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-antibody(s) directed against desmogleins (Dsg) and Dg1. Binding of Dsg specific auto-antibodies to target structures induces an interruption of the desmosomal integrity which ultimately results in clinical manifestations of flaccid blisters and erosions in PV patients. Underlying mechanisms inducing blister formation upon binding of Dsg-specific auto-antibodies are largely unknown. Numerous studies demonstrated the patho-physiology of auto-antibodies 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 described the pathogen-icity 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 charac-terized EC1-specific auto-antibody AK23 without the Ca2+ dependency. Our results reveal new aspects of a more defined understanding of auto-antib-induced blister formation in PV.

Melanocyte-targeted Bispecifc PD-1 Agonist 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 repigmentation in many patients. Some emerging therapies, e.g. IL-17 and TNF-alpha inhibitors, show promising activity but carry potential systemic safety risks. Tissue-restricted immune modulation is a promising approach to over-come many issues associated with systemic immunosuppressants. In vitiligo, localized sup-pression of auto-reactive 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 can-cer patients has shown to cause immune related disease in a minority of 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 auto-reactive 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 inflammatory cyto- kine production. Importantly, in the absence of target cell binding, these molecules are un-able to inhibit T cell. In conclusion, the immune modulating bispecifics described here have the potential to deliver potent melanocyte-restricted T cell inhibitors that avoid immunosuppression and could improve skin repigmentation in vitiligo patients.

Differentiation of Therapeutic Antibodies Targeting Interleukin-23

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Cutaneous human papillomavirus (HPV) infection typically manifests with isolated warts. However, some patients in familial clustering develop extensive and protracted HPV infec-tions, primarily the β-HPV types 5 and 8, with distinct cutaneous presentations. This clinical entity, epidermodysplasia verruciformis (EV), with autosomal recessive inheritance, is char-acterized 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 TMC1B, TMCG, or TMCG, 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 genotypes and genetic determinants of cutaneous wart lesions in a cohort of 50 EV patients. This method, VirPlex, can detect 926 viruses, including more than 400 HPV's, and the corresponding human mutations. Nine distinct mutations in TMCG (n=2), TMCG (n=5) and CB1 (n=2) in 12 distinct families, including 14 patients were detected. The most pre-dominant 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 β-16, β-29 and one γ-HPV(VP1128) in a patient with TMCG 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 virome of EV patients.

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

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by itch, dry skin, plaques, nodules, abscesses and sinus tracts, 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 AD skin. This has revealed striking differences in comparison to healthy skin and other inflammatory skin diseases such as psoriasis and eczema. We find over-representation of cells in the plasma immune infiltrate in comparison with healthy and other inflammatory skin diseases. However, AD skin is a sub-type of cells with an ‘activated memory’ profile, which have been described in autom-immune diseases. The presence of this B cell population, which express markers associated with regulatory function, potentially limits the potential of acting as tertiary lymphoid tissue. There is also expansion of 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 in comparison to the highly infiltrated epidermis of AD. We have also observed that cell interactions and subsequent pathway analysis suggests that immune cells drive the transition into this IF-E-like profile. My findings support the paradigm of HS as an auto-immune keratinization 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.