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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 (auto-ab) against the desmosomal adhesion molecules desmoglein3 (Dsg3) and Dsg1. Binding of Dsg specific auto-ab to target structures induces an interruption of the desmosomal integrity which ultimately results in the clinical manifestation of flaccid blisters and erosions in PV patients. Underlying mechanisms inducing blister formation upon binding of Dsg-specific auto-ab are largely unknown. Numerous studies demonstrated the pathogenicity of auto-ab specific for the amino-terminal region (extracellular domain 1, EC1) of Dsg3. However, the Dsg3 specific auto-ab response in PV patients is polyclonal, including auto-ab directed against both amino- and membrane proximal epitopes. In this study, 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 murine auto-ab specifically binds human Dsg3 and is capable of inhibiting intercellular keratinocyte adhesion accompanied by the activation of the p38 MAPK signal transduction pathway. Here, for the first time, we can demonstrate that a specific auto-ab directed against the membrane-proximal region EC5 of human Dsg3 exhibit pathogenic activity similar to the well characterized EC1-specific antibody AK23 without the Ca^{2+} dependency. Our results deliver new aspects of a more defined understanding of auto-ab-induced blister formation in PV.

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Metatranscriptomics reveals association of α-, β-, and γ-HPVs with typical epidermodysplasia verruciformis in a large cohort of patients with *CIB1*, *TMC6*, or *TMC8* mutations

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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 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, TMC6, or TMC8, 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 viral and human genetic determinants of cutaneous wart lesions in a cohort of 50 EV patients. This method, VirPy, can detect 926 viruses, including more than 400 HPVs, and the corresponding human mutations. Nine distinct mutations in TMC8 (n=2), TMC6 (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(HPV128) in a patient with TMC8 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.

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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 repigmentation in many patients. Some emerging therapies, e.g. JAK 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 been shown to cause autoimmune related diseases, including vitiligo. 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, potently inhibit TCR mediated signalling, suppress T cell activation and inflammatory cytokine production. Importantly, in the absence of target cell binding, these molecules are unable to inhibit T cells. 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.

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

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Eczema herpeticum (EH) is a disseminated, potentially life-threatening HSV infection in atopic dermatitis (AD) patients. Recently, we showed a type-2 skewed immune response to viruses in EH patients. 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 IgE 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 to display the polarization. Cytokine profiles of HSV-reactive T cell lines (TCL) were examined by cytokine bead assay and MHC-I tetramer$^+$ (HSV-UL25) CD8$^+$ T cells were stained for IL-4 and IFN-gintracellularly. HSV-specific IgE was increased in ADEH+ and significantly reduced in dupilumab treatment patients. Frequencies of HSV-specific T_C1 cells were elevated in ADEH- patients treated with dupilumab compared to untreated patients. HSV-specific T_H cells 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 IgE production.

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Differentiation of Therapeutic Antibodies Targeting Interleukin-23

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Clinically relevant differences amongst therapeutic antibodies against the same target may relate to their unique molecular attributes. Differences in therapeutic profile across the domains of psoriatic disease between guselkumab (GUS) and risankizumab (RIS) have been observed. To explore potential mechanisms underpinning this, we studied GUS, a fully human IgG1 specific for interleukin (IL)-23 with a native Fc region, and RIS, a humanized anti-IL-23 IgG1 with a mutated Fc region. We compared binding and functional characteristics of the antigen-binding and Fc regions of these antibodies. GUS and RIS displayed comparable picomolar affinities for binding IL-23 by KinExA and surface plasmon resonance assays, and equivalent potency (IC_{50}=0.2 nM) for inhibition of IL-23-induced STAT-3 phosphorylation in human peripheral blood mononuclear cells. However, in cells transfected with individual Fcg receptors (FcgRs), GUS showed strongest binding to CD64 (FcgR1), while RIS showed negligible binding to any FcgRs, by virtue of its mutated Fc region. Furthermore, in interferon (IFN)g-primed human monocytes, labeled GUS showed dose-dependent binding to CD64 by flow cytometry, while RIS did not. GUS binding to CD64 on monocytes did not trigger activation as shown by lack of cytokine or chemokine production. Importantly, CD64-bound GUS was able to bind IL-23, as detected by anti-p40 staining. In conclusion, compared with RIS, GUS uniquely binds both CD64$^+$ myeloid cells and IL-23. CD64$^+$ mononuclear phagocytes are enriched in psoriatic skin and serve as the dominant IL-23 source. Taken together, GUS presence may be enriched within the inflamed tissue microenvironment by binding to CD64, neutralizing IL-23 at its cellular source, potentially leading to durable response and observed therapeutic differences within the class. Further studies are warranted to generate additional evidence supporting this hypothesis.

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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 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 axillary HS 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 plasma cells in the immune infiltrate in comparison with healthy and other inflammatory skin diseases. Transcriptional profiling of the B cell population suggests that in HS tissue 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 class switch recombination, supports the concept of diseased skin as having 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 that of the interfollicular epidermis in hyperkeratinised states. Modelling of the cell-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 auto-inflammatory 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.