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Bermekimab, Anti-Interleukin-1α Antibody, Inhibits Skin Injury Induced Response in Healthy Volunteers

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Interleukin-1 alpha (IL-1α) is constitutively expressed in epithelial cells located at barrier sites, such as skin, lung, and gut. Upon cellular death, injury or infection, IL-1α is released. Serum levels of IL-1α are largely undetectable in healthy volunteers (HV), or atopic dermatitis (AD) and hidradenitis suppurativa (HS) patients, while levels in the skin, especially in the epidermis and exudates from HS tunnels, are highly concentrated. Bermekimab (BMK) is a first-in-class fully human anti-IL-1α monoclonal antibody that has been tested in Phase-2A AD and HS studies. To support understanding of PK/PD relationships, we developed a human skin explant model to assess proteomic and transcriptomic effects of IL-1α blockade on injury-induced inflammation. After 24-hours culture, IL-1α was detected in the media. *Ex vivo* IL-1α blockade resulted in significant decreases of CXCL1, IL-8, GCSF and IL-6 levels compared to untreated 24-hours samples ($p<0.05$ for all analytes), and consistent reduction of 73 genes (N=10 donors). Next, we utilized this model to measure post-treatment skin PD effects in HV receiving a single dose of BMK in a Phase 1 study (NCT04544813). BMK exhibited linear PK following a single IV (400-1200mg) or SC (200-800mg) administration. Consistent with *ex vivo* blockade, CXCL1, IL-8, GCSF and IL-6 were reduced in culture media from post-dose versus pre-dose skin explants with significantly higher % reduction of IL-8 and IL-6 observed in the 800mg compared to the 200mg SC cohorts ($p<0.05$ for both analytes). Down-regulation of the same 73 genes in skin explants were also observed. These data support the relevance of IL-1α as a key skin alarmin driving tissue injury inflammation, and BMK reduces the downstream skin injury responses. Clinical research evaluating BMK in inflammatory skin diseases is ongoing.

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Autoantibody landscape of cutaneous disorders revealed by wet protein array

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Autoantibodies are found in various pathological conditions such as autoimmune diseases, infectious diseases, and malignant tumors. However, their clinical implications have not yet been fully elucidated. Herein, we employed our proteome-wide cDNA library (HuPEX) which covers approximately 90% of the human transcriptome for proteome-wide autoantibody evaluation. In the first step, proteome-wide autoantibody screening was conducted upon pooled serum. In the second step, the concentration of selected autoantibodies within individual sera was quantified. Primary screening identified a total of 565 autoantibodies in three representative inflammatory disorders: systemic sclerosis (SSc), psoriasis, and cutaneous arteritis. Quantitative analysis revealed that each autoantibody level either positively or negatively correlated with serum levels of C-reactive protein. In SSc, we discovered 18 autoantibodies whose serum levels positively correlated with serum levels of C-reactive protein. Enrichment analysis revealed that significantly high proportion of the proteins targeted by these 18 autoantibodies was regulated by SNAI1, a transcription factor known to be upregulated in SSc skin. Sum of these autoantibodies was significantly associated with the clinical manifestations of SSc. Similar results were obtained for psoriasis and cutaneous arteritis. In addition, 488 autoantibodies were detected from the sera of malignant melanoma. Notably, patients with metastases had increased overall autoantibody production compared to those with tumors limiting to the primary site. Collectively, our novel technology can reveal the "autoantibody landscape" of human subjects and may provide novel clinical biomarkers.

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Functional profile of circulating eosinophils in atopic dermatitis

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Atopic dermatitis (AD) may be accompanied by an increase in blood eosinophil (Eos) levels. A role for Eos in the pathophysiology of this disease is therefore suspected but not yet elucidated and justifies the need to clarify their functional profile. We conducted a prospective monocentric study that assessed the Eos phenotype between patients with moderate to severe AD (aged over 18 who fulfilled with AD UK working party criteria and presented a moderate or severe form of AD defined by SCORAD score $\geq$ 25 and/or EASI score $\geq$ 7) and healthy subjects (aged over 18, without atopic comorbidities or immunomodulatory treatment). Patients were included between December 2020 and April 2021. We included 23 patients (mean age at 33 y-o) and 14 healthy control subjects (HCS). Mean SCORAD was at 56.9. DA patients had a mean blood Eos level of 322.7/mm3 (vs 130.2/mm3 in HCS, $p<0.0001$). Mean fluorescence intensity of surface expression of CD125 (p=0,04), CD63 (p=0.01), CRTH2 (p=0.0002) and CCR3 ($p<0.0001$) were lower on Eos in AD patients compared to HCS. Conversely, CD69 surface expression was increased in AD patients (p=0.006). This profile was consistent with a higher level of Eotaxin-2 (p=0,0003), IL-3 (p=0,02) and TARC ($p<0,0001$) in the serum of AD patients. IL-4, IL-13 and IL-5 serum levels tended to be significatively higher compared with HCS. The phenotypic profile of circulating Eos from AD patients is distinct from HCS, reinforcing the concept of "priming" profile of Eos which are being functionally programmed to act in a tissue. This specific profile of Eos in AD justifies a better understanding of the exact role of Eos in AD.

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Vildagliptin administration alters mouse skin proteome

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Bullous pemphigoid (BP) is a subcutaneous blistering skin disease in which IgG autoantibodies are formed against the hemidesmosomal protein BP180. Epidemiological studies have revealed a strong association between an increased risk of BP and the use of dipeptidyl peptidase 4 inhibitors (DPP4i/gliptins), especially vildagliptin. We performed a proteomic study on vildagliptin-exposed mice to reveal factors for the development of DPP4i-associated BP. We administered vildagliptin via drinking water to mice (control group, n = 5, treatment group n = 6) for three months and used minimum two-dimensional difference gel electrophoresis (2D-DIGE) to study the effects of vildagliptin on the skin proteome. We identified protein spots with mass spectrometry (MS) and validated results using label-free shotgun MS, immunoblotting (IB), qPCR, and immunohistochemistry (IHC). We detected an upregulation of beta-actin (treatment/ctrl ratio of 1.76) and moesin (1.74) in vildagliptin-treated mice in 2D-DIGE and IB, and an increase of galectin-1 (4.18) in MS. A downregulation of DPP4 (0.83) was detected in IB. IHC showed that vildagliptin decreased staining of moesin, galectin-1, and DPP4 in the epidermal keratinocytes and dermal fibroblasts. In addition, IB showed a decrease of BP180 (0.68) in vildagliptin-treated mice. The discrepancies between 2D-DIGE and IHC results may arouse from the partition by the solubility in the former and/or epitope masking/modification in the latter. The skin morphology of vildagliptin-treated mice was normal. No differences were observed in mRNA level in qPCR analysis. We conclude that vildagliptin administration alters mouse cutaneous protein expression of beta-actin, moesin, galectin-1, DPP4, and BP180. These proteins participate e.g., in cytoskeleton-cell membrane interaction, and T-lymphocyte regulation, which could have a role in the breakage of immunotolerance against BP180 in DPP4i-associated BP.

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Increased Interleukin-36γ in keratinocytes may induce epithelial-mesenchymal transition in patients with systemic sclerosis

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Systemic sclerosis (SSc) is a connective tissue disease characterized by autoimmunity, vasculopathy, and fibrosis. Several studies demonstrated the involvement of various cytokines including IL-1 in SSc. IL-36 cytokines, a subgroup of IL-1 family, comprise IL-36α, β, γ, IL-36Ra, and IL-38. IL-36α, β, γ are known to promote inflammatory response, which is mainly produced by epithelial cells. Whereas, IL-36Ra as well as IL-38 may have anti-inflammatory effect. Recent reports suggest that epidermis may play an important role in the pathogenesis of SSc, although the involvement of IL-36 family is still unclear. Thus, we focused on IL-36 family regarding its level and function in SSc. IL-36α, β, γ and IL-38 expressions were evaluated by immunohistochemistry in skin tissue from SSc patients and healthy controls, and for IL-36γ by western blot analysis. Serum IL-36γ levels were evaluated by ELISA in 61 patients with SSc and controls. As results, IL-36γ expression levels were significantly higher in epidermis of SSc patients compared to that in controls and sera of SSc patients also had increased levels of IL-36γ. To analyze a role of IL-36γ in SSc, cultured healthy keratinocytes were stimulated with IL-36γ, and expression levels of epithelial-mesenchymal transition (EMT)-related factors were analyzed by qRT-PCR and Immunofluorescence. Expression levels of E-cadherin were significantly downregulated while that of Slug and Vimentin were increased in IL-36γ-stimulated keratinocytes with or without TGFβ than that in controls. Furthermore, skin fibroblasts in the presence of IL-36γ significantly induced Egr-1 expression. In conclusion, increased levels of IL-36γ in epidermis may be involved in the fibrotic pathogenesis of SSc by inducing EMT.

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Epidermal leucotriene B4 receptor expression as a marker of regulatory T-cell–driven responses in resolved psoriasis

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Pathogenic memory T cells are implicated in the local memory of chronic skin diseases. The factors underlying their persistence and function in human resolved epidermis are not understood. Lipid composition and transcriptional programs of lipid metabolism are known to be altered in active and non-lesional psoriasis, and the faculty of resident T cells to uptake fatty acids is essential to their local survival. Here, we wished to correlate the functionality of epidermal T cells in resolved skin of psoriasis patients to the lipid metabolism using transcriptomic data. Skin biopsies from patients with psoriasis were collected both on resolved and non-involved (non-lesional) areas, as well as in healthy controls. Flow cytometry was performed on epidermal cell suspension of healthy controls, with assessment of T cell surface markers and cytokine production. T cells were activated in skin explants with the pan T-cell agonist OKT-3 compared to an IgG2a control. The RNA purified from the epidermal compartment was analyzed by a custom panel from Nanostring focused on inflammatory markers and lipid metabolism. In healthy epidermis, the proportion of cells expressing the checkpoint inhibitor PD1+ among T cells was negatively correlated to the expression of the transcripts *LTB4R* and *PTGES2* suggesting their involvement in the immune regulation. LTB4R is a receptor of the pro-inflammatory leukotriene LTB4 and its expression was decreased in resolved epidermis compared to non-lesional skin. Furthermore, *ex vivo* activation of T cells in resolved psoriasis but not in non-lesional epidermis induced *IL10* and *IDO1* and the level of this upregulation was positively correlated to the baseline level of *LTB4R*. Altogether, our data suggest a link between the level of expression of *LTB4R* and the immune regulation present in the resolved psoriasis epidermis.