Innate immunity in rheumatic diseases

POS0364

EFFECT OF BIOLOGIC DMARDS TREATMENT ON GENE EXPRESSION AND ACTIVITY OF NATURAL KILLER CELLS IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is the most common inflammatory type of arthritis, with various immune players implicated in its pathogenesis. Natural killer (NK) cells are innate lymphocytes that showed controversial roles in RA, whether pathogenic or protective (Shegarfi, H. et al. 2012, Yap, H.-Y. et al. 2018). Previously, we were able to identify a gene signature in NK cells of RA patients that can aid in understanding the state of NK cells in RA disease and identify RA patients from healthy controls (Elemam, N.M. et al. 2019, Elemam, N.M. et al. 2020). Furthermore, this signature might facilitate the selection of biomarkers that can be used for early detection of RA and prediction of effectiveness of RA treatment.

Objectives: In this study we aimed at exploring the effect of several RA therapeutic agents such as tocilizumab, rituximab and anti-TNF α (adalimumab, etanercept and golimumab) on the previously identified gene signature in NK cells of RA patients.

Methods: Whole blood transcriptomic data from publicly available dataset (GSE93272) was used to predict the percentage of activated NK cells in the blood of RA patients using the software CIBERSORT. Then, a correlation analysis was done between the percentage of NK cells and number of days of receiving tocilizumab treatment. Whole blood samples were collected from the recruited 17 RA patients (satisfying the 2010 ACR/EULAR classification criteria for RA). NK cells were isolated using RosetteSep negative selection method and RNA was extracted and gene expression was assessed using qRT-PCR. RA patients taking tocilizumab, rituximab, or anti-TNF α (adalimumab, etanercept or golimumab but none of the patients received infliximab) were compared to those not receiving any biological DMARDs. Statistical analysis was done using Student's t-test.

Results: In silico analysis has shown that the percentage of activated NK cells is positively correlated with the number of days of tocilizumab therapy in RA patients, suggesting a direct enhancing effect of tocilizumab on NK cell activity. Then, it was crucial to investigate the effect of different biological DMARDs on NK gene expression in RA patients. All the investigated chemokines (CCL2, CXCL10, CXCL16, CXCR1, CXCR2, CXCR6 and CCR4) in the identified gene signature showed a significant change in RA patients receiving tocilizumab, rituximab, or anti-TNF α therapies. Furthermore, the other genes including RELA, ICAM, IL1RN, TLR3 and TLR10 were significantly changed in NK cells of RA patients receiving biological DMARDs in comparison to patients not receiving the treatments. However, some of the genes including CD56, BTK, IBTK, ITGB7, IL1B, PECAM-1, IL12RB2, IFNG and CKLF did not show a significant change upon receipt of biological DMARDs.

Conclusion: In conclusion, NK cell activity and gene expression could be affected by the type of biological DMARDs received by RA patients. Therefore, this identified gene signature of NK cells could be used as a diagnostic tool to identify RA patients and a target for biological DMARDs in RA.

REFERENCES:

- Elemam, N. M., M. Y. Hachim, S. Hannawi and A. A. Maghazachi (2019). "Natural Killer Cells Gene Expression Can Differentiate Rheumatoid Arthritis Patients from Healthy Controls [abstract]." ACR/ARP Annual Meeting, supplement of *Arthritis & Rheumatology* **71**(suppl 10).
- Elemam, N. M., M. Y. Hachim, S. Hannawi and A. A. Maghazachi (2020). "Differentially Expressed Genes of Natural Killer Cells Can Distinguish Rheumatoid Arthritis Patients from Healthy Controls." *Genes* (Basel) **11**(5).
- Shegarfi, H., F. Naddafi and A. Mirshafiey (2012). "Natural killer cells and their role in rheumatoid arthritis: friend or foe?" *TheScientificWorldJournal* **2012**: 491974-491974.
- Yap, H.-Y., S. Z.-Y. Tee, M. M.-T. Wong, S.-K. Chow, S.-C. Peh and S.-Y. Teow (2018). "Pathogenic Role of Immune Cells in Rheumatoid Arthritis: Implications in Clinical Treatment and Biomarker Development." *Cells* **7**(10): 161.

Figure 1. In silico analysis and correlation of NK cell activity with the number of days of Tocilizumab therapy in RA patients.

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ANTI-TNF GLUCOCORTICOID RECEPTOR MODULATOR ANTIBODY DRUG CONJUGATE FOR THE TREATMENT OF AUTOIMMUNE DISEASES

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Background: Glucocorticoids (GC) are potent drugs used for treating many inflammatory diseases. While GCs are effective in many immune diseases, dose and duration of administration is limited due to significant side effects. Resting immune cells have very little TNF expression on the cell surface and it is only in an activated state that TNF expression is upregulated. Upon immune cell stimulation, TNF is upregulated and although a significant amount of TNF is cleaved from an activated cell, a portion remains on the cell surface. We have observed that anti-TNF antibodies bind to transmembrane TNF (tmTNF) and undergo endocytosis to the lysosome (1). We have developed a stable antibody drug conjugate (ADC), ABBV-3373, that has a proprietary, highly potent, glucocorticoid receptor modulator (GRM) payload linked to an anti-TNF monoclonal antibody (mAb) that is able to deliver the GC payload to activated immune cells.

Objectives: We hypothesized that a TNF ADC with a GRM payload would be able to deliver increased efficacy through both TNF inhibition and targeted GRM payload delivery to activated immune cells while sparing systemic glucocorticoid side effects.

Methods: A mouse surrogate TNF GRM ADC was characterized in an acute *in vivo* contact hypersensitivity model of inflammation (CHS) and in a mouse model of collagen induced arthritis (mCIA). Additionally, the human anti-TNF GRM ADC, ABBV-3373 has been characterized in healthy volunteers.

Results: In the CHS model the anti-TNF GRM ADC significantly inhibited the inflammatory response with minimal effect on systemic GC biomarkers. In mCIA a single dose of an anti-TNF GRM ADC, administered at disease onset, was able to completely inhibit arthritis for greater than 30 days while an anti-TNF mAb only partially inhibited disease. ABBV-3373, a human anti-TNF GRM ADC with a GC payload, was evaluated in a Phase 1 study in healthy volunteers. ABBV-3373 demonstrated antibody-like PK profile and ABBV-3373 did not impact cortisol levels at predicted efficacious doses while control subjects that received a single oral dose of 10 mg prednisone demonstrated expected decreases in cortisol levels.

Conclusion: These data suggest that an anti-TNF ADC delivering a GRM payload into activated immune cells may provide improved efficacy in immune mediated diseases, while minimizing systemic side effects associated with standard GC treatment.

REFERENCES:

- Deora, A. et al. *MABs*. 2017;9(4):680-695.

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POS0366

PRO-INFLAMMATORY EFFECTS OF HUMAN APATITE CRYSTALS EXTRACTED FROM PATIENTS SUFFERING FROM CALCIFIC TENDINOPATHY

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Background: Calcific tendonitis of the rotator cuff is due to carbonated apatite deposits in the shoulder tendons. During the evolution of the disease, an acute inflammatory episode may occur leading to the disappearance of the calcification. Although hydroxyapatite crystals-induced inflammation has been previously studied with synthetic crystals, no data are available with calcifications extracted from patients suffering from calcific tendinopathy. The objective of the study was to explore the inflammatory properties of human calcifications and the pathways involved.

Objectives: The objective of the study was to explore the inflammatory properties of human calcifications and the pathways involved.

Methods: Human calcifications were obtained from patients treated for their shoulder pain related to a calcific tendinopathy of the rotator cuff. Calcifications were extracted by ultrasound-guided lavage and aspiration as previously described [1]. Human calcifications and synthetic hydroxyapatite (sHA) were used *in vitro* to stimulate human monocytes and macrophages, the human myeloid cell line THP-1 and human tenocytes. The release of IL-1 β , IL-6 and IL-8 by cells was quantified by ELISA. Gene expression of pro- and anti-inflammatory cytokines was evaluated by quantitative PCR. NF- κ B activation and NLRP3 involvement was assessed in THP-1 cells using a NF- κ B inhibitor and a Caspase 1 inhibitor. The inflammatory properties were then assessed *in vivo* using a mouse air pouch model. The membrane thickness and infiltrate were assessed 6 and 24 hours after the injection of human calcifications or synthetic hydroxyapatite using hematoxylin and eosin staining. Macrophages, neutrophils and lymphocytes infiltrates were assessed by immunohistochemistry. Total RNA was extracted from the membranes and expression of IL-1 β , IL-6 and TNF β was quantified by PCR.

Results: Human calcifications were able to induce a significant release of IL-1 β when incubated with monocytes, macrophages and THP-1 only if they were first primed with LPS (lipopolysaccharide) for monocytes and macrophages or PMA (Phorbol 12-myristate 13-acetate) for THP-1. No IL-1 β was detected in tenocytes' supernatants. Stimulation of THP-1 by human calcifications led to similar levels of IL-1 β when compared to synthetic hydroxyapatite although these levels were significantly inferior in monocytes and macrophages. IL-6 and IL-8 levels were not increased in the supernatants after crystal stimulation. Patient's crystals enhanced mRNA expression of pro-IL-1 β , as well as IL-18, NF- κ B and TGF β when IL-6 and TNF α expression were not. IL-1 β production was reduced by the inhibition NF- κ B as well as Caspase 1 indicating the role of NLRP3 inflammasome. *In vivo*, injection of human calcifications or synthetic hydroxyapatite in air pouch led to significant increase in membrane thickness with an infiltrate mainly composed of macrophages. Significant overexpression of IL-1 β was only observed in the synthetic hydroxyapatite group.

Conclusion: As synthetic hydroxyapatite, human calcifications were able to induce an inflammatory response resulting in the production of IL-1 β after NF- κ B activation and through NLRP3 inflammasome. In some experiments, IL-1 β induction was lower with human calcifications compared to synthetic apatite. Differences in size, shape and protein content may explain this observation.

REFERENCES:

- [1] Darrieutort-Laffite C, Arnolfo P, Garraud T, Adrait A, Couté Y, Louarn G, et al. Rotator Cuff Tenocytes Differentiate into Hypertrophic Chondrocyte-Like

Cells to Produce Calcium Deposits in an Alkaline Phosphatase-Dependent Manner. J Clin Med. 2019 Sep 26;8(10):1544. doi: 10.3390/jcm8101544.

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POS0367

NLR4 AND FC- γ -R CROSSTALK ON CD1c+ DENDRITIC CELLS DIFFERENTIALLY CONTRIBUTES TO RHEUMATOID ARTHRITIS IMMUNOPATHOLOGY

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Background: Rheumatoid arthritis (RA) is an autoimmune disorder in which Th17 cells, B cells and inflammatory cytokines (1-3) contribute to joint tissue damage, however the role of specific myeloid populations to immunopathogenesis of RA remains unclear.

Objectives: To address this question, we studied transcriptional, phenotypical and functional characteristics of monocytes (Mo), CD1c+ and CD141+ conventional dendritic cells (cDC) from RA patients.

Methods: Frequencies and maturation patterns of Lin-CD14-HLADR+ plasmacytoid (CD11c-), CD1c+ and CD141+ cDC (CD11c+) subsets and CD14+ Mo from n=25 RA patients at baseline were analyzed by multicolor flow cytometry. In addition, longitudinal studies on the evolution of these populations after treatment initiation were conducted on a smaller group of RA patients. Moreover, CD1c+ and CD141+ cDC subsets and total Mo were sorted from the peripheral blood from n=4 untreated RA and healthy individuals and the synovial fluid from n=3 RA and chondrocalcinosis patients. Differential transcriptional patterns within each population were analyzed by RNAseq. Functional validation of targets were performed *in vitro* with cDC subsets isolated from the synovial fluid of RA patients. Finally, silencing of expression of NLR4 and NLRP3 on CD1c+cDCs was performed with specific siRNAs.

Results: Both CD1c+ (p=0.0001) and CD141+ (p=0.0008) cDCs were significantly depleted from the blood and enriched in the synovial fluid from untreated RA patients, but proportions of CD1c+ cDCs were more significantly recovered after treatment initiation and associated with improved clinical parameters. In addition, specific increased expression levels of the IgG-Fc receptor CD64 on CD1c+ cDC was associated with higher DAS28 (p=0.0002). Moreover, differential transcriptional patterns of circulating CD1c+cDCs from RA patients were characterized by genes linked to toll-like receptor, Fc-receptor, inflammasome pathways and elevated CCR2 expression (p=0.016), while CD141+cDCs transcribed interferon-related genes. Importantly, CCR2^{hi} CD1c+cDCs from the synovial fluid from RA patients transcribed proinflammatory cytokines such as IL-1 β , CCL3 and IL-8, actively expressed the inflammasome mediator caspase 1 and were more effective activating pathogenic IFN γ IL-17⁺ CD4⁺ T cells *in vitro* than CD141+ cDC (p=0.0019). These functional profiles could be artificially induced stimulating CD1c+ cDCs with dsDNA in the presence of IgGs and was dependent on caspase 1 and the NLR4 inflammasome.

Conclusion: Our data provides novel insights about specific activation and functional patterns on CD1c+cDC contributing to RA pathogenesis and identifies new sensors that could represent novel therapeutic target to treat RA.

REFERENCES:

- [1] Alvandpur N, Tabatabaei R, Tahamolli-Roudsari A, Basiri Z, Behzad M, Rezaeepoor M, et al. Circulating IFN-gamma producing CD4+ T cells and IL-17A producing CD4+ T cells, HLA-shared epitope and ACPA may characterize the clinical response to therapy in rheumatoid arthritis patients. Human immunology. 2020.
- [2] Nistala K, Adams S, Cambrook H, Ursu S, Olivito B, de Jager W, et al. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(33):14751-6.
- [3] Chapuy-Regaud S, Nogueira L, Clavel C, Sebbag M, Vincent C, Serre G. IgG subclass distribution of the rheumatoid arthritis-specific autoantibodies to citrullinated fibrin. Clinical and experimental immunology. 2005;139(3):542-50.

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