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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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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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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

Pyrophosphate Deposition Disease (CPPD), 8 Reactive Arthritis (REA) and 45 Osteoarthritis patients. Gout and CPPD diseases were confirmed by the presence of crystals in SF. Clinical data were collected. The concentration of soluble MIC-A (sMIC-A), interleukin (IL)-1, IL-6 and IL-8 was measured by ELISA. Murine Rae-1, H60 and Mult1 transcripts were quantified by real-time quantitative PCR (RT-qPCR) in 3 models of joint inflammation: Serum Transfer Arthritis (STA), Collagen-induced arthritis (CIA) and Collagenase-Induced Osteoarthritis (CIOA).

Results: Significantly higher levels of sMIC-A were found in the synovial fluid of RA patients in comparison with all others diseases ($p < 0.001$, figure n.1). sMIC-A levels were correlated to white blood cell counts and levels of inflammatory cytokines IL-1, IL-6 and IL-8. Similarly, higher expression levels of Rae-1, H60 and Mult1 were found in chronic arthritis mouse models in comparison with osteoarthritic mice.

Abstract AB0069 – Figure 1

Conclusions: Our data identifies synovial sMIC-A as an important player in rheumatoid arthritis compared to other rheumatic diseases and osteoarthritis. Investigations in mouse models are in agreement with this finding

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AB0070

ADIPOKINES AND CYTOKINES IN THE PATHOGENESIS OF PSORIASIS AND METABOLIC DISORDERS

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Background: Psoriasis is a systemic immune-associated disease with a specific comorbidity. The manifestations of the metabolic syndrome in this category of

patients are changes that develop on systemic immune-associated inflammatory psoriatic process background and contribute to the progression of chronic inflammation.

Objectives: The aim of the study was determination of the main adipokines and cytokines content in the serum of peripheral blood, severity and activity of the disease in patients with psoriasis; clinical and laboratory evaluation of metabolic disorders. The interconnection between the production of adipokines and cytokines in psoriasis was analysed depending on the severity and activity of the psoriatic process and the nature of metabolic disorders.

Methods: Serum levels of adipokines (C-peptide, ghrelin, insulin, glucagon, leptin, visfatin, resistin, GIP, GLP-1 and PAI-1) and cytokines (IL-1 β , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, FGF-2, G-CSF, GM-CSF, IFN- γ , IP-10, MCP-1, MIP-1 α , MIP-1 β , PDGF-BB, RANTES, TNF- α and VEGF) were measured in 36 patients with moderate and severe psoriasis, psoriatic arthritis. There was control group of 15 basically healthy persons. Clinical and laboratory evaluation of metabolic disorders (BMI, dyslipidemia, carbohydrate metabolism disorders) and cardiovascular diseases was performed for all examined patients. The duration, severity and the amount of body surface area involved in psoriasis were evaluated in all patients using recommended indices (BSA, PASI).

Results: Patients with psoriasis showed an increase in the production of glucagon, leptin, visfatin, GLP-1 ($p < 1.0E-03$) and a decrease in the level of C-peptide, insulin, GIP, PAI-1, resistin ($p < 1.0E-06$) compared with the control group. The difference of ghrelin concentrations in both group was not statistically significant. Patients with psoriasis showed an increase in the level of IL-1 β , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, FGF-2, GM-CSF, IFN- γ , IP-10, MCP-1, MIP-1 β and TNF- α compared to control group ($p < 0.01$). Positive correlations between the level of adipokines and cytokines were revealed.

Conclusions: The obtained data allow us to define adipokines as mediators between immune and endocrine systems. The imbalance between the proinflammatory and anti-inflammatory effects of adipokines observed in psoriasis demonstrates lipid metabolism dysfunction as one of the possible provoking factors of chronic inflammation determining the severity of the underlying disease. According to the results of the study, the following biological adipokines and cytokines should be classified as early biological markers of severity of the psoriatic immune-associated inflammatory process, with all its comorbid risks: GLP-1, glucagon, leptin, visfatin, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12, IL-13, IL-17, eotaxin, FGF-2, GM-CSF, IFN- γ , IP-10, MCP-1, MIP-1 β , TNF- α . The levels of adipokines and cytokines are probably the earliest biological markers in patients with metabolic syndrome and psoriasis, the control of adipokines and cytokines level can be used to optimise therapy.

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AB0071

EFFECTS OF CHONDROITIN SULPHATE AND GLUCOSAMINE ON INFLAMMATORY CYTOKINES IN MACROPHAGES

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Background: The combination of pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), and endogenous danger-associated molecular patterns (DAMPs), such as hyaluronan (HA) fragments, are known to be able to induce an inflammatory response from macrophages characterised by the release of pro-inflammatory cytokines¹. We have previously shown that pharmaceutical grade chondroitin sulphate (CS), commonly used in the symptomatic treatment of osteoarthritis (OA), can attenuate the inflammatory response.

Objectives: To evaluate the synergistic effects of CS and glucosamine (GLU) in combination on LPS/HA mediated inflammatory responses of an immortalised human macrophage cell line (THP-1) and primary synovial fluid cells.

Methods: THP-1 monocyte cells were grown and differentiated into mature macrophages by the addition of 200 nM of phorbol 12-myristate 13-acetate (PMA) as previously described¹. Cells were cultured with a physiologically achievable range of concentrations of CS and GLU (0, 10, 50, 200 μ g/ml of each, Bioibérica, S.A. U.) for 6 hours, then primed with physiologically relevant concentrations of LPS (10 ng/ml) (n=12/group). After 24 hours, cell culture media were replaced with serum free Opti-MEM supplemented with the previously mentioned concentrations of CS and GLU, LPS, and 10 μ g/ml HA fragments (ultra-low molecular weight, Lifecore). After a further 24 hours, supernatants were harvested for NF- κ B activity and pro-inflammatory cytokine (IL-1 β , IL-6, IFN- γ , and TNF- α) assessment. Cell viability was measured using PrestoBlue reagent. The human knee