A novel tool to analyse the pathogenic impact of IgG binding to extracellular domain 5 of Desmoglein 3

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Pemphigus vulgaris (PV) is a severe autoimmune blistering disease characterized by auto-antibodies that target desmoglein (Dsg) and Dsg1. Binding of Dsg-specific auto-antibody to target structures induces an interruption of the desmosomal integrity which ultimately results in the clinical manifestation of flaccid blisters and erosions. Understanding mechanisms inducing blister formation upon binding of Dsg-specific auto-antibodies are largely unknown. Numerous studies demonstrated the pathogeneity of auto-antibody specific for the amino-terminal region (extracellular domain 1, EC1) of Dsg3. However, the Dsg3 specific auto-antibody response in PV patients is polyclonal, including auto-antibodies directed against both amino- and membrane proximal epitopes. Here, we investigated the pathogenicity of a murine monoclonal antibody directed against the membrane-proximal region (EC5) of the Dsg3 ectodomain was analysed. This Dsg3-specific antibody was isolated from the supernatant of a Dsg3-specific B-cell hybridoma and tested in various specificity and functional assays as well as in vivo. Results clearly demonstrate that this specific auto-antibody directed against the membrane-proximal region EC5 of human Dsg3 exhibit pathogenic activity similar to the well-characterized EC1-specific antibody AK23 without the Ca2+ dependency. Our results deliver new insights into aspects of a more defined understanding of auto-antibody induced blister formation in PV.

Melanocyte-targeted Bispecific PD-1 agonists as Localized Immune Suppressants against Vitiligo

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Vitiligo is an autoimmune skin disease mediated by autoreactive T cells that destroy epidermal melanocytes, causing depigmentation. Current treatments, e.g., corticosteroids or UV light therapy, have limited efficacy with inconsistent remission in many patients. Some emerging therapies, e.g., IAk inhibitors, show promising activity but carry potential systemic safety risks. Tissue-restricted immune modulation is a promising approach to overcome many issues associated with systemic immunosuppressants. In vitiligo, localized suppression of autoreactive CD8 T cells may be achieved by engaging inhibitory receptors on these cells as they attack melanocytes. The PD-1 pathway is a key immune checkpoint that inhibits T cell responses and helps to maintain peripheral tolerance. Blocking this pathway in cancer patients has shown to cause autoimmune related disease reactions in vitiligo patients. Furthermore, a recent report described defective PD-L1 up-regulation in melanocytes from vitiligo patients, suggesting that PD-1 driven tolerance is impaired in this disease. Therefore, designing melanocyte-targeted PD-1 agonists that trigger this pathway in attacking autoreactive T cells and inhibit their activity is an attractive approach to treat vitiligo. Here we describe targeted PD-1 agonist bispecifics that consist of an affinity enhanced TCR targeting domain specific for a melanocyte pHLA complex, fused to a PD-1 agonist moiety. These novel molecules, once bound to melanocytes, activate the PD-1 pathway on interacting T cells, potentially inhibit TCR mediated signalling, suppress T cell activation and inhibit cytokine production. Importantly, in the absence of target cell binding, these molecules are unable to inhibit T cell activation. In conclusion, the immune modulating bispecifics described here have the potential to deliver potent melanocyte-restricted T cell inhibitors that avoid systemic immunosuppression and could improve skin repigmentation in vitiligo patients.

Metatranscriptomics reveals association of α-, β-, and γ-HPVs with typical epidermodysplasia verruciformis in a large cohort of patients with CIBI, TMCI, and TMC2 mutations

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Human papillomavirus (HPV) infection typically manifests with isolated warts. However, some patients in familial clustering develop extensive and protracted HPV infections, primarily the β-HPV types 5 and 8, with distinct cutaneous findings. This clinical entity, epidermodysplasia verruciformis (EV), with autosomal recessive inheritance, is characterized by numerous cutaneous flat warts in childhood, which progress into squamous cell carcinomas later in life. The typical “form of EV, not vulnerable to other infections, is caused by mutations in CIB1, TMCI, or TMCI, which impair keratinocyte-intrinsic immunity to β-HPV infection. Mutations in other genes related to T-cell development or function, have been associated with “atypical” EV in patients with other infections. We developed a whole-transcriptome sequencing-based method on RNA isolated from skin biopsies for concomitant detection of HPV and human genetic determinants of cutaneous wart lesions in a cohort of 50 EV patients. This method, VirPlex, can detect 926 viruses, including more than 400 HPVs, and the corresponding human mutations. Nine distinct mutations in TMCI (n=2), TMCI (n=5) and CIB1 (n=2) in 12 distinct families, including 14 patients were detected. The most predominant HPV in this cohort was HPV14. In addition, the RNA-seq data were examined for variant detection and prioritization, pathogenicity confirmation, and RNA expression profiling. Besides, we identified a total of 20 different HPVs including 16 β-, three α- and one γ-HPV (HPV19/128) in a patient with TMC2 mutation. In summary, the utilization of RNA-Seq as a first-tier diagnostic method allowed us to simultaneously profile the transcriptome of host for mutation detection and exploring the consequences of variants of unknown significance as well as to profile the cutaneous virion of EV patients.

Influence of dupilumab on HSV-specific immune response in atopic dermatitis

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Dupilumab is a monoclonal antibody targeting the IL-4Ra chain, and therefore acts directly against type-2 inflammation. Molecular investigations examining the impact of dupilumab on the immune defense against viruses are lacking so far. We aimed to analyze the effect of dupilumab on the antiviral response in AD patients with (ADEH+) and without (ADEH-) a history of EH. Blood from 56 AD patients of which 25 were treated with dupilumab as well as from 10 healthy controls was taken and IgG binding HSV-1 gD was measured. PBMCs were stimulated with HSV-1 protein, and peptides, respectively, and antigen-reactive T cell enrichment via CD154 or CD137 was performed followed by flowcytometry. Inhibition of display of virus specific T cell epitope by TCR gene on HSV-UL125 C1D T cells were examined for IL-4 and IFN-g in vitro. HSV-specific IgG was increased in ADEH+ and significantly reduced in dupilumab treated patient families. Frequencies of HSV-specific T cell families were elevated in ADEH- patients treated with dupilumab compared to untreated patients. HSV-specific T cell families showed stable frequencies under dupilumab therapy. Raised IFN-γ and reduced IL-4 production was shown in HSV-UL25 epitope-specific T cells of dupilumab treated AD patients compared to untreated ones. These results suggest that dupilumab strengthens the HSV-specific defense in AD increasing the specific type-1 immune response and attenuating type-2 related reactions including HSV-specific IgG production.

The transcriptional landscape of hidradenitis suppurativa at single-cell and spatial resolution

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Hidradenitis suppurativa (HS) is a chronic inflammatory dermatosis characterised by painful, fluctuating abscesses and sinuses, typically in flexural sites. It has an estimated prevalence of 2% and can be severely debilitating. The inflammatory and immune landscape underlying HS remains poorly understood and treatment options are limited or only partially effective. We have performed global transcriptional profiling at single-cell and spatial resolution on HS skin. This has revealed striking differences in comparison to healthy skin and other inflammatory skin diseases such as psoriasis and eczema. We find overlap-representation of cells in the plasma immune infiltrate in comparison with healthy and other inflammatory skin diseases such as psoriasis and eczema. In HS, there is a subtype of cells with an ‘activated memory’ profile, which have been described in autoimmune diseases. The presence of this B cell population, which express markers associated with ‘trained’ T cells, potentially lies behind the potential of acting as tertiary lymphoid tissue. There is also expansion of a fibroblasts subpopulation associated with inflammation and vascular endothelial cells, and the follicular units show a range of transcriptional profiles, ranging from a profile similar to normal follicular epithelial cells to keratinocytes. In summary this work shows the importance of cell interactions and subsequent pathway analysis suggests that immune cells drive the transition to this IFE-like profile. My findings support the paradigm of HS as an autoimmune keratinisation disorder. It also supports the idea of investigating the repurposing of drugs that have been developed for other autoimmune or autoinflammatory diseases with similar underlying immune cell profiles.