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Keratinocyte-mediated activation of TGFβ maintains skin-recirculating memory CD8$^+$ T cells

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Tissue-derived factors are critical for the development and persistence of skin-resident memory CD8$^+$ T cells. Regulated activation of TGFβ by integrins $\alpha_v\beta_6$ and $\alpha_v\beta_8$ expressed on keratinocytes is required for residence of epidermal T_{RM} that are lost in mice lacking these integrins (*Itgb6*$^{-/-}$*Itgb8*$^{\Delta KC}$ mice). However, whether skin-derived signals also affect recirculating memory cells that are only transiently in skin have been never noted. Here, we show that after resolution of skin vaccinia virus (VV) infection, antigen-specific circulating memory CD8$^+$ T cells migrate into skin in an antigen- and inflammation-independent manner. In *Itgb6*$^{-/-}$*Itgb8*$^{\Delta KC}$ mice, the absence of activated TGFβ results in normal expansion and differentiation of CD8$^+$ T cells but a gradual loss of E- or P-selectin binding central and peripheral memory populations from secondary lymphoid organs that results in reduced protection to VV skin challenge. Notably, pertussis toxin inhibition of skin entry of the memory cells rescues the loss of memory cells in *Itgb6*$^{-/-}$*Itgb8*$^{\Delta KC}$ mice. These data demonstrate that skin migration can persist after resolution of local skin infection and, surprisingly, the cytokine environment within this nonlymphoid tissue shapes the differentiation state and persistence of the central and peripheral memory T cell pool.

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IFN-γ enhances cell-mediated cytotoxicity against keratinocytes via JAK2/STAT1 in lichen planus

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Lichen planus (LP) is a chronic debilitating inflammatory disease of unknown etiology affecting the skin, nails, and mucosa. LP is histologically characterized by dense infiltration of T cells and epidermal keratinocyte death. However, little is known about the pathogenesis of LP, and there is urgent need for more effective treatments. Here, using global transcriptomic profiling of LP samples(n=37) and healthy controls (n=24), we demonstrate that LP is characterized by type II, but not type I, interferon (IFN) inflammatory response. The type II IFN, IFN-γ, is demonstrated to prime keratinocytes and increase their susceptibility to cytotoxic responses. We further demonstrate that the promotion of cytotoxic responses to IFN-γ-in keratinocytes are MHC class I dependent and reliant upon JAK1/STAT1 but not JAK1 or STAT2 signaling. Thus, *JAK2* or *STAT1* knock-outs by CRISPR/Cas9 completely inhibit cell-mediated cytotoxic responses to IFN-γ primed keratinocytes. Lastly, using drug prediction algorithms on our transcriptomic data JAK inhibitors are identified as promising therapeutic agents in LP, which we confirm using the JAK1/2 inhibitor baricitinib, which fully protects keratinocytes against cell-mediated cytotoxic responses. In summary, this work elucidates the role and mechanisms of IFN-γ in LP pathogenesis and provides evidence for the therapeutic use of JAK-inhibitors in patients with LP.

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B cell cytokine analysis by single cell analysis reveals therapeutic reactivity of systemic sclerosis-associated interstitial lung disease

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Purpose: Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis. SSc-associated interstitial lung disease (ILD) is a severe complication of SSc. The cyclophosphamide (CYC) treatment is the only therapy found to be effective in improving lung function. However, not every SSc-ILD patients treated with CYC recover. Recent studies indicate that B cells play a critical role in SSc. However, the relationship between response to CYC therapy and B cell function remains unknown. In this study, we assessed the role of interleukin (IL)-10-producing regulatory B cells and IL-6-producing pathogenic B cells in the responders or non-responders of the CYC treatment. Methods: We cultured human lung endothelial cells (LECs) in a microchannel. B cells from patients were loaded into the microchannel. After each of adhered B cells produced cytokines, we measured IL-6 and IL-10 production from single B cells by our original micro fluidic-ELISA system. Similarly, we assessed B cells from topoisomerase (topo) I-induced SSc model mice. Results: In SSc-ILD patients, the number of B cells adhering to LECs significantly increased relative to healthy controls. Adhered B cells from CYC-responders produced significantly higher amounts of IL-10 and lower amounts of IL-6 than those from non-responders. In the mouse study, the number of topo I-specific B cells which adhere to LECs significantly increased compared to non-specific conventional B cells. Topo I-specific B cells also showed significantly higher production of IL-6 and lower production of IL-10 than conventional B cells. Conclusion: These results suggested that the effectiveness of CYC to SSc-ILD patients is associated with the cytokine profile of B cells which interact with LECs.

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Progranulin promotes bleomycin-induced skin sclerosis by enhancing TGF-β/Smad3 signaling through up-regulation of TGF-β type I receptor

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Progranulin (PGRN) is an autocrine growth factor with multiple physiological and pathological functions. Previous reports demonstrated PGRN could increase dermal fibroblasts in wound healing and activate cancer associated fibroblasts in some cancers. Because systemic sclerosis (SSc) is a prototypical fibrosis-related disorder, here, we aimed to clarify the role and mechanism of PGRN in bleomycin (BLM)-induced model of SSc for the first time. We observed that the serum PGRN levels were increased in Chinese SSc patients compared with healthy controls. Immunohistology and RT-qPCR demonstrated that PGRN was also elevated in the lesion from mice model of BLM-induced dermal fibrosis. Additionally, in BLM-treated mice, PGRN deficiency not only attenuated dermal fibrosis but also decreased the differentiation of myofibroblasts. The reduced progression of skin sclerosis in PGRN-deficient mice was associated with downregulation of TGF-β receptor I (TβR I) and decreased level of p-Smad3, with correspondingly impaired the expression of its downstream target gene connective tissue growth factor(CTGF) in skin lesion. This study demonstrates that PGRN plays a promoting role in the development of dermal fibrosis through the activation of the TGF-β/Smad3 signaling via upregulation of TβR I. PGRN may be a new therapeutic target in SSc.

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Autoantigen Keratin 17 presented by keratinocytes directs T cell auto-reactivity in psoriasis

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Keratin 17, an intermediate filament protein, is overexpressed in psoriasis and is not found in healthy epidermis. Studies have shown that K17 could serve as a major autoantigen recognized by autoreactive T cells in psoriasis because it shares similar epitopes with streptococci and promotes the proliferation and cytokines secretion of T cells. However, as a cytoskeleton protein, how K17 are presented and promoted T cell auto-reactivity remains unclear. Previous studies and our works confirmed that keratinocytes (KCs) can express the antigen presentation related molecules (MHC-II, ICAM-1) and function as non-professional antigen-presenting cells (APCs) in the state of disease or directly be stimulated by cytokines. We first identified the sequences of the peptides MHC presented by Co-immunoprecipitation (Co-IP) and Mass spectrum (MS). Strikingly, we found that three K17 epitopes loaded in the antigen-binding groove of MHC-I and MHC-II in IFN-γ activated KCs. Also, *in vitro*co-incubation assay indicated that KCs stimulated by IFN-γ can specifically activate the CD4$^+$T cells of HLA matched psoriasis patient, while the counterpart with K17 silence cannot activate neither CD4$^+$T cells nor CD8$^+$T cells. Further, we developed an intradermal injection model in which CD4$^+$ or CD8$^+$ T cells from mice immunized with K17 protein are delivered directly to the dermis of WT, Langerin-DTR mice or K17$^{-/-}$ mice after tapes. Unexpectedly, K17-specific CD4$^+$ or CD8$^+$T cells injected into taped WT and Langerin-DTR mice resulted in psoriatic-like inflammation or skin GVHD respectively. Meanwhile, depletion of K17 in epidermis cannot induce keratinocytes-directed, T cell mediated effector immune responses*in vivo*. Furthermore, K17 peptide shared same sequence in human and mice could induced K17-specific T cell proliferation and Th1/Th17 polarization *in vitro*. These results indicate that keratinocytes may function as accessory APC to present K17 epitopes and prime auto-reactive T cells in skin inflammation of psoriasis.

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Calcitonin gene-related peptide (CGRP) signaling through endothelial cells (ECs) is a key component of the contact hypersensitivity (CHS) response

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Using in vitro techniques, we found that ECs respond to CGRP by modifying immune function. To examine the in vivo relevance of this finding, we engineered an inducible, conditional knockout (KO) mouse in which administration of tamoxifen (TX) results in deletion of RAMP1 (an essential component of the CGRP receptor) in ECs. Groups of TX-treated Cre$^+$ mice (RAMP1 deleted) and TX-treated, RAMP1 floxed Cre$^-$ (no RAMP1 deletion), were immunized to dinitrofluorobenzene (DNFB) by application of 25 µl of 0.5% DFNB to the shaved dorsum or were mock-immunized with vehicle alone. One week later, mice were challenged by application of 5 µl of 0.2% DNFB to each side of each ear and 24-hr ear swelling assessed; mice were then euthanized and ears harvested for histology. The CHS swelling response in the KO mice was significantly attenuated compared to Cre$^-$ control mice and the inflammatory infiltrate in the ears of the KO mice was significantly less than in the controls. To provide proof of principle that an approach targeting the CGRP receptor might modify CHS, wild-type mice were injected with the competitive inhibitor of CGRP BIBN 4096 BS (BIBN) intraperitoneally (IP) 30 min before sensitization to DNFB and again 60 min after immunization. One week, later mice were injected IP with BIBN and 30 min later DNFB applied to the ears. Control mice were treated identically except that they were injected with diluent without BIBN. Treatment of mice with BIBN resulted in a significant decrease in the expression of CHS, similar to that seen in the inducible, conditional RAMP1 KO mice. In a preliminary experiment, the same protocol was followed except that, instead of administering BIBN IP, mice received BIBN intradermally at the site of immunization (shaved dorsum) and at the site of challenge (base of each ear); very similar results were seen. Peripheral nerves may regulate cutaneous immune responses through release or non-release of CGRP acting on ECs and this pathway may be a therapeutic target for the treatment of skin diseases.