031 Development of a mouse model of Pemphigus Vulgaris as a tool to evaluate naïve antengen-specific tolerance
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Pemphigus is an autoimmune blistering disease caused by autoantibodies mainly directed against desmoglein (Dsg). So far, only broad systemic immunosuppression and B-cell depletion are being applied in PV patients. In this study, we aim at investigating a Dsg-specific CD4+ T-cell directed therapy in PV. We apply human leucocyte antigen (HLA)-transgenic mice crossed with the PV-associated ELRBBR2-/- mutation, a human CD4 coreceptor and lack mouse MHC class II (I-As/-). Immunization with recombinant human Dsg3 protein leads to induction of Dsg3-specific CD4+ T and Dsg3-reactive IgG producing B cells. For induction of Dsg3-specific T cell tolerance, we applied a set of immunodominant Dsg3 CD4+ T epitopes linked to nanoparticles in a preventive setting prior to i.p. immunization with human Dsg3. Anti-Dsg3 antibody titers were by 74% and 80%, respectively, reduced compared to control animals. To summarize, we are able to detect antigen specific T and B cells upon Dsg3-immunization, and can efficiently promote a CD4+ T-cell specific tolerance induction based on the application of peptide-potent nanoparticles in a precinical model of PV. These preliminary results hold great promise to translate the concept of Dsg3-specific T cell tolerance induction into the clinic.

032 Expansion of BCL2+ lymphocytes in cutaneous graft-versus-host disease is associated with steroid resistance and poor prognosis
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Graft-versus-host disease (GVHD) remains a major cause of mortality and alloimmune hematopoietic stem cell transplantation (HSCT) and response to first-line therapy with glucocorticoids is often limited. To identify novel therapeutic targets for treatment and prophylaxis of GVHD, we are exploring the mechanisms involved in the development of the disease. Different Expressed Genes (DEG) analysis on sequentially isolated T cells of HSCT recipients. In recipients who later developed GVHD, we observed early up-regulation of the anti-apoptotic molecule BCL2, which is targeted in chronic lymphocytic leukemia with a recently approved small molecule inhibitor. Furthermore, gastrointestinal tract, liver and skin affected by acute and chronic GVHD showed higher BCL2 mRNA expression compared to matched control groups. BCL2 protein levels were elevated in overall leukocytes and pathogenic cell subsets including CD4+ T lymphocytes in peripheral blood and skin of GVHD patients. Notably, BCL2 expression levels correlated to steroid-refractory GVHD and increased transplant-related mortality. In vitro inhibition of BCL2 in allo-reactions led to dose-dependent apoptosis of T cells and increase of CD4/CD8 ratio. Our results highlight the role of BCL2 as survival factor for GVHD-mediating lymphocytes. Selective inhibition of BCL2 may present a novel and urgently needed targeted therapy in treatment of steroid-refractory GVHD.

033 CD4+ resident memory T cells colocalize with CD103+ dendritic cells in perifollicular lymphocyte clusters in a murine delayed-type hypersensitivity model
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Tissue-resident memory T (T eff) cells play an important role in local immunity by inducing rapid immune responses upon re-exposure of the antigen. However, how CD4+ T eff cells retain and alter their proliferation of skin inflammation is largely unknown. To elucidate a possible mechanism, we used a delayed-type hypersensitivity (DTH) model, an established model mediated by CD4+ T cells. T eff cell receptor β-deficient (TCR β-) mice were transferred with CD4+ T cells from GFP-expressing, ovalbumin (OVA)-specific TCR-transgenic (OTU) mice and sensitized with subcutaneous injection of OVA emulsion (day -7). In these mice, all T eff cells were OT2 mouse-derived and could be detected by GFP expression. TCR β- KO mice exhibited significant ear swelling upon the initial challenge (day 0) with intradermal OVA injection compared to WT KO mice. Moreover, with vehicle control placebo (day 2), CD4+ T eff cells remained in the dermis at day 35 after the resolution of ear swelling and expressed CD69, a marker of T eff cells. At the same time (day 35), re-challenge with OVA induced a more rapid response, marked by a peak of ear swelling at 6 hours along with the production of IFN-γ by CD4+ T eff cells. Two-photon microscopy revealed two modes of CD4+ T eff cells' behaviour; some were retained in non-cluster form around blood vessels, and some were in cluster form around hair follicles. The T eff cell infiltration into skin was non-innervated and IFN-γ producing T eff cells in non-clustered form (DCs) were colocalized with CD4+ T eff cells around hair follicles. Additionally, these CD1c+ cells also expressed CD103, a marker of conventional DC subset 2 (cDC2). Taken together, these results indicate that CD4+ T eff cells retain after clinical resolution of skin inflammation and induce site-specific proliferation. This finding led to the hypothesis that T eff cells are retained in a DTH model. Retention of CD4+ T eff cells in skin is characterized by cluster formation around hair follicles where they colocalize with cDC2 cells.

034 Characterization of novel TMEM173 mutation causing a lupus- and SAVI-like phenotype, modified by polymorphisms in TMEM173 and IFIH1
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Monogenic STING gain-of-function mutations cause early-onset type I interferonopathy, with disease presentation ranging from fatal vasculopathy to mild chilblain lupus. Upon binding to pathogen or self-derived cytosolic nucleic acids, cyclic GMP-AMP synthase (cGAS) triggers the production of cGAMP that further activates TMEM173-encoded transmembrane protein STING, which in turn activates the variable protein phosphatase 2A (PP2A). The current understanding of interferonopathy is presently unclear. We report a novel gain-of-function mutation in C20GTE STING mutation causing a distinct phenotype with alopecia, photosensitivity, thyroid dysfunction and features of STING-associated vasculopathy with onset in infancy (SAVI), such as early-onset livedo reticularis, skin vasculitis, facial erythema and bacterial skin infections. Single residue polymorphism in TMEM173 and two IFIH1 autoimmune-related risk alleles showed variable penetrance, implying contribution to the disease presentation and phenotype. The C20GTE mutation causes constitutive activation of interferon-related pathways in in vitro cell models, as well as aberrant interferon signature and inflammammasome activation in patient PBMCs. Protein-protein interaction analyses propose impaired cellular trafficking of C20GTE mutant STING. Clinically, the index patient has benefited from the JK inhibitor bircanib therapy. These findings of STING-mediated lymphocytes, Selective inhibition of BCL2 may present a novel and urgently needed targeted therapy in treatment of steroid-refractory GVHD.

035 Targeted inhibition of complement at the basement-membrane zone in pemphigoid diseases
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Bullous pemphigoid (BP) and mucous membrane pemphigod (MMP) are blistering skin diseases in which autoantibodies against basal keratinocyte antigens cause loss of cell adhesion and detachment from the basement-membrane zone (BMZ). Biosurfaces usually show BMZ-bound IgG and C3 in direct immunofluorescence (IF), indicating complement activation that results in attraction and activation of inflammatory cells and subsequently dermal-epidermal separation. We cloned more than 30 anti-BP180/BP230 single chain variable fragment (scFv) antibodies for passive immunotherapy of BP patients. All mAbs were validated at the human monoclonal platform when addressing and competing BMZ deposition of human IgG. Importantly, we demonstrated that targeting a C1s, C4b, C2, and C3 convertase (CC3) inhibiting gigastasin domain we were able to test for targeted inhibition of the classical complement activation pathway at the BMZ, in an BP ex vivo model. This recombinant protein inhibiting complement C3 activation on target cells were tested for clinical validation at the BMZ in an ex vivo model. This recombinant molecule bound to normal human skin sections pre-incubated with BP sera and inhibited C3 deposition on BMZ, demonstrating a clinical relevant inhibition of C3 activation. This finding led to the hypothesis that targeting CC3 levels, as detected by ELISA. Another fusion protein proposed for inhibition of the alternative pathway at the BMZ was not effective in our model, illustrating the dependence on the classical complement activation pathway in BP. Because BP180-NC16A is expressed at the BMZ of skin, mucous membranes and the retina, this innovative approach could be translated to other complement-dependent diseases affecting these tissues.