POS0444 PROFILING OF CIRCULATING MIRNAS IN DIFFICULT-TO-TREAT RHEUMATOID ARTHRITIS

J. Baloun[1,2], A. Pekacova[1,2], H. Mann[2,3], J. Vencovský[2,4], K. Pavelka[2,4], L. Šenolt[1,2]. *[1]Institute of rheumatology, Department of Experimental Rheumatology, Prague, Czech Republic; [2]1st Faculty of Medicine of Charles University, Department of Rheumatology, Prague, Czech Republic; [3]Institute of rheumatology, Inpatient department, Prague, Czech Republic; [4]Institute of rheumatology, Research Center, Prague, Czech Republic*

Background: Biologic (b-) and targeted synthetic (ts-) disease-modifying antirheumatic drugs (DMARDs) have brought significant progress in the treatment of rheumatoid arthritis (RA), but a significant proportion of RA patients still remain symptomatic despite treatment according to current recommendations. These patients have recently been defined as "difficult-to-treat (D2T)" RA (1). There is evidence that miRNA expression may play a role in the diagnosis and therapy of RA (2).
Objectives: In a retrospective study, we analyzed patients' blood samples prior to b-/ts-DMARD treatment and profiled circulating miRNAs to predict the development of D2T-RA.
Methods: A total of 36 patients fulfilling the EULAR definition of D2T-RA (1) (mean age 59.1±10.7 yrs, 78% females), 36 patients with RA in sustained clinical remission on b-/ts-DMARDs at two consecutive examinations 12 wks apart (mean age 66.3±9.6 yrs, 78% females), and 36 healthy controls (mean age 61.1±7.7 yrs, 68% females) were included in the study. Blood samples were collected before initiation of b-/ts-DMARD. We profiled circulating miRNAs using the sequencing approach and differential expression analysis was performed using DESeq2 algorithm.
Results: The massive parallel sequencing of circulating miRNAs detected 814 quantifiable miRNAs and DESeq2 algorithm revealed 35 miRNAs with different concentrations in patients who developed D2T-RA compared to patients with RA who achieved sustained remission or healthy controls. Out of these miRNAs, miR-16-5p (1.5x) and miR-451a (2.1x) were downregulated and miR-126-3p (1.4x) was upregulated in D2T RA patients compared to controls. In addition, miR-101-3p (1.5x) was downregulated in D2T RA compared to RA patients. Except for miR-101-3p, these miRNAS have been previously associated with RA and might predict development of D2T disease prior to initiation of b-/ts-DMARD therapy.
Conclusion: We found four miRNAs as potential biomarkers differentiating patients who are at risk to develop difficult-to-treat disease compared to patients who have a chance of sustained remission even before initiation of biological or targeted synthetic DMARDs. Further studies with larger sample size are needed to validated these data.
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POS0445 DISSECTING THE ROLE OF PLATELET FUNCTION IN INFLAMMATORY ARTHRITIS

D. Kimpel[1], K. Kannan[2]. *[1]University of Virginia, Rheumatology & Immunology, Charlottesville, United States of America; [2]University of Arkansas, Medicine, Little Rock, United States of America*

Background: Inflammatory autoimmune diseases such as rheumatoid arthritis (RA) show an increase in atherosclerotic cardiovascular disease, and are commonly associated with an increase in platelet counts. Thus, platelets fill a central role in the tenet: Inflammation begets coagulation, and coagulation begets inflammation. We have previously reported that induction of arthritis by the Streptococcal cell wall component peptidoglycan-polysaccharide (PGPS) results in increased platelet numbers, aggregation, activation, and increased expression of P-selectin (CD62-P) and of the costimulatory molecule CD40. These platelets are also more sensitive to stimuli such as ADP and thrombin, which induce dense granule release of serotonin and aggregation. We have also described increased P-selectin expression limited to the joints in mice with acute arthritis. Platelets, generally considered elements of the hemostatic system, are underappreciated for their pro-inflammatory potential.
Objectives: To further understand the role of platelets in inflammatory arthritis, and distinguish their biochemical and adhesive features we set out to characterize the impact of modulating platelet activity in acute and chronic rodent models of arthritis.
Methods: Chronic inflammatory arthritis was induced in Lewis rats by a single i.p. injection of PGPS which results in an early phase non-T cell dependent arthritis, and a chronic T cell dependent phase after day 10. An acute inflammatory arthritis was induced in Balb/c mice by the same method. Joint scoring and volume measurement were carried out daily to determine an arthritis severity score. The role of platelets on PGPS arthritis was assessed using a) depletion of platelets using periodic intraperitoneal (IP) anti-platelet antibody, b) blockade of the integrin GPIIb/IIIa with the monoclonal antibody (mAb) abciximab to inhibit activation and aggregation via vWF and fibrinogen. In the acute murine arthritis c) P-selectin deficient mice were assessed for severity of arthritis, and d) wild-type mice were pre-treated with a single IP injection of anti-CD41 (GPIIb) antibody.
Results: Depletion of platelets in rats during the PGPS treatment resulted in amelioration of both the early and chronic phases of disease. We previously demonstrated that TNF is elevated in this model following PGPS injection, so for comparison animals were treated with infliximab, a monoclonal antibody to TNF, with equivalent suppression of both acute and chronic phases of disease. Abciximab, targeting the dual function adhesion and signaling molecule GPIIb/IIIa, did not decrease arthritis, and in fact increased the severity of the chronic phase of arthritis. In the acute murine model, despite the previously described expression of CD62-P in joints and on platelets, the CD62-P knockout mice had no significant difference in arthritis severity. Conversely treatment of wild-type mice with the anti-CD41 to block GPIIb resulted in suppression of the arthritis.
Conclusion: Platelet depletion had a dramatic impact on both acute and chronic phases of inflammatory arthritis, which is not unexpected given that platelets carry an array of pro-inflammatory and procoagulant mediators including IL-1, chemokines, vWF, and fibrinogen, as well as an array of adhesion molecules for binding to endothelium, leukocytes, and to other platelets. The increase in chronic arthritis severity despite inhibiting platelet aggregation by blockade of GPIIb/IIIa was unexpected, but may have been due to increased platelet-endothelial binding or platelet-leukocyte aggregation thus exacerbating the chronic T cell phase. Lack of CD62-P appeared to have no influence on acute phase arthritis development. Interestingly anti-CD41, which blocks one part of the GPIIb/IIIa integrin, was effective at ameliorating arthritis in the murine model. Platelets play an often underappreciated role in inflammatory processes, but understanding the mechanisms will require further dissection of the complex nature of these cellular elements which have dual inflammatory and hemostatic roles.
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POS0446 ARP2/3 AS A LASP1 INTERACTION PARTNER REGULATES CELL-TO-CELL CONTACT FORMATION OF FIBROBLAST-LIKE SYNOVIOCYTES IN RA

D. Beckmann[1], A. Krause[1], U. Hansen[1], H. Kiener[2], J. Kremerskothen[3], H. Pavenstädt[3], T. Pap[1], A. Korb-Pap[1]. *[1]University Hospital Muenster, Institute of Musculoskeletal Medicine, Muenster, Germany; [2]Medical University of Vienna, Department of Medicine III, Division of Rheumatology, Vienna, Austria; [3]University Hospital of Muenster, Department of Nephrology and Rheumatology, Internal Medicine D, Muenster, Germany*

Background: In rheumatoid arthritis (RA), fibroblast-like synoviocytes (FLS) undergo a stable transformation resulting in an aggressive phenotype mediating cartilage damage by increased levels of adhesion molecules. In this context, Lasp1 and the Arp2/3 complex are of interest because they modulate actin organization and focal adhesion turnover.
Objectives: In this study, the effects of Arp2/3 on cadherin-11 mediated cell-to-cell contact formation have been investigated using the arthritic hTNFtg mouse model.
Methods: Expression levels of Lasp1 and Arp2/3 protein complex were investigated in synovial tissue of wild type (wt) and hTNFtg hind paws by immunohistochemistry. Primary FLS were analysed, respectively and co-immunoprecipitation experiments were performed. In addition, *lasp1*$^{-/-}$ mice were interbred with hTNFtg animals and offspring were evaluated for disease progression and joint destruction. To further study the role of Arp2/3 in the function of the cadherin-11 adhesion complex, the effects of an Arp2/3 inhibitor (CK666) on cell-to-cell contact formation in FLS derived from hTNFtg and *lasp1*$^{-/-}$hTNFtg mice were investigated by stainings. To assess signaling pathway activation, cells were stimulated with the growth factor PDGF.

Results: Upregulated Lasp1 levels were found in synovial tissue and FLS of hTNFtg compared to wt mice. Assays showed that Arp2/3 is part of the adherens junction (AJ) machinery in FLS although Arp2/3 expression levels were not changed between the genotypes. *In vivo* evaluation of *lasp1*$^{-/-}$hTNFtg mice revealed a milder arthritis score, less cartilage degradation and reduced FLS attachment to articular cartilage compared to hTNFtg mice. *In vitro*, the loss of Lasp1 led to clear alterations in AJ arrangement indicated by altered β-catenin pattern. As expected, β-catenin expression was mainly located at adhesion sites between adjacent cells. In hTNFtg FLS, these structures were characterized by a zipper-like pattern. In contrast, these structures were disrupted in *lasp1*$^{-/-}$hTNFtg FLS. Interestingly, CK666 induced zipper-like structures in hTNFtg FLS comparable to the pattern found in *lasp1*$^{-/-}$hTNFtg cells. Furthermore, *lasp1*$^{-/-}$hTNFtg FLS showed decreased Src phosphorylation following PDGF stimulation in comparison to hTNFtg FLS.
Conclusion: Lasp1 represents an interesting target involved in RA-caused joint destruction, because its loss results in significantly reduced cartilage destruction and altered FLS contacts mediated by Arp2/3.
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POS0447 TREATMENT WITH UPADACITINIB IN RA PATIENTS FOR WHICH BIOLOGIC THERAPY FAILED

L. Robles Kirkegard[1], E. Rubio Romero[1], P. León[1]. [1]*Servicio Andaluz Salud, Hospital Universitario Virgen del Roció, Sevilla, Spain*

Background: Upadacitinib is a JAK inhibitor recently approved for rheumatoid arthritis treatment with promising results in its studies for severe moderate RA for which conventional therapies failed.
Objectives: To study and describe the evolution of patients diagnosed with RA and treated with Upadacitinib who had inadequate response to biological therapy in association or not with DMARDs and GC for 6 months.
Methods: A population of 23 patients (19 of them female) with RA on treatment with Upadacitinib was analyzed over 6 months. Using patient-reported outcomes (PROs) to measure disease activity by visual analogue scale (VAS), the HAQ Disability Index (HAQ-DI), Disease Activity Score (DAS28), and morning stiffness duration. The age´s (average ± SD) = 53 ± 10 years and time of disease evolution (average ± SD) = 17.6 ± 23.3 years. Pretreatment HAQ (average ± SD) = 2.2 ± 0.5. All patients received previous biologic treatments and 61% (14 patients) combined therapy with DMARDs. Only 2 cases had no glucocorticoid treatment prior to treatment with Upadacitinib.
Results: In 3 months' time, most patients (81%, n = 21) treated with Upadacitinib were able to reduce GC dose, and this reduction was maintained 6 months from the beginning of the treatment. After 3 months of treatment, most patients experienced an enhancement in DAS28 (89%, n = 18), with an average improvement in DAS28 of (± SD) 1.87±1.09 units. Regarding the pain (VAS), 67% of the patients showed improvement after 3 months (n=18), reaching 71% after 6 months (n=17). Sixty-eight percent of treated patients showed a reduction in morning stiffness after 3 months (n = 19), and this improvement increased up to 84% of treated patients at 6 months (n = 19). Side effects were observed in five patients, consisting of dizziness and nausea. In no case were they a reason for the withdrawal of the drug. Treatment was withdrawn in three patients due to primary failure.
Conclusion: Treatment with Upadacitinib allows GS dose reduction, as well as an improvement in DAS28, VAS and morning stiffness at three months and six of treatment. These data are in line with the evidence published in Upadacitinib pivotal studies, meaning a good alternative in the treatment of patients with moderate or severe RA.
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POS0448 SYNOVIAL TRANSCRIPTOMIC PROFILES CORRELATE WITH DISEASE ACTIVITY IN EARLY UNTREATED RHEUMATOID ARTHRITIS

C. Traille[1], T. Sokolova[1], S. De Montjoye[2], A. Nzeusseu Toukap[2], L. Meric de Bellefon[2], A. Loriot[3], B. Lauwerys[1], P. Durez[1], N. Limaye[4]. [1]*UCLouvain Brussels Woluwe, IREC RUMA, Woluwe-Saint-Lambert, Belgium;* [2]*Cliniques universitaires Saint-Luc (UCLouvain), Service de rhumatologie, Bruxelles, Belgium;* [3]*De Duve Institute UCLouvain, Computational Biology, Woluwe-Saint-Lambert, Belgium;* [4]*De Duve Institute UCLouvain, Genetics of Autoimmune Diseases and Cancer, Woluwe-Saint-Lambert, Belgium*

Background: Synovitis is the common feature across all individuals with a diagnosis of rheumatoid arthritis (RA). Yet, cellular and transcriptomic alterations occuring in RA synovium are highly variable amongst patients. So far, most data on clinical-tissue correlations either rely on hypothesis-driven approaches or are potentially biased by heterogeneous clinical characteristics (e.g. disease duration or disease-modifying antirheumatic drugs).
Objectives: We used transcriptomic profiling of synovial tissue from early, untreated rheumatoid arthritis patients (ERA) to 1/ identify the genes with the most variable expression amongst patients and 2/ explore the ability of unbiased (data-driven) approaches to define clinically relevant ERA subgroups.
Methods: Synovial biopsies were harvested from clinically involved joints of ERA patients using needle arthroscopy or ultrasound-guided biopsy. Data on disease activity were collected at inclusion. For each sample, 350ng total RNA was sent for RNAsequencing using a standardized protocol (Macrogen Europe). After quality control (Fast QC) and genome alignement (HiSat2), normalized read counts were analyzed on Qlucore Omics Explorer. To focus on inter-sample heterogeneity, genes were filtered based on variance (σ/σ_{max}). Unbiased approaches (Principal Component Analysis, Unsupervised Clustering) were applied to define patients' clusters. Pathway enrichment analysis were performed on Metascape. CibersortX was used to extrapolate the immune cell subsets relative composition from gene expression data. All other statistical analyses were performed on GraphPad Prism v9.
Results: Total RNA was obtained from synovial biopsies from 74 patients. We first applied variance filtering to identify the genes whose expression showed the greatest variation between patients (n = 894 most variable genes). PCA analysis on the level of expression of these genes did not divide samples into distinct groups, instead yielding a continuous distribution broadly associated with baseline disease activity, as measured by DAS28CRP. Consequently, we used unsupervised clustering to allow for unbiased definition of two patient clusters (PtC): PtC1 (n=52) and PtC2 (n=22) based on their expression of these 894 genes. Pathway analysis of these genes revealed significant enrichment of immune system genes, in the *Inflammatory response* and *Rheumatoid Arthritis* pathways (gene cluster 1: GC1), B cell & plasma cell-related pathways (GC2) and metabolic processes-related genes (GC3). Interestingly, PtC1 and PtC2 were characterized by very different clinical features. More specifically, patients from the group with a strong B & plasma cell signature (PtC1) displayed higher baseline indices of all disease activity score components (median DAS28CRP: 5.56 *vs* 4.09; p-value = 0.0003). They also had higher rates of baseline radiological erosions (erosive disease in 34.6 % *vs* 10%; p-value = 0.0252) but similar rates of seropositive disease. In line with our pathway analyses, we found a higher signature (inferred relative frequency) of B & plasma cells, T cells and M1-like macrophages in PtC1 compared to PtC2 synovia. PtC2 synovia instead had relatively higher M2-like macrophage and resting mast cell signatures.
Conclusion: In this large synovial biopsy study, we found that synovial transcriptomic profiles in ERA patients distribute continuously based on the expression of inflammatory and immune cell transcriptomic pathways. These synovial transcriptomic signatures correlate strongly with systemic disease activity.
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