The resulting files were used for unsupervised clustering of cell populations after thresholding each channel. Statistical intensity (MPI) was gathered and analyzed using histoCAT. Positive and negative cell populations were performed using an app-based algorithm in Visiopharm. Per object mean pixel intensity (MPI) was gathered and analyzed using autoplate. Positive and negative comparisons were identified using a sliding scale for each channel. Phenograph analysis was used for unsupervised clustering of cell populations after thresholding each channel. Statistical analysis between groups was performed using the Mann-Whitney test all values reported as mean ± SFA. Skin lesions of DM patients contain an increased number of CD163+ cells compared to normal skin from HC patients (14.4±4.7 vs 2±1 cells/ROI, p<0.05). CD163+ cells had increased MPI of key inflammatory pathways: pSTING (34.4±4.9 vs 9±1.9), IFNβ (8.2±0.8 vs 4.4±2.7), and β8.17 (6±5.1 vs 1.3±1.0), all p<0.05. A population of CD4 cells was identified that produced higher IFNγ MPI compared to HC CD4 cells (16.4±4.5 vs 8±0.08β, p<0.01). Lesional DM skin also contained more FOXP3+ CD4 cells when compared to HC (6±4±23 vs 6±1±3 cells/ROI, p<0.05). The function of these cells is unclear. Compared to HC CD163+ cells in DM appear to be an important source of IFNγ via activation of the STING pathway. IFNγ is produced by both CD163+ and a subset of CD4 cells.

System approach to evaluate disease factors in pemphigus

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Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune blistering diseases characterized by oral or mucosal lesions in the presence of autoantibodies (autoAb) targeting the cell-adhesion proteins desmoglein (Dsg)1 and Dsg3. Lesion location has been elegantly explained by the Desmoglein Compensation Hypothesis (DCH), which utilizes the epidermal distribution of Dsg subtypes as well as autoAb profiles. According to this theory, PF presents with subcorneal lesions in the presence of anti-Dsg1 Abs only, while lesions in PV are suprabasilar and accompanied by anti-Dsg1 only in mucosal PV, or anti-Dsg1 and -Dsg3 in mucocutaneous PV. While the validity of this hypothesis has been supported in the literature, logical inconsistencies have been noted and exceptions have been published in several small-scale studies. We sought to develop a system to evaluate how often patients contradict the DCH and characterize these contradictions in a large sample size of 289 pemphigus patients. We find that roughly half of the PV and PF patients with active disease at time of visit present with a combination of lesion morphology and anti-Dsg levels that contradict the DCH. The most common contradiction is cutaneous only PV at time of enrolment (n=14), including 7 patients who report no mucosal lesions at any time in their history. Other categories in which lesion morphology does not align with the predicted autoAb status include mucocutaneous disease in the absence of either Dsg1, Dsg3, or both (n=23). We find stark differences based on ethnicity, with the highest proportion of patients that follow the DCH among the Ashkenazi Jewish population (63.5%) and the lowest for African Americans (25%). These findings demonstrate clear challenges to expand our understanding of pemphigus morphology beyond the DCH, in particular for populations that have not been the focus of previous studies.

Distinct Chronicity Accessiblility Profiles of CD8+ Tissue Resident Memory T Cells

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Skin is a uniquely accessible and responsive target for vaccine delivery. Emerging evidence suggests that keratinocytes can modulate skin immunity in response to diverse stimuli, producing either proinflammatory or immune suppressive mediators depending on the nature of the exogenous stress. To improve the immunogenicity of skin targeted vaccines, we engineered keratinocytes to support a proinflammatory local environment. Keratinocytes were genetically engineered to express the stress response transcription factor x-box binding protein 1 (XBP1). In a mouse model, keratinocyte-specific overexpression of XBP1 was transient and induced a proinflammatory skin microenvironment characterized by increased expression of proinflammatory cytokines, localized inflammatory infiltrates, including localized increases of dermal CD103+ DCs, XCR1+DCs, plasmacytoid DCs, γδ T cells, and group 1 innate lymphoid cells. Simultaneous non-viral delivery of plasmids driving expression of XBP1 and antigen OVA resulted in increased antigen expression and increased the induction of antigen-specific cellular and humoral responses, including durable antigen-specific skin-resident memory CD8 T cells and efficacious protective immunity, compared to delivery of antigen by traditional routes. This translated to improved survival in a ZIKV infection model, and improved therapeutic immunity in a clinically reflective endogenous melanoma model. Further, overexpression of XBP1 in keratinocytes in human skin resulted in a proinflammatory skin microenvironment. These findings support the feasibility of keratinocyte-targeted DNA vaccines to induce a proinflammatory skin microenvironment for effective immunization.