Objectives: Here, we investigated the therapeutic potential of ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor used in B cell malignancies, to alter B cell pathology in SSc in an *in vitro* model of autoimmunity.

Methods: PBMCs and sorted B cells of 24 patients with SSc were used for functional testing after stimulation with hypomethylated DNA fragments (CpG) to induce an innate immune response. The effects of ibrutinib on cytokine production, autoantibody release and activation of the transcription factor NFκB were evaluated via multiplex cytokine assay, ELISA and flow cytometry.

Results: Ibrutinib was able to reduce the production of the profibrotic hallmark cytokines IL-6 and TNF-α, which are mainly released by the effector B cell population, in response to TLR9-stimulation. Importantly, small doses of ibrutinib (0.1 μM) preserved the production of immunoregulatory IL-10 and IFN-γ while effectively inhibiting the cardinal cytokines of hyperactivated profibrotic effector B cells in SSc. Intracellular cytokine staining of IL-6 in B cell subsets further endorsed the potential of ibrutinib to inhibit B cells in a subset-specific manner, reducing IL-6⁺ naïve B cells significantly but not IL-6⁺ memory B cells. The subset specificity was abolished when high doses of ibrutinib (10 μM) were applied. In a flow cytometry analysis of phosphorylated NFκB, an important transcription factor in the induction of innate immune responses in B cells, significantly less activation was observed with ibrutinib treatment (0.1 μM). Higher doses of ibrutinib were unable to further reduce the abundance of pNFκB.

Conclusion: Our data could pave the avenue for a clinical application of ibrutinib for patients with SSc as a novel treatment option for the underlying pathogenetic immune imbalance contributing to disease onset and progression.

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AB0030 INCREASED CIRCULATING CD19+CD24^{hi}CD38^{hi} REGULATORY B CELLS ARE BIOMARKERS OF RESPONSE TO METHOTREXATE IN EARLY RHEUMATOID ARTHRITIS

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Background: The protagonism of regulatory B cells seems to vary along the course of the disease in murine models of inflammatory conditions. Decreased numbers of circulating regulatory CD19+CD24^{hi}CD38^{hi} transitional B cells (cTrB) have been described in patients with longstanding RA.

Objectives: To examine the frequency and evolution of cTrB cells in the peripheral blood of early RA (ERA) patients.

Methods: Freshly isolated PBMCs from 48 steroid and DMARD-naïve ERA patients with a disease duration below 24 weeks and 48 healthy controls (HC) were examined by flow cytometry. Cocultures of isolated memory B cells were established with autologous T cells, in the absence or presence of TrB cells.

Results: As compared with HC, ERA patients demonstrated an increased frequency of cTrB cells. cTrBs of ERA and HC displayed an anti-inflammatory cytokine profile and were able to downregulate T cell IFNγ and IL-21 production, together with ACPA secretion in autologous B/T cell cocultures. Basal frequencies of cTrBs above the median value observed in HC were associated with a good EULAR response to MTX at 12 months (RR=2.91; 95% CI, 1.37-6.47). A significant reduction of cTrBs was observed 12 months after initiating MTX, when the cTrB cell frequency was no longer elevated but decreased, and this was independent of the degree of clinical response or the intake of prednisone.

Conclusion: An increased frequency of regulatory cTrB cells is apparent in untreated ERA, and the baseline cTrB cell frequency is associated with the clinical response to MTX at 12 months.

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AB0031 T HELPER 17 CELLS WERE SIGNIFICANTLY DECREASED BY MITOCHONDRIAL ELECTRON TRANSPORT CHAIN COMPLEX INHIBITOR IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Reactive oxygen species (ROS) and T helper 17 (TH17) cells have been known to play an important role in the pathogenesis of rheumatoid arthritis (RA). However, the interrelationship between ROS and TH17 remains unclear in RA

Objectives: To explore whether ROS affect TH17 cells in peripheral blood mononuclear cells (PBMC) of RA patients, we analyzed ROS expressions among T cell subsets following treatment with mitochondrial electron transport chain complex inhibitors.

Methods: Blood samples were collected from 40 RA patients and 10 healthy adult volunteers. RA activity was divided according to clinical parameter DAS28. PBMC cells were obtained from the whole blood using lymphocyte separation medium density gradient centrifugation. Following PBMC was stained with Live/Dead stain dye, cells were incubated with antibodies for CD3, CD4, CD8, and CD25. After fixation and permeabilization, samples were stained with antibodies for FoxP3 and IL-17A. MitoSox were used for mitochondrial specific staining.

Results: The frequency of TH17 cells was increased by 4.83 folds in moderate disease activity group (5.1>DAS28≥3.2) of RA patients compared to healthy control. Moderate RA activity patients also showed higher ratio of TH17/Treg than healthy control (3.57 folds). All RA patients had elevated expression of mitochondrial specific ROS than healthy control. When PBMC cells were treated with 2.5μM of antimycin A (mitochondrial electron transport chain complex III inhibitor) for 16h, the frequency of TH17 cells was significantly decreased.

Conclusion: The mitochondrial electron transport chain complex III inhibitor markedly downregulated the frequency of TH17 cells in moderate disease activity patients with RA. These findings provide a novel approach to regulate TH17 function in RA through mitochondrial metabolism related ROS production.

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AB0032 ABNORMAL STATUSES OF PERIPHERAL CD4+T CELL SUBSETS IN PATIENTS WITH GOUT AND THEIR CHANGES AFTER RECEIVING COMBINED IMMUNOMODULATORY THERAPY

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Background: Gout is a chronic systemic inflammatory disease that results from the deposition of monosodium urate crystals in joints and the associated activation of the innate immune system associated with hyperuricemia¹. As the pathogenesis of gout is still a matter of speculation and debate, accumulating evidence converges on inflammasome activation and immunological dysregulation². However, the detailed statuses of lymphocyte subsets in patients with gout are unknown and influence of immunomodulatory combination therapies on the lymphocyte subsets remain to be clearly evaluate³.

Objectives: To evaluate the quantitative statuses of peripheral CD4⁺T subpopulations in patients with gout and further investigate the effects of immunomodulatory combination therapies on those cells.

Methods: Total 247 patients who met the clinical criteria of gout from the American College of Rheumatology and 206 healthy controls (HCs) were enrolled in this retrospective cross-sectional study. Among those patients, 70 follow-up patients donated their peripheral blood after receiving immunomodulatory drugs (e.g., low-dose interleukin-2, rapamycin, metformin, retinoic acid, etc). The absolute numbers of Th1, Th2, Th17 and Tregs in peripheral CD4⁺T subsets were detected by flow cytometry combined with standard absolute counting beads.

Results: Compared with HCs, the absolute numbers of Th1 and Th17 were evidently increased in gout patients ($P < 0.001$), while the level of Tregs was significantly decreased ($P < 0.05$) (Figure 1). After immunomodulatory combination treatments, there were dramatical increases in a wide variety of CD4⁺T subsets such as Th1, Th17 and Tregs ($P < 0.05$). Interestingly, the increased amount of Tregs was much more than that of other T cells, leading to the decrease ratios of T cells/Tregs such as Th2/Tregs, restoring immune homeostasis (Figure 2).

Conclusion: This cross-sectional study clarified the abnormal statuses of CD4⁺T subsets in gout patients, suggesting that CD4⁺T subsets, especially Tregs, might be relevant and play a crucial role in the pathogenesis of gout, thus providing a potential therapeutic target for gout patients. Immunomodulatory combination therapies effectively increase the number of Tregs and may help for gout patients' symptom remission.

Figure 1: Comparison of absolute counts of peripheral CD4⁺ lymphocyte subsets between patients with gout (n=247) and healthy controls (n=206). All the CD4⁺ lymphocyte subpopulations were analyzed by detected by flow cytometry combined with standard absolute counting beads. Data were presented as mean±SD and statistical analysis was determined by two-tailed unpaired t-test. Patients had lower levels of Tregs and T cells/Tregs such as Th1 and Th17 compared with those of healthy controls. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2: Changes of peripheral CD4⁺ lymphocyte subpopulations after immunomodulatory combination treatments (n=70). Data were presented as mean±SD and statistical analysis was determined by two-tailed paired t-test. Absolute counts of Tregs and T cells/Tregs such as Th1 and Th2 in gout patients were significantly increased after immunomodulatory combination treatments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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AB0033

CHARACTERIZATION OF THE PERIPHERAL B CELL COMPARTMENT IN PATIENTS WITH EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS

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Background: Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare form of systemic vasculitis, which is characterized by bronchial asthma, hypereosinophilia, and systemic vasculitis. B-lymphocytes play a key role in EGPA as producers of IgE and anti-neutrophil cytoplasmic antibodies (ANCA). Indeed, the neutrophils that are targeted by these antibodies are widely described as the mechanism of endothelial damage in this disease. On the other side, the therapeutic response to rituximab in EGPA patients provides evidence for a role of B-cells in the pathogenesis of EGPA. Therefore characterizing B cell subpopulations may help in understanding the disease and the treatment.

Objectives: To characterize the peripheral B cell compartment in patients with EGPA and to analyze the in vivo potential of B lymphocytes to class-switch to IgE and to assess in vitro the differentiation potential of naïve B cells of EGPA patients into IgE-secreting plasmablasts.

Methods: Clinical characteristics of the patients, including organ involvement and treatment regimen were evaluated. Laboratory work-up included ANCA-status, eosinophils, IgE, IgG, IgA, IgM, and peripheral CD19⁺ B-cell count. For immunophenotyping isolated PBMCs were stained with monoclonal or polyclonal antibodies and B cells were classified into: naïve, marginal zone, class-switched memory B cells, unconventional memory B cells, transitional and plasmablasts. Furthermore, the expression of IgG+ and subclasses IgG1-4, IgA⁺, IgE⁺ B cells, BAFFR and TACI was quantified. For in vitro differentiation assays magnetically isolated B lymphocytes from EGPA patients and age-matched healthy controls were stimulated with CD40L, IL-21 and IL-4. Starting the culture with equal number of B cells, the absolute number of plasmablasts, and IgE class switched cells after 9 days was determined by counting the events in the CD27^{high}CD38^{high} gate or the IgG/A/D-IgE⁺ gate by flow cytometry. IgE secretion in the supernatant was measured by ELISA. JAK-STAT signalling pathway was analyzed in response to IL-4 and IL-21 stimulation and phosphorylation of STAT5 and 6 measured by flow cytometry.

Results: 34 patients with EGPA diagnosed according to ACR and CHC-criteria were included into the study. Ten of these patients were analysed separately because they received rituximab therapy. Peripheral B cell numbers in EGPA patients were markedly diminished. B cell subpopulation phenotyping showed in average 57.9% naïve B cells, 12.5 % marginal zone like B cells and 19.2% switched memory B cells. Plasmablasts constituted in average 1.15% of the peripheral B cell compartment, transitional B cells 2.0%. Interestingly, the expression of BAFF receptor and TACI in the memory B cell subset was significantly decreased in EGPA patients when compared with healthy donors. In vitro assays of isolated B cells from EGPA patients demonstrated an increased proportion of IgE-class-switched B cells after 9 days of culture under IL4 stimulation compared with controls. However, no differences were observed in the phosphorylation of STAT5 and STAT6 after stimulation with IL-4 or IL-21.

Conclusion: In the EGPA-patients we observed markedly diminished B-cells despite of normal lymphocyte counts. B cells showed a reduced expression of BAFF-R and TACI. Class switch to IgE is enhanced in EGPA patients.

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AB0034

EARLY IMMUNIZATION AGAINST TREATMENT IS ASSOCIATED TO POOR CLINICAL RESPONSE AT 6 MONTHS AND LOW NUMBER OF TRANSITIONAL B CELLS AT BASELINE IN RHEUMATOID ARTHRITIS PATIENTS TREATED BY ADALIMUMAB

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Background: Circulating anti-drug antibodies (ADAs) are detectable approximately in 33% of adalimumab treated rheumatoid arthritis (RA) patients, often within the first 6 months of therapy^{1,2}. Classically, circulating ADAs associate with their specific drugs to form immune complexes, increasing drug clearance, and by this mechanism reducing therapeutic effect³. B cell involvement leading to ADAs production is not yet well established.