Targeting keratinocytes to potentiate non-viral DNA skin immunization

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ABSTRACTS | Adaptive and Auto-Immunity

Skin is a uniquely accessible and responsive target for vaccine delivery. Emerging evidence suggests that skin is equipped to 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 mediators, localized inflammatory infiltrates, and localized infiltration of dermal CD103+ DCs, XCR1+ DCs, plasmacytoid DCs, -3 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 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 alone. This translated to improved survival of B10D2 mice infected with a Zika virus model, and improved therapeutic immunity in a clinically reflective endogenous antigen encoding plasmid alone. This translated to increased titers of ZIKV-ENV antibody in a resident memory CD8 T cell population and efficacious protective immunity, compared to delivery of ZIKV-ENV plasmid alone. 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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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 (IFNβ). To investigate the type 1 interferon signature, we identified pathways and cellular phenotypes in a subset of DM patients. Healthy controls (HC) and 3 DM patients, with paraffin-embedded (FFPE) samples obtained from trunk, arm, or leg were stained with a panel of 15 mAbs conjugated antibodies. Regions of interest (ROI) of 500x500µm were acquired at a frequency of 2008Hz on the Hyperion Imaging System (Fluidigm). The resulting files were MCD files were converted to 16-5 bit TIFF files using MCD Viewer™ (Fluidigm). Cell segmentation was performed using an app-based algorithm in Visiopharm. Per object mean pixel intensity (MPI) was gathered and analyzed using ImageJ. Permutation and negative comparison statistics were identified using a sliding scale for each channel. Phenograph algorithm 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 median ± standard deviation. Skin lesions of DM patients contain an increased number of CD163+ cells compared to normal skin from HC patients (14±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 β1 (8.17 ± 5.6 ± 1.0 vs 1.3 ± 0.1), all p<0.05. A population of CD4 cells was identified that produced higher IFNβ MPI compared to HC CD4 cells (16±4.4 vs 8±0.8; p<0.01). Lesional DM skin also contained more FOXP3+ CD4 cells when compared to HC (64±20.3 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 IFNβ via activation of the STING pathway. IFNβ is produced by both CD163+ and a subset of CD4 cells.