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Antibodies to the BP180 C-terminus cause autoimmune blistering disease through activation of the C5a/C5aR1 axis and Fcγ receptors

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Bullous pemphigoid (BP) is a rare autoimmune blistering disease characterized by autoantibodies targeting BP180 with the non-collagenous 16A (NC16A) domain representing the immunodominant site. The role of additional extracellular targets of the BP180 C-terminus has not yet been unequivocally demonstrated. Here, we showed that BP180 ectodomain-reactive sera depleted for NC16A IgG induced dermal-epidermal separation in control skin after incubation with normal human leukocytes indicating the pathogenic potential of anti-BP180 C-terminal IgG. To further corroborate our *in-vitro* findings, we generated a new pemphigoid mouse model by transfer of rabbit IgG against a murine fusion peptide consisting of the NC1−14 domains of BP180, downstream of NC15A (murine NC16A homologue). Following subcutaneous injection of anti-NC1−14 IgG over 12 days, C57BL/6J mice presented with erythematous lesions, erosions, and crusts, particularly on the head/ neck, recapitulating a BP-like phenotype. Direct immunofluorescence and histopathological analyses of skin biopsies showed linear IgG and complement C3 deposits along the basement membrane and subepidermal cleavage with inflammatory infiltrates. Disease development was significantly abrogated in Fcγ receptor-deficient vs control mice. Following administration of anti-NC1−14 IgG to C5aR1-deficient mice, a significant reduction of skin lesions was also observed in C5aR1 knock-out vs. wildtype mice. Our data demonstrate the ability of IgG targeting the BP180 C-terminus downstream of NC16A/ NC15A to exert tissue damage driven by Fcγ receptor- and C5aR1-mediated mechanisms. The new mouse model will be instrumental to further investigate the role of BP180 C-terminal epitopes in pemphigoid diseases and identification of more specific therapies for BP.

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Metabolic and functional dysregulation of tissue-specific human regulatory T cells in chronic inflammatory diseases

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Regulatory T cells (T_{reg}) are a critical immune component guarding against excessive inflammatory responses, but current understanding of T_{reg} heterogeneity and function in non-lymphoid tissue in humans is limited. In this project we are characterising unique types of T_{reg} signatures in the context of tissue-confined inflammatory responses in human skin and gut. We are hypothesising that a common ground between these pathologies may be found in a dysfunction of immune regulation. To investigate this, we are utilizing transcriptomic, phenotypic and metabolomic methods, using an integrative bioinformatical approach of single-cell (sc) sequencing data and global metabolomic screens of T_{regs} from blood and tissue of patients with chronic inflammatory diseases. ScRNA-sequencing data from patients diagnosed with psoriasis, chronic cutaneous sarcoidosis, and ulcerative colitis have shown that diseased tissue-T_{regs} have a strong tissue-resident signature. We found an exacerbated expression of polyamine catabolic genes linked to a more activated phenotype of T_{reg} compared to their healthy counterparts. Additionally, we performed untargeted metabolomics of blood-derived T_{regs}, identifying further changes in metabolites downstream of several nitrogen metabolic pathways and nucleotide synthesis. Together, our results indicate a novel interface of polyamine catabolism and pyrimidine synthesis found in tissue T_{regs} in chronic inflammation. Ultimately, our findings will significantly contribute to basic understanding of T_{reg} function and shed light on immune regulation during chronic inflammation.

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Single cell RNA sequencing reveals specific subsets in Systemic Sclerosis fibroblast cultures

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Systemic sclerosis (SSc) is a chronic autoimmune disease causing vasculopathy and fibrosis in the skin and vital organs. Following an inflammatory reaction, fibroblasts in the connective tissues become activated myofibroblasts and secrete excessive amounts of ECM related proteins, such as collagen, creating fibrosis. Many studies have been carried out in fibroblasts in culture, however, the information obtained remains limited due to high potential heterogeneity of these fibroblasts. We therefore used single cell RNA sequencing to better understand fibroblast heterogeneity in SSc to search for potential new therapeutic targets and new biomarkers. We conducted single cell RNA-sequencing (scRNA-seq) analysis of human dermal fibroblasts from 4 healthy and 4 SSc donors. We identified several up- and down-regulated genes in defined cell populations; we also confirmed significant upregulation of tetraspanin CD9 in all SSc fibroblast subsets when compared to controls. CD9 upregulation at the cell surface of SSc fibroblasts was confirmed by flow cytometry. Additionally, we identified strong upregulation of Four and a half LIM domains 1 (FHL1) in a small cluster of SSc fibroblasts at the RNA and protein level. These cells did not express α-SMA and thereby constitute a small subpopulation of activated fibroblasts. CD9 was found in exosomes of SSc fibroblasts and FHL1 has been implicated in myofibroblast differentiation; there is not much known about their role in fibrosis. We are therefore focusing on the direct impact of both CD9 and FHL1 using specific deletion experiments.

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Skin dysbiosis promotes autoimmune inflammation via neutrophil activation and the IL-23/IL-17 axis

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Systemic lupus erythematosus (SLE) is an autoimmune disease, but its etiology is not completely understood. Recently, the microbiotas in the nasal cavity and gut have been shown to be involved in the development of SLE, but the influence of skin microbiota is still unclear. Here, we demonstrated that *Nfkbiz*$^{\Delta K5}$ mice treated with *Staphylococcus aureus* develop SLE-associated autoantibodies and glomerulonephritis with IgG deposition. Epicutaneous *S. aureus* application significantly increases staphylococcal colonization in the skin of *Nfkbiz*$^{\Delta K5}$ mice with reduced expression of antimicrobial peptides, which promotes caspase-mediated keratinocyte apoptosis and neutrophil activation, inducing the IL-23/IL-17 immune response by activating dendritic cells and T cells. As a consequence, *Nfkbiz*$^{\Delta K5}$ mice develop an SLE-like autoimmune disease. Furthermore, the administration of anti-IL-23p19 antibody and anti-IL-17A antibody, but not anti-IL-12p40 antibody, alleviated the systemic autoimmune response. Our results provide a novel murine model for SLE and reveal the fundamental importance of skin microbiota in immune homeostasis.

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Targeted inhibition of complement C3 deposition at the basement membrane zone in pemphigoid disease

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Autoantibodies against structural proteins of the basement membrane zone (BMZ) cause dermal-epidermal adhesion loss in pemphigoid diseases. Biopsies usually show IgG and C3 at the BMZ, indicating complement (C) activation that facilitates attraction and activation of inflammatory cells and blister formation. We generated 23 anti BP180 antibody (ab) clones from two bullous pemphigoid (BP) patients by phage display. All ab clones were validated by ELISA and immunofluorescence (IF). Unexpectedly, our monovalent scFv abs displaced patient IgG from immobilized antigen in vitro. When injected into human skin organ culture, anti BP180 scFvs were non pathogenic, because for pathogenicity bivalent abs with cross linking and C fixing properties are required. As a proof of concept we exploited this non pathogenicity by designing proteins that allow targeted C inhibition at the BMZ: An anti BP180 scFv was fused with a C1s inhibiting domain and tested for targeted inhibition of the classical C activation pathway at the BMZ, in an ex vivo assay. Our recombinant molecule bound to normal human skin sections preincubated with BP sera and efficiently inhibited C3 fixation and C5a anaphylatoxin liberation. Another fusion protein designed for targeted inhibition of the alternative pathway was not effective in our model, illustrating the dependence on the classical C activation pathway in human BP. Because BP180 is expressed at the BMZ of skin, mucous membranes, and the retina, this innovative approach may be translated to various C dependent diseases affecting these tissues, e.g., mucous membrane pemphigoid, epidermolysis bullosa acquisita, or age related macular degeneration. Alternatively, the compound domain may be exchanged for other pharmaceutically active drugs, allowing for targeted delivery to the BMZ in a broad range of other diseases of the skin and mucous membranes.

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Transcriptomic profiling reveals a pronounced TH22 signature in dupilumab-associated face and neck dermatitis

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Dupilumab, a therapeutic antibody that blocks the eczematous type 2 immune response in atopic dermatitis (AD), has shown efficacy in many clinical trials and real-life observational studies. Besides blepharitis and conjunctivitis, the de novo appearance of head-neck dermatitis is recognized as a distinct side effect, occurring in up to 10% of patients at any time after dupilumab initiation. Histopathological features distinct from conventional AD such as psoriasiform hyperplasia or increased numbers of ectatic capillaries suggest a drug effect, but exact underlying mechanisms remain largely unknown. We have thus profiled punch biopsies from dupilumab-associated face and neck dermatitis (DAFND) by using single-cell RNA sequencing, and compared data with untreated AD of the same region and of the trunk, as well as healthy control individuals. We found that dupilumab treatment was accompanied by normalization of IL-4/IL-13 activity markers such as *CCL17*, *CCL18* and *CCL26* in antigen-presenting cells and myofibroblasts, confirming effective inhibition of type 2 inflammation within DAFND lesions. Nevertheless, *IL13* itself was still considerably increased in all AD groups, including DAFND skin. In addition, *IL22*, a type17/type22-associated cytokine, was significantly increased in helper T cells of DAFND compared to untreated head-neck AD and AD from the trunk. By contrast, other type 17-associated mediators such as *IL17A* or *IL26* were neither elevated in DAFND nor untreated head-neck dermatitis, while lesional skin of the trunk showed upregulation of *IL26*. Our data suggest that dupilumab effectively dampens conventional type 2 inflammation downstream of the IL4 receptor in a broad range of leukocytes and stromal cells within DAFND lesions, but with concomitant upregulation of type 22-associated T-cell derived inflammation, which might be the key drivers of this dupilumab-associated side effect.