EFFECT OF IN VIVO HYDROXYCHLOROQUINE AND EX VIVO ANTI-BDCA TREATMENT ON PDC IFNA PRODUCTION FROM PATIENTS AFFECTED WITH CUTANEOUS LUPUS ERYTHEMATOSUS

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Background: BII8059 (aka 24F4A) is a monoclonal antibody that targets BDCA2, an inhibitory receptor expressed on pDCs. Plasmacytoid dendritic cells (pDCs) are a major source of Type-I Interferon (IFN-I), which is considered to be a key pathogenic driver in Cutaneous Lupus Erythematosus (CLE). Recent results from a Phase I clinical trial suggest that BII8059 may ameliorate skin lesions in CLE patients. BII8059 is currently evaluated in Phase II clinical trial for CLE with or without SLE.

Objectives: Given that Hydroxychloroquine (HCQ), a widely-used CLE therapy, and BII8059 are both able to inhibit pDC-derived IFN-I production; this study aimed to determine whether BII8059 would show an additional inhibitory effect on pDCs response after ex-vivo and in-vivo treatment with HCQ.

Methods: The effect of BII8059 on pDC-derived IFNa was measured from peripheral blood mononuclear cells (PBMC) either from healthy donors in presence or absence of HCQ or from CLE patients clinically exposed to various levels of HCQ, TLR7, TLR7/8, and TLR9 agonists (ssRNA, RA84, and Cpg-A) were used for pDC stimulation.

Results: PDCs were the only producers of IFNa in response to Cpg-A, RA84, and ssRNA stimulation in PBMC cultures. CLE patients with high blood HCQ levels showed lower ex-vivo pDC responses to Cpg-A, but not RA84 or ssRNA. In contrast, BII8059 reduced the amount of IFNa produced by pDCs from CLE patients in response to all TLR agonists, irrespective of the blood HCQ level. This effect was observed in patients with low or high blood IFN signature and in patients with or without concomitant SLE diagnosis.

Conclusion: Clinically-relevant HCQ concentrations partially inhibit the pDC response to TLR9 and weakly affect the response to TLR7/8 stimulation. BII8059 robustly inhibits pDC responses even in the presence of HCQ, highlighting its unique potential to disrupt pDC disease relevant biology, which could provide additional therapeutic benefit for CLE patients.


PHARMACOLOGICAL MANIPULATION OF SIRTUIN 1 ACTIVITY IN EXPERIMENTALLY INDUCED ARTHRITIS

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Background: Sirtuin 1 (Sirt1) is a member of the sirtuin family of NAD+ dependent protein deacetylases. This nuclear enzyme with deacetylase activity serves as a metabolic sensor and transcriptional regulator, and exerts its beneficial effects by promotion of cell viability, tissue regeneration, and inhibition of inflammation.

Objectives: 1) To investigate the effect of pharmacological modulation of Sirt1 enzymatic activity on disease outcomes of experimental rheumatoid arthritis in mice; and 2) To characterize surface marker and pro-inflammatory cytokine expression in neutrophils following Sirt1 activation or inhibition.

Methods: Arthritis was induced by intra-peritoneal administration of a cocktail of monoclonal antibodies against collagen type II in Balb/c mice (CAIA). We utilized synthetic compounds which specifically activate or inhibit Sirt1 activity - SRT2183 and EX527, respectively. We investigated the effect of Sirt1 activity modulation on clinical scores of arthritic mice after subcutaneous seven-day treatment initiated at Day 7 of CAIA. We used flow-cytometry for phenotyping and functional analysis of blood, and bone marrow (BM) derived neutrophils stimulated in vivo, and in vitro. The effects of the two compounds on Sirt1 activity and on cell viability were assessed using ELISA-based colorimetric assays.

Results: Activation and inhibition of Sirt1 activity led to CD11b down-regulation and CCR2 up-regulation on healthy blood and BM neutrophils, suggestive of neutrophil mobilization from the BM to the periphery following treatment in vivo. Both SRT2183 and EX527 had a positive effect on clinical scores in CAIA mice. However, in EX527-treated mice the re-emergence of signs of joint inflammation was observed 14 days after cessation of treatment. Administration of SRT2183 was able to up-regulate IL-1beta in healthy and CAIA mice, which we also observed in un-stimulated neutrophils in vitro. Iso-nicotinamide, a compound which activates sirtuins via an alternative mechanism, had a similar effect on IL-1beta expression in vitro. Finally, Parp1 cleavage, a marker for cell death, was reduced in purified BM neutrophils following Sirt1 activation.

Conclusion: Sirt1 activity modulation, whether activation or inhibition, improved clinical scores in arthritis. This corresponds to the mobilization of neutrophils from the BM to the periphery. The selective increase of IL-1beta following Sirt1 activation indicates that while activation or inhibition achieves a similar outcome, this is done through different molecular pathways. Our results underscore that systemic pharmacological modulation of Sirt1 activity for a complex disease, such as rheumatoid arthritis, is complicated and attention should be paid to the schedule and duration of treatment, as well as to the progressive involvement of various cell types, in order to maximize the beneficial effects.

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MESENCHYMAL STEM CELLS INHIBIT THE ACTIVATED COMPLEMENT C5 BY CLUSTERING IN LUPUS NEPHRITIS

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Background: Dysregulation of clusterin (CLU) and over-activated complement C5 was involved in the development and progression of lupus nephritis (LN). Allogeneic mesenchymal stem cells (MSCs) transplantation has achieved good clinical efficacy for refractory LN, however, the exact mechanisms remain to be elucidated.

Objectives: To investigate the clinical effect of MSCs on SLE model mice (B6. 1pr), and explore the mechanisms of MSCs inhibiting the activated complement C5 in vivo and in vitro.

Methods: 26-week-old female B6.1pr mice were randomly allocated in three groups, which were given the following treatments, CTX (200mg/kg), MSCs (1 x 10^6), and an equal volume of PBS. 24 hours urine and peripheral blood were collected periodically. All mice were sacrificed at 40 weeks of age. Urine protein to creatinine ratio and plasma creatinine were quantified to evaluate renal disease.

Results: Compared to the control mice, both proteinuria and plasma creatinine were significantly improved in each treatment group. Plasma C3 was significantly elevated in mice of MSCs and CTX groups. There were decline trends in plasma creatinine and C5b-9 expression in MSCs were detected by real-time PCR and ELISA. MSCs-derived CLU on September 15, 2023 by guest. Protected by copyright.http://ard.bmj.com/ Ann Rheum Dis: first published as 10.1136/annrheumdis-2019-eular.4230 on 27 May, 2019. Downloaded from

Disclosure of Interests: None declared

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Levels of C3, soluble C5b-9 (SC5b-9), CLU, and anti-dsDNA antibody were determined in the plasma by ELISA. Histopathological evaluation of renal lesions was undertaken by HE, PAS, PASM and Masson staining under light microscopy. Podocyte foot processes were assayed by the transmission electron microscopy. Accumulation of immunocomplexes (IC), C3, C5b-9, and CLU were detected in renal specimens by immunofluorescence or immunohistochemistry. Expressions of CLU in MSCs were detected by real-time PCR and ELISA. MSCs-derived CLU was purified and functional analysis was performed accordingly.

Results: Compared to the control mice, both proteinuria and plasma creatinine were significantly improved in each treatment group. Plasma C3 was significantly elevated in mice of MSCs and CTX groups. There were decline trends in plasma levels of anti-dsDNA and SC5b-9 in treated mice when compared to the control mice. Notably, plasma CLU was only significantly elevated in MSCs treated mice. Pathological analysis showed that the proliferation of glomerular cells and foot process fusion were significantly alleviated in MSCs treated mice. Immunofluorescence and immunohistochemistry showed that depositions of IC, C1q, C3 and C5b-9 were significantly decreased in the MSCs group, although the expression of CLU was obviously increased in these mice. Mechanistically, interferon-α promoted the secretion of functional CLU by MSCs in vitro.

Conclusion: Allogeneic MSCs transplantation can effectively improve the clinical outcome of lupus mice. Possible mechanisms of MSCs might be related to inhibit the activated C5 via clusterin, which would be a potential treatment target in the future.

Disclosure of Interests: None declared

REFERENCE

COMPARATIVE ANALYSIS OF TLRS AND INFLAMMASOMES GENES EXPRESSION IN DIFFERENT ETHIOLOGY ARTHRITIS AFTER STIMULATION WITH INFLAMMATORY STIMULI AND VITAMIN D

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Background: It has been shown that a variety of environmental and genetic factors, as well as deficiency of vitamin D plays a key role in outcomes of arthritis. Many studies have shown that the central feature of synovitis in rheumatoid arthritis (RA) is activated synovium fibroblasts (SF) that play a key role in expression and secretion of different patterns of inflammatory factors. Recent studies demonstrated that nucleotide-binding oligomerisation domain-like receptor (NLR) containing a PYRIN domain 1 (NLRP1) and NLRP3 inflammasomes as well as Toll-like receptors (TLR), may be important in pathogenesis of chronic autoimmune joint diseases such as RA and potentially in development of osteoarthritis (OA). Therefore, better understanding of the role of SF, TLRS and inflammasomes inflammatory pathways in different early disease progression, monitoring, and therapy.

Objectives: A pilot study, to evaluate the effects of tumour necrosis factor α (TNFα), lipoteichoic acid (LTA), lipopolysaccharide (LPS), vitD on expression levels of TLRs, inflammasomes, and vitD receptor (VDR) in human SF different ethiology knee damage: OA, RA, early arthritis (EA) (duration <12 months), healthy controls (HC) (after meniscus tear due to trauma).

Methods: Synovial tissue and blood samples for vitD analysis were collected from patients undergoing joint replacement/arthroscopic synovectomy surgery, following informed consent according to the permission Lithuanian Ethics Commit-tee. The isolated cells were expanded in a monolayer and used between passages 2 and 4. The expression of TLR1, TLR2, TLR4, NLRP1, NLRP3 inflammasomes genes was analysed by qRT-PCR after 24h of stimulation with LTA, LPS, TNFα, vitD.

Results: Analysis of gene expression results revealed that TNFα, LPS or LTA have no effect on TLR4 and TLR1 genes expression levels in SF. Downregulation of NLRP1 expression and upregulation of NLRP3 accompanied by enhanced expression of TLR2 was determined after stimulation with all factors, particularly TNFα. Highest upregulation of TLR2 was observed in RA and early arthritis patients, levels of other genes showed high variation between all patients, disrepectful to diagnosis. Stimulation with TNFα resulted in 8-fold downregulation of VDR gene expression only in RA group, but not in OA, EA or HC. Stimulation with vitD had no effect on expression levels of studied genes in SFs in vitro, while the blood levels of vitD were neither associated with the ethiology of arthritis nor with VDR responses to stimulation with TNFα, LPS, LTA.

Conclusion: We demonstrated downregulated expression of NLRP1, associated with increased levels of NLRP3 and TLR2 upon inflammatory stimuli in human articular SF from patients with arthritis of different ethiology. These data further support the active involvement of those cells in inflammatory responses. Downregulated expression of VDR by TNFα in SF of RA patients implies altered signalling of vitD in the disease.

REFERENCES

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ACTIVATION OF TLR2 BY ADAMTS-5-MEDIATED DEGRADATION FRAGMENTS OF CARTILAGE EXPLANTS IS INHIBITED BY THE ANTI-TLRS5 NANOBODY®, M6495

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Background: Patients with joint diseases such as osteoarthritis (OA) are believed to have an abundance of endogenous Toll-like receptor (TLR) ligands in their joints, which might be responsible for activating TLRS that may ultimately initiate a self-perpetuating inflammatory loop in the disease. TLR ligands may be generated from the breakdown of articular cartilage, which in turn arises from the activity of two key enzymes: A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) and Matrix metalloproteinases (MMPs).

Objectives: In this study, we investigate the effect of M6495, a novel anti-ADAMTS-5 inhibiting nanobody® on TLR2 activation by ADAMTS-5 derived cartilage cleaved fragments

Methods: Human cartilage biopsies were retrieved from OA patients undergoing total knee replacement. The tissue cartilage was snap frozen in liquid nitrogen to eliminate underlying metabolic activity and then digested with recombinant human ADAMTS-5 (4 µg/ml) for 24, 48 and 72 hours respectively at 37 °C. The undigested remaining cartilage was discarded after each timeframe and the digested cartilage solution (DS) was used for further testing. Tissue cleavage was assessed by measuring the release of aggrecan degradation biomarkers: AGgnecanase mediated aggrecan degradation (AGN1) and MMP mediated aggrecan degradation (FFGV). DS was tested for TLR activation in a secreted embryonic alkaline phosphatase (SEAP) reporter gene based HEK hTLR2 (human Toll like receptors) cell line. Cartilage tissue in buffer alone was used as control at each time point.

Results: Aggrecan degradation in cartilage was confirmed by increased release of AGN1 (p<0.0001) (Fig. 1a) and FFGV (p<0.01 at 48 hours and p<0.001 at 72 hours) (Fig. 1b) in the DS compared to control. M6495 inhibited release of ADAMTS-5-mediated AGN1 (p<0.0001, Fig. 1a) and FFGV (p<0.05 at 48 hours and p<0.05 at 72 hours, Fig. 1b). ADAMTS-Smediated DS showed TLR2 activation in the SEAP based reporter system when compared to control (p<0.05) (Fig. 1c). Adding M6495 blocked the ADAMTS-5 mediated DS TLR2 activation (p<0.01) (Fig. 1c).

Conclusion: ADAMTS-5-mediated cartilage degradation leads to release of aggrecan fragments, which activate the TLR2 receptor in vitro in a specialised reporter system. Anti-ADAMTS-5 inhibiting nanobody®, M6495, showed a suppression in release of degradation biomarkers leading to limited activation of TLR2. The data suggest a potential chondro-protective effect by M6495.

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