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 pathway in SSc in an in vitro model of autoimmunity.

Methods: PBMCs were isolated from B cells of 24 SSc 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 NFkB were evaluated via multiplex cytokine assay, ELISA and flow cytometry.

Results: Ibrutinib was able to reduce the production of the proinflammatory 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 IFNγ 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.

References:

Disclosure of Interests: Jakob Einhaus: None declared, Ann-Christin Pecher: None declared, Elisa Asteriti: None declared, Hannes Schmid: None declared, Irene Monjo: None declared, Amaya Puig-Kröger: None declared, Reinhild Klein: None declared, Corina Schneidawind: None declared, Jörg Hennes Grant/research support from: Novartis, Roche, Cellgene, Pfizer, Abbvie, Sanofi, Sandokan, Speaker’s bureau: Abbvie, Lilly, Sanofi, Novartis, Pfizer, UCBB, Roche, Nordic, Sandokan, Maria-Eugenia Miranda-Carús Grant/research support from: BMS, Roche DOI: 10.1136/annrheumdis-2020-eular.930

Objectives: To examine the frequency and evolution of cTrB 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 FoxP3. 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 2.5uM 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.

References:

Disclosure of Interests: None declared DOI: 10.1136/annrheumdis-2020-eular.3441

Objectives: In order to investigate the potential of ibrutinib to inhibit B cells in a subset-specific manner, reducing 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.

References:

Disclosure of Interests: Jakob Einhaus: None declared, Ann-Christin Pecher: None declared, Elisa Asteriti: None declared, Hannes Schmid: None declared, Kathy Ann-Scruggs: None declared, Hildegard Keppeler: None declared, Reinhild Klein: None declared, Corina Schneidawind: None declared, Jörg Hennes Grant/research support from: Novartis, Roche, Cellgene, Pfizer, Abbvie, Sanofi, Sandokan, Boehringer-Ingelheim,; Dominik Schneidawind: None declared DOI: 10.1136/annrheumdis-2020-eular.1791

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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 FoxP3. 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 2.5uM 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.

References:

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