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 distinct 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 disease condition could make a significant contribution to the early disease diagnosis, 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 TLR, inflammasomes, and vitD receptor (VDR) in human SF different ethiology joint diseases like 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/artroscopic synovectomy surgery, following informed consent according to the permission Lithuanian Ethics Committee. The isolated cells were expanded in a monolayer and used between passages 2 and 4. The expression of VDR, 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, discreditably 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 (Fig. 1a) and 48 and 72 hours respectively at 37 °C. The undisturbed 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: Aggreca- nase mediated aggrecan degradation (AGN1) and MMP mediated aggrecan degradation (FGFG). 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.

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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ACTIVATION OF TLR2 BY ADAMTS-5-MEDITATED DEGRADATION FRAGMENTS OF CARTILAGE EXPLAINS IS INHIBITED BY THE ANTI-ADAMTS-5 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 undisturbed remaining cartilage was discarded after each timeframe and the digested cartilage solution (DS) was used for further testing.

Results: Aggrecan degradation in cartilage was confirmed by increased release of AGN1 (p<0.0001) (Fig. 1a) and FGFG (p<0.01 at 48 hours and p<0.01 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 FGFG (p<0.05 at 48 hours and p<0.05 at 72 hours, Fig. 1b). ADAMTS-5-mediated 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.

Disclosure of Interests: Neha Sharma: None declared, Christian Thudium: Employee of: I am a full time employee of Nordic Bioscience, Morte...