Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.3407

AB0067

ALLOSTERIC RECEPTOR MODULATION OF A FREE FATTY ACID RECEPTOR TURNS NATURAL AGONISTS INTO POTENT ACTIVATORS OF THE SUPEROXIDE GENERATING NEUTROPHIL NADPH-OXIDASE

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Background: Short chain fatty acids are generated in the colon by bacterial fermentation of dietary fibres and serve as natural agonists for the free fatty acid receptor 2 (FFA2R/GPR43) which belongs to the large family of G-protein coupled receptors. We have earlier shown that acetate triggers an increase in the cytosolic concentration of free Ca^{2+} in neutrophils without any assembly/activation of the superoxide generating NADPH-oxidase (Mol Cell Biol. 2016 Sep 26;36(20):2583–95). Allosteric modulators bind to receptors at sites topographically distinct from the agonist/antagonist binding site and can regulate receptor functions positively or negatively.

Objectives: We undertook this study to determine whether an FFA2R selective modulator affects the neutrophil response induced by natural FFA2R agonists.

Methods: Neutrophils were collected from healthy blood donors. Release of superoxide anions generated by the assembled/activated NADPH-oxidase was recorded by sensitive isoluminol/HRP amplified chemiluminescence method. Intracellular Ca^{2+} transients were measured with FURA 2-AM labelled neutrophils.

Results: The allosteric modulator lacked a direct activating effect on neutrophils, but turned natural FFA2R agonists into potent activating agonists that triggered not only a transient rise in the cytosolic concentration of free Ca^{2+} ions but also an assembly of the NADPH-oxidase. The NADPH-oxidase activity induced by the combined effect of the allosteric modulator and the natural agonist acetate could be further increased in neutrophils treated with the pro-inflammatory cytokine TNF- α . The receptor selectivity was demonstrated through the inhibition of the neutrophil activity by the novel FFA2R antagonist CATPB. In addition, the allosteric modulator lacked effect on neutrophil responses triggered by a novel and selective agonist for the closely related GPR84, a receptor that recognises medium chain fatty acids.

Conclusions: Allosteric modulators that positively co-operate with natural FFA2R agonists and prime neutrophils in their response to such agonists may serve as good tools for further unravelling the physiological functions of the FFA2R and its involvement in various diseases. In this study, allosteric modulation of FFA2R is introduced as a receptor selective mechanism to prime neutrophils to produce increased amounts of reactive oxygen species.

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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.3211

AB0068

ALARMIN S100A8 AND S100A9 MODULATE THE INFLAMMATORY MICROENVIRONMENT IN EARLY TENDINOPATHY

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Background: Alarmins- also referred to as damage associated molecular patterns (DAMPs)- are endogenous molecules mobilised in response to tissue damage known to activate the innate immune system in the early stages of disease. The molecular mechanisms that regulate inflammatory and remodelling pathways

in tendinopathy are largely unknown therefore identifying early immune effectors is essential to understanding the pathology. S100A8 and S100A9 are constitutively expressed by cells of myeloid origin; under pathological conditions they are induced in other cell types in response to environmental triggers and cellular damage.

Objectives: Based on previous investigations we sought evidence of S100A8/A9 expression in human tendinopathy and thereafter, to explore mechanisms whereby S100 proteins may regulate release of inflammatory mediators and matrix synthesis in human tenocytes.

Methods: Torn supraspinatus tendon (established pathology) and intact subscapularis tendon (early pathology) biopsies were collected from patients undergoing arthroscopic shoulder surgery. Control samples of healthy hamstring tendon were collected from patients undergoing hamstring tendon ACL reconstruction. S100A8/A9 expression was analysed at transcript and protein level using quantitative RT-PCR and immunohistochemistry, respectively. Primary human tenocytes were cultured from hamstring tendon tissue. The *in vitro* effect of recombinant human S100A8/A9 on human tenocytes was measured using quantitative RT-PCR and release of inflammatory mediators was measured at protein level by ELISA.

Results: Immunohistochemical staining of tendinopathic tissues indicated the presence of S100A8 and S100A9 in tendinopathy with early diseased tissue displaying a distinct increase in S100A8 and S100A9 expression compared with control and established pathology. These findings were mirrored by data obtained at transcript level from both early and late pathology. Treating tenocytes with exogenous S100A8/9 significantly increased release of IL-6 and CCL2; however, no alterations in genes associated with matrix remodelling were observed at a transcript level.

Conclusions: The presence of S100A8 and S100A9 in early tendinopathic lesions suggests expression is upregulated in response to cellular damage. We have confirmed the presence of S100A8, S100A9, CCL2 and IL-6 in tendinopathy and propose that S100A8 and S100A9 participate in early pathology by modulating the stromal microenvironment and influencing the inflammatory profile of tenocytes. S100A8 and S100A9 may participate in a positive feedback mechanism involving enhanced leukocyte recruitment and release of pro-inflammatory cytokines from tenocytes that perpetuates the inflammatory response within the tendon in the early stages of disease. This, in turn, may contribute aberrant matrix remodelling and associated morphological deficiencies within the tendon. We propose S100A8 and S100A9 are active alarmins in early tendinopathy that indirectly influence matrix remodelling by perpetuating the stromal inflammatory environment. Selective targeting of DAMP signalling may offer novel therapeutic approaches in the management of human tendon disorders.

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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.7019

AB0069

INCREASED EXPRESSION OF SOLUBLE MIC-A IN THE SYNOVIAL FLUID OF RHEUMATOID ARTHRITIS PATIENTS

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Background: MIC-A (Major histocompatibility complex class I chain-related gene A)¹ is a transmembrane or soluble protein that interacts with the activating NKG2D receptor. MIC-A stimulates effector responses mediated by NK and CD8 +T cells under cellular stress conditions, like cancer or infections.² MIC-A is also associated with autoimmune diseases (such as rheumatic disorders) characterised by immune dysregulation triggered by environmental factors, and plays important roles in immune activation and surveillance.³ In mice, various NKG2D ligands were discovered: Rae-1, H60 and MULT1 families.⁴

Objectives: This study aims at investigating the potential pathological relevance of soluble MIC-A (sMIC-A) protein in inflammatory rheumatic diseases involving articular structures in humans. The expression of orthologous NKG2D ligands in mouse models of experimental joint inflammation is also quantified.

Methods: We collected synovial fluid (SF) from 118 subjects: 22 Rheumatoid Arthritis (RA), 13 Psoriatic Arthritis (PSOA), 12 Gout Disease (GOUT), 18 Calcium