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AB0065 N-CADHERIN IS DOWN-REGULATED BY DECOY RECEPTOR 3 IN SPECIFICALLY RHEUMATOID SYNOVIAL FIBROBLASTS

K. Fukuda¹, Y. Miura², S. Hayashi¹, T. Maeda³, R. Kuroda¹. ¹Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan; ²Division of Orthopedic Science, Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, Kobe, Japan; ³Department of Immunology and Rheumatology, Stanford University, Stanford, USA

Background: Decoy receptor 3 (DcR3) is a secreted decoy tumour necrosis factor receptor and competitively binds and inhibits the TNF family including Fas ligand, LIGHT, and TL1A. We previously reported that DcR3 overexpressed in rheumatoid synovial fibroblasts (RA-FLS) stimulated by TNF α protects the cells from Fas-induced apoptosis.¹ We recently reported that DcR3 binds to TL1A expressed on RA-FLS resulting in the negative regulation of cell proliferation induced by inflammatory cytokines.² Further, we newly revealed the gene expression profiles in RA-FLS regulated by DcR3 by using microarray data analysis.³ Among the genes in the profile, we demonstrated the possible involvement of tryptophan hydroxylase 1 down-regulated,⁴ interleukin 12B up-regulated by DcR3⁵ and centrosomal protein 70 kDa⁶ in the pathogenesis of RA. The profiles also indicated that Cadherin 2/type 1 N-cadherin (CDH2) was up-regulated by DcR3 (fold change 1.93).³ CDH2 has been reported to be associated with cell attachment and migration,⁷ osteoblast differentiation,⁸ and the proliferation of RA-FLS.⁹ The hemophilic interaction of CDH2 suppresses the proliferation of RA-FLS through increasing the P27^{Kip1} that inhibit cell-cycle progression.⁹

Objectives: In this study, we analysed CDH2 expression in RA-FLS stimulated with DcR3 in detail to reveal the involvement of CDH2 and DcR3 in the pathogenesis of RA.

Methods: Real-time polymerase chain reaction (real-time PCR). RA-FLS were stimulated with various concentration of DcR3-Fc or 1,000 ng/ml of IgG1, or left untreated in serum-free Opti-MEM for 12 hour. The relative expression levels of CDH2 mRNA were quantified by real-time PCR.

Immunohistochemistry.

Anti-CDH2 antibody was applied to frozen sections of synovial tissues from patients with RA or OA for overnight. After that, the expression of CDH2 protein was evaluated by immunohistochemical analysis.

Results: Real-time PCR demonstrated that DcR3-Fc significantly increased the expression of CDH2 mRNA in RA-FLS (104% with 10 ng/ml, 112% with 100 ng/ml, and 200% with 1000 ng/ml). Immunohistochemistry revealed that CDH2 was expressed more in the sublining layer of rheumatoid synovium than OA synovium.

Conclusions: In the gene expression profiles, we focused on CDH2 as the gene was highly up-regulated and belonged to major functional clustering categories; protein complex assembly, cell motility, regulation of transcription, cell membrane and glycosylation. In this study, we showed that the expression of CDH2 mRNA in RA-FLS was induced by DcR3 and that CDH2 was increased in the sublining layer of rheumatoid synovium. In this study, we demonstrated that DcR3 significantly induced CDH2 expression in RA-FLS. Considering the fact that CDH2 inhibits RA-FLS proliferation, DcR3 signalling may control the hyperplasia of RA synovium.

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AB0066 COMBINATION OF IL-10 AND IL-18 BUT NOT IL-6 AND IL-18 INDUCES IFN-GAMMA PRODUCTION AND SURFACE EXPRESSION OF TRAIL ON NK CELLS

K. Sato, Y. Aizaki, H. Yazawa, T. Mimura. Department of Rheumatology and Applied Immunology, Saitama Medical University, Saitama, Japan

Background: Adult-onset Still's disease (AOSD) is a systemic inflammatory disease, the cause of which is largely unknown. AOSD has been recently classified as one of the autoinflammatory diseases, in which innate rather than acquired immunity plays an important role. Serum IL-18 has been shown to be significantly high in AOSD patients.

Objectives: The aim of this study was to quantify the levels of multiple cytokines in the serum of AOSD patients, and compare the serum cytokine profile with that of healthy controls. We also attempted to evaluate the effects of the cytokines that were upregulated in the AOSD serum on natural killer (NK) cells, since NK cells are cells of innate immunity and IL-18 has been shown to enhance their cytotoxicity.¹

Methods: We quantified the serum levels of 10 cytokines (IFN- α , IFN- γ , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A and TNF- α) in patients with AOSD and healthy controls using multiplex bead array assays and IL-18 using ELISA. We next sorted NK cells from peripheral blood mononuclear cells (PBMCs) of healthy controls, stimulated them *in vitro*, and quantified the level of IFN- γ in the culture supernatant by ELISA and also assessed the surface expression level of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) on the cells.

Results: Compared to samples from healthy controls, the mean serum levels of IL-6 and IL-18 from AOSD patients were significantly higher. IL-10 was detectable in some of the patients. Originally, IL-18 was identified as a stimulator of IFN- γ production², however, serum IFN- γ was below the detection limit. When NK cells were stimulated *in vitro* with IL-18 alone, the protein level of IFN- γ in the culture supernatant was still below the detection limit. When we further added IL-6 and/or IL-10 to the culture, the combination of IL-10 and IL-18, but not IL-6 and IL-18, induced IFN- γ . As IL-6 is a classic pro-inflammatory cytokine and IL-10 is considered to be an anti-inflammatory cytokine, this result was rather unexpected. Thus, we evaluated the expression of IL-6 and IL-10 receptors on NK cells and found that IL-10 receptors (IL-10R and IL-10R β) were present, but IL-6 receptors (IL-6R and gp130) were absent. We also assessed TRAIL expression on NK cells. Here, too, the combination of IL-10 and IL-18 induced TRAIL expression the most potently. Although a previous research showed that the combination of TLR3 and IL-18 signalling synergistically induced TRAIL on NK cells in an IFN- γ dependent manner³, the addition of an anti-IFN- γ antibody did not diminish the TRAIL expression.

Abstract AB0066 – Figure 1

Conclusions: A combination IL-10 and IL-18, not IL-6 and IL-18, induced the production of IFN- γ and surface expression of TRAIL in NK cells. This TRAIL expression did not evidently depend on IFN- γ . TRAIL was expected to be useful for the treatment of malignancy, but it turned out to be toxic to hepatocytes⁴. Since NK cells are rich in the liver and the abnormality of liver function is among the major symptoms in AOSD, we suggest that the combination of IL-10 and IL-18 may cause liver dysfunction by inducing TRAIL on NK cells in the liver.

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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

L. Björkman, J. Mårtensson, A. Holdfeldt, H. Forsman, M. Gabl, M. Sundqvist, C. Dahlgren. *Rheumatology and inflammation research, University of Gothenburg, Gothenburg, Sweden*

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

L.A.N. Crowe¹, M. Akbar¹, K. Patommel¹, S.M. Kitson¹, E. Garcia Melchor¹, D. S. Gilchrist¹, G.A. Murrell², I.B. McInnes¹, N.L. Millar¹. ¹Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK; ²Department of Orthopaedic Surgery, St. Georges Hospital Campus, University of New South Wales, Sydney, Australia

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

L. Bernardi^{1,2}, A. Mariotte¹, A. Scanu², D. Noël³, L. Punzi², S. Bahram¹, P. Georgel¹, J. Sibilia¹. ¹INSERM UMR_S 1109, Fédération de Médecine Translationnelle de Strasbourg, Université de Strasbourg, Strasbourg, France; ²Rheumatology Unit, Department of Medicine-DIMED, University of Padova, Padova, Italy; ³INSERM UMR_S 1183, Montpellier University, Montpellier, France

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