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SAT0049

INFECTION WITH CITRULLINATING PORPHYROMONAS GINGIVALIS CAN INDUCE AUTOIMMUNITY TO HUMAN RIBOSOMAL PROTEINS AND TNF ALPHA TREATMENT NONRESPONSE

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Background: *Porphyromonas gingivalis* (P.g.) is involved in triggering self-reactive immune responses when citrullinating bacterial or human proteins. However, first evidence to link anti-ribosomal T and B cells responses to rheumatoid arthritis (RA) has been published but the mechanism and its influence on therapy is not clear (1). Infection based autoimmunity induced by citrullination of human proteins with P.g. peptidyl arginine deiminase from RA patient (RA-PPAD) and crossreactivity binding induced by P.g. was investigated using patient sera, affinity purified RA patient antibodies and monoclonal antibodies to cit-RA-PPAD.

Objectives: Antibodies to RA-PPAD isolated from an RA patients (RA-PPAD) was first time linked to target specific citrullinated ribosomal proteins and therapy.

Methods: Screening of RA sera was conducted on 37.830 unique human proteins on protein microarrays (<http://www.engine-gmbh.de>) with 30 RA sera. The autoantibody response to 840 different proteins was recorded and bioinformatically evaluated. Protein arrays were citrullinated with human peptidyl arginine deiminase PAD 2, 4, rabbit PAD and RA-PPAD from P.g. Sera and affinity purified antibodies were used to detect reactivity to 840 autoantigens and 15aa CCP peptide from RA-PPAD. Sera from TBA treated sera anti-TNF (adalimumab, etanercept, certolizumab) treatment were tested with the cit-PPAD-peptide of 15aa (CPP).

Results: A human protein microarray consisting of 840 identified autoantigens from RA patients was modified by human PAD2 and PAD4, rabbit PAD, and RA-PPAD from P.g. Using cit specific monoclonal antibodies we identified the ribosomal proteins (RP), RPL18a, RPS27a, modified by PAD2, RPL18a and MRPS11 modifies by PAD4, and RPL7L1 modified by rabbit PAD specifically targeted. In addition 6 RA patient sera and 3 osteoarthritis (OA) control sera were used to identify the citrullinated RA-PPAD specific modified autoantigens not targeted when modified by human PAD2 or PAD4 or rabbit PAD. We identified the RA-PPAD citrullinated ribosomal Proteins RPL3, RPL21, RPS24, RPL9, RPL15, RPS24, RPS3a, MRPL28 specifically targeted by RA patients. This identifies ribosomal proteins as major specific RA-PPAD citrullination targets. Moreover, affinity purified antibodies bound to native and citrullinated RA-PPAD from 6 RA patient sera and 3 OA patient sera were tested for crossreactivity on citrullinated human proteinarray. Antibodies to citrullinated ribosomal proteins MRPS11, RPL21, RPS3a, RPL18a, RPS27a, MRPL28 were detected in the RA group but not in the OA control group. High antibody titre against the cit-PPAD-peptide of 15aa (CPP) derived from the autocitrullination site (R63) of RA-PPAD correlates with TNF α -inhibitor (TBA) non-response (n=61). DMARD patients refractory to different treatment regimes (n=61), receiving anti-TNF (adalimumab, etanercept, certolizumab), do not respond when maintaining high α -CPP IgG level.

Conclusion: Failure of *Porphyromonas gingivalis* clearance in RA patients leads to infection induced enzymatic mimicry based autoreactivity targeting

evolutionary conserved human ribosomal proteins. Autoimmunity to ubiquitous self-antigens may trigger localized tissue damage in RA. TBA non-response leads to the suggestion to clear *Porphyromonas gingivalis* infection before α -TNF treatment.

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SAT0050

TNF-A INDUCES MITOPHAGY FORMATION IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLAST AND INHIBITION OF MITOPHAGY AMELIORATES SYNOVITIS IN COLLAGEN ANTIBODY-INDUCED ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a systemic and chronic autoimmune disease that primarily targets synovial membranes. Mitophagy is a selective form of autophagy which removes damaged mitochondria. When mitochondria become damaged, PINK1 (PTEN-induced putative kinase 1) is accumulated on the outer membrane of damaged mitochondria and induces mitophagy. The study of mitophagy in lung fibrosis and parkinson's diseases has recently been described. However, the role of mitophagy in RA has not been reported yet.

Objectives: The aim of this study was to determine the roles of mitophagy in rheumatoid arthritis synovial fibroblast (RASf) and collagen antibody-induced arthritis (CAIA) mice model.

Methods: We studied the mitophagy marker, PINK1, in RASf treated with TNF- α by western blotting and immunofluorescence. Arthritis was induced in PINK1 knockout mice by intraperitoneal (i.p.) injection of anti-type II collagen antibody cocktail, followed by i.p. injection of lipopolysaccharide. The severity of rheumatoid arthritis was histopathologically assessed

Results: PINK1 expression and damaged mitochondria increased in RASf treated with TNF- α . TNF- α produced Intracellular ROS in RASf and mitophagy was regulated by ROS. PINK1 knockdown RASf decreased migration and invasion function. Histopathological examination revealed that the paws from PINK1-knockout mice showed markedly reduced swelling and inflammation compared with those of wild type mice

Conclusion: Taken together, the results suggest that regulation of PINK1 expression in RA may represent potential therapeutic and diagnostic targets for RA

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SAT0051 BARICITINIB IMPROVES JOINT MOBILITY AFTER INJURY IN A RODENT FORCED-AMBULATION MODEL

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Background: Movement-evoked pain and impaired joint mobility are common comorbidities in inflammatory diseases such as Rheumatoid Arthritis (RA) and Osteoarthritis. The Janus kinase (JAK) pathway has been implicated in both inflammation and chronic pain. Clinical data suggests that baricitinib, a selective JAK 1/2 inhibitor, can robustly and rapidly alleviate pain in RA.¹ Utilizing a rodent forced-ambulation model, we have previously shown attenuation of gait deficits with analgesics such as opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), and an anti-NGF (Nerve Growth Factor) antibody.² However, in this same model a fusion protein blocker of TNF α (tumor necrosis factor alpha) signaling failed to demonstrate efficacy though inflammation was decreased. The present work investigated the potential of baricitinib, recently approved for treatment of RA, to reduce inflammation-induced joint pain and gait impairment in this model of joint inflammation mediated pain.

Objectives: To determine if JAK 1/2 pathway inhibition is effective in treating inflammation-induced joint pain and gait impairment.

Methods: Unilateral joint injury was induced in female Sprague Dawley rats (Harlan, Indianapolis, IN, USA) by unilateral intra-articular injection of 20 μ g Complete Freund's Adjuvant (CFA). Using the GaitScan (CleverSys Inc., Reston, VA) treadmill system, a composite gait score comprising of range of motion, normalized stance distance, stance/swing ratio, and paw print size was evaluated over 3 days post-injection.² Rats were treated with vehicle, positive control (40 mg/kg Tramadol), or clinically relevant (based on plasma levels) increasing doses of baricitinib (1, 3, or 10 mg/kg p.o., 2-hrs prior to each test, q.d.). Dorsal root ganglion (DRG) were harvested post-gait evaluation and Total STAT3 (Cell Signaling, #4904) and phospho-STAT3 (Y705) (Cell Signaling, #9131) protein levels were examined via immunoblotting. The p-values were derived from repeated measures ANOVAs.

Results: In rat DRG homogenates, baricitinib significantly decreased phospho-STAT3 (Y705) protein levels in a dose-dependent manner ($p < 0.01$) with a significant effect after a 3 mg/kg dose and a maximal response after a 10 mg/kg in both the ipsilateral (right) and contralateral (left) sides. Total STAT3 protein levels remained unchanged. Similarly, treatment with baricitinib significantly improved composite gait score at the 10 mg/kg dose ($p < 0.05$) by Day 3.

Conclusion: These data indicate that treatment with baricitinib attenuates CFA-induced joint deficits, a surrogate measure of joint pain. This effect correlated with the pharmacodynamic inhibition of JAK-STAT signaling in DRGs. These data support a role for JAK-STAT signaling in pain

signaling and provide an opportunity to investigate the potential mechanism of action of baricitinib in joint pain.

Figure 1. Representative still images from video recordings of animals during gait analysis at baseline (Top Panel), 3 Days post-CFA and vehicle treatment (Middle Panel) and 3 Days post-CFA with 10 mg/kg daily baricitinib treatment (Bottom panel). The ventral image of the rat illustrates the same part of the gait cycle (ipsilateral stance phase initiation) across treatment groups to highlight impairment in the CFA-injected limb (Red Box) vs Baseline and apparent improvement following 10 mg/kg baricitinib treatment. The panels on the left provide examples of software-detected paw prints for each treatment group which show that CFA + vehicle treatment produced a small, closed-toed paw print (Middle) which appears restored to near baseline levels following baricitinib treatment (Bottom).

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SAT0052 PHOTODYNAMIC THERAPY TARGETING ACTIVATED FIBROBLASTS INDUCES SYNOVIAL CELL DEATH IN EXPERIMENTAL ARTHRITIS

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Background: Activated synovial fibroblasts (SF) contribute to rheumatoid arthritis (RA) by producing a multitude of cytokines, chemokines and proteases thus aggravating disease. Activated SF can be distinguished from quiescent fibroblasts by their expression of fibroblast activation protein (FAP). Selective depletion of FAP+ SF in inflamed joints could decrease their contribution to the arthritis process and thus constitute a viable treatment option. Further focussing of the treatment to only those areas affected by the disease can be accomplished by applying targeted photodynamic therapy (tPDT). In tPDT a light sensitive molecule, a photosensitizer (PS), is conjugated to a targeting moiety. Upon activation by light this construct produces reactive oxygen species, killing the targeted cells.

Objectives: To this end we developed and tested a therapy that selectively depletes activated SF by targeting FAP on these cells with an