019

Single cell level protein analysis of autoantigen-reactive B cells in systemic sclerosis and the murine model

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Since the antigen affinity of B cells varies from cell to cell, functional analysis in each B cell has been difficult from technical aspects. Especially in autoimmune diseases, promising pathogenic B cells have not been adequately studied to date because of the rarity. Here, we analyzed the single-cell function of autoantigen-reactive B cells in systemic sclerosis (SSc). Topo I-reactive B cells were extracted from peripheral blood of SSc patients. mRNA expression and protein expression analysis, co-culture analysis with T cells in micro-space, and cytokine and antigen binding capacity analysis were performed. Functional, cytokine, and antigen binding capacity analysis were also performed in the SSc murine model. We also confirmed the pathogenicity by adoptive transfer experiments. The significance of Bruton's tyrosine kinase (BTK) inhibitors in this murine model was also investigated. Topo I-reactive B cells with high affinity for topo I antigen produced large amounts of inflammatory cytokines, such as interleukin (IL)-6 and IL-23, and the frequency of those cells was high. Those with low affinity produced a large amount of the inhibitory cytokines, such as IL-10 and IL-35, and the percentage of those cells was high. The former induced T helper 17 cells and resulted in fibrosis. The latter induced regulatory T cells and suppressed fibrosis. Furthermore, the more B cells with higher antigen affinity, the more skin sclerosis and the worse lung function. Neutralization or deficiency of inflammatory cytokines, such as IL-6 or IL-23, reduced antigen affinity of topo I-reactive B cells and inhibited fibrosis. BTK inhibitor reduced topo I-reactive B cells with high affinity, increased topo I-reactive B cells with low affinity, and inhibited fibrosis. Thus, it might be that autoantigen-reactive B cells produce inflammatory cytokines or inhibitory cytokines according to their antigen affinity, which in turn affect T cells through cytokines from autoantigen reactive B cells, and contributes to fibrosis.

020

Cytokine imbalance during targeted therapies for psoriasis and atopic dermatitis – the yin yang of Th17 and Th2

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Biologics targeting specific cytokines and pathways have revolutionized the management of patients with chronic inflammatory diseases such as psoriasis. Although generally very safe, these treatments have their limitations and, surprisingly, can aggravate pre-existing or induce novel inflammatory diseases. Here, we present a cohort of psoriasis patients developing new onset eczema during anti-IL17 treatment, an intriguing side effect of IL-17 blockade with a largely unknown pathogenesis. The coexistence of psoriasis and eczema in a single patient is uncommon given their distinct and opposing immune mechanisms. Upon collection of their anamnestic data and evaluation of clinical and histopathological phenotypes, we performed gene expression analyses of skin biopsies using Nanostring technology in order to better define the inflammatory signatures involved. All patients showed very good clinical response of the underlying psoriasis to anti-IL17 treatment prior to developing eczematous lesions. Moreover, they carried elevated IgE levels, indicating an atopic predisposition. Gene expression analyses revealed that the predominant Th17 signature in plaque psoriasis is entirely replaced by a strong Th2 signature after the morphologic switch to eczema, a signature seen in atopic dermatitis. Intriguingly, two patients with atopic dermatitis receiving anti-IL4R treatment developed psoriasiform lesions and showed a shift from a Th2 towards a Th17 signature in the gene expression analysis. Our findings indicate a dynamic equilibrium between Th2 and Th17, with these two pathways controlling each other. Thus, we propose the "yin-yang" of Th2 and Th17, with IL-4 and IL17 representing antipodal vectors. This explains that blockade of these cytokines can lead, in predisposed patients, to the induction of the opposing inflammatory pathway via lifting the controlling mediator.

021

IL-4 Receptor α Blockade Attenuates Allergic Conjunctivitis in a Novel Murine Ocular Model

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Conjunctivitis, a common comorbidity in atopic dermatitis (AD), occurs at an increased frequency in AD patients given treatments that target interleukin-4 receptor alpha (IL-4Rα) or IL-13. However, the mechanisms driving this development of conjunctivitis remain unknown for AD. Wild-type BALB/c mice were treated epicutaneously with MC903 (calcipotriol)+ ovalbumin (OVA) to evoke AD-like disease and challenged ocularly with OVA or with vehicle control (phosphate-buffered saline [PBS]) once daily for 7 days. Induction of conjunctivitis was measured through ophthalmologic clinical outcomes, based on eyelid edema, secretion, squinting and redness (scored 0−12). Skin-sensitized mice were treated with an antibody specific for CD200R3 or CD4 (GK1.5) to deplete basophils and T cells, respectively, or with an IL-4Rα blocking antibody, prior to daily ocular challenge and clinical score assessment. RNA sequencing was performed on conjunctival tissue following pharmacologic IL-4Rα blockade. Data are reported as mean ± SEM. At Day 7, mice challenged ocularly with OVA showed significantly higher ocular clinical scores compared with vehicle-treated mice (7.7±0.5 vs 2±0.2). Basophil-depleted mice showed decreased ocular clinical scores compared with control antibody-treated mice (4.6±0.2 vs 7.8±0.6; $P<0.01$). Furthermore, treatment with GK1.5 led to a dramatic reduction in ocular clinical scores (3.1±0.3 vs 7.3±1.5; $P<0.0001$). Anti-IL-4Rα-treated mice displayed attenuated ocular inflammation (4.9±0.7 vs 6.7±0.7; $P<0.0001$) and normalization of ocular gene expression compared with isotype-treated mice. Our study unveiled anti-IL-4Rα-augmented genes that encode tear proteins and enzymes. In this novel murine model of allergic conjunctivitis, both basophils and CD4+ T cells were necessary for development of conjunctivitis, while IL-4Rα blockade attenuated conjunctivitis development. Anti-IL-4Rα-augmented genes may represent candidate mechanisms for the increased frequency of conjunctivitis in AD patients given treatments targeting IL-4Rα or IL-13.

022

Using single-cell transcriptomics to characterise early mechanisms of disease remission in psoriasis

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Biologic drugs have revolutionised outcomes in psoriasis, with a growing number of patients achieving clear skin ('remission'). However, treatment involves expensive regular injections, and the long-term impact remains uncertain. An improved understanding of the early mechanisms underpinning biologics-induced remission may help inform strategies around drug withdrawal. This promises to overcome the burden of long-term continuous therapy. Here, we performed single-cell RNA sequencing on skin biopsies from 5 individuals with psoriasis who achieved remission upon treatment with the exemplar IL23p19 inhibitor risankizumab. We profiled whole skin at baseline, day-3, and -14 of treatment. Data was filtered, integrated, scaled, and normalized with the Seurat package, yielding a final dataset of 176,967 cells. A graph-based clustering approach identified 39 cell types, including keratinocyte, fibroblast, myeloid, and T cell subsets. Differential expression analysis demonstrated marked differences in transcript levels between day 0 and day 3, with the largest number of modulated genes observed among fibroblast and myeloid populations. Changes in the relative abundance of cell types became apparent at day 14, and could be verified by deconvolution of published bulk-RNA sequencing data. Finally, trajectory analysis suggested that the inflammatory cell states that characterise lesional skin are reversed soon after risankizumab treatment. Our high-resolution atlas indicates that myeloid and fibroblast populations are 'early responders' to risankizumab therapy, potentially driving biologic-induced remission.

023

Tick feeding on human skin represses the local immune response facilitating pathogen transmission

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As a consequence of global temperature rise, ticks (*Ixodida*) and tick-borne diseases are emerging. During tick attachment to human skin, the feeding cavity becomes a site of transmission for tick salivary compounds, which can exert immunosuppressive effects. Tick-borne pathogens including *Borrelia burgdorferi* may benefit from dampened immune activation at the bite site. We therefore assessed local and circulating human immune cells and cytokine secretion upon tick bite and developed a human skin explant model mimicking *Ixodes ricinus* bite. Analysis of tick feeding sites revealed a rapidly occurring pattern of immunomodulation in human skin. Neutrophil, B and T cell populations increased, suggesting an initial inflammatory response independent from pathogen transmission. We observed higher expression of T cell tissue-residency markers following tick bite, whereas T cell and innate lymphoid cell cytokine production was stunted. Furthermore, tick bite biopsies contained decreased numbers of dermal dendritic cells and Langerhans cells. In our explant model, local immunomodulation was replicated upon injection of tick salivary gland extracts with and without *B. burgdorferi* spirochetes. In early stages of *B. burgdorferi* model infections, we detected strain-specific immune responses and close spatial relationships of macrophages and spirochetes. Importantly, pre-incubation of spirochetes with tick salivary gland extracts hampered local accumulation of immune cells and increases spirochete loads. Collectively, we show that tick feeding exerts profound changes on the skin immune network, which interfere with the primary response against tick-borne pathogens.

024

Antifibrotic effects of a calpain inhibitor ALLN on bleomycin-induced systemic sclerosis model via antagonizing TGF-β/Smad signaling pathway

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Systemic sclerosis (SSc) is a connective tissue disorder representing fibrosis in the skin and internal organs such as lungs. An activated differentiation of local progenitor cells to myofibroblasts is likely a key mechanism underlying overproduction of extracellular matrix and resultant tissue fibrosis in SSc. Calpains are family members of Ca^{2+}-dependent cysteine proteases for which the biological action may contribute to fibrosis in various organs. However, the precise mechanism of calpain-dependent fibrosis and therapeutic utility of their inhibitors in SSc remain unclear. This study aimed to investigate if a potent calpain inhibitor ALLN could possess the antifibrotic effects on a bleomycin-induced SSc model mice and human cultured cells. Normal human dermal fibroblasts pretreated with ALLN were stimulated with recombinant TGF-β1, followed by assessment for expression properties of TGF-β1/Smad signaling and fibrogenic molecules. ALLN (3mg/kg/day) was intraperitoneally administered 3 times a week in bleomycin-induced SSc model mice. ALLN treatment significantly inhibited over-phosphorylation and nuclear transport of Smad2/3 in TGF-β1-stimulated dermal fibroblasts. TGF-β1-dependent increase of α-smooth muscle actin, collagen type 1, fibronectin 1, and representative mesenchymal markers were attenuated in mRNA and protein expression by ALLN. Likewise, ALLN reverted a TGF-β1-dependent change of epithelial/mesenchymal markers in human lung epithelial cells. Consistent with these, ALLN remarkably suppressed the development of skin and lung fibrosis, following decrease of infiltrating $CD3^{+}$T cells, in bleomycin-induced SSc model mice. No obvious side effects were observed. Our data provide evidence that calpains may be a primary contributor and novel therapeutic target for skin and lung fibrosis in SSc, with a treatment perspective of its inhibitor ALLN.