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FRI0160 INTERLEUKIN-17A INDUCES INFLAMMATORY RESPONSE VIA NLRP3 INFLAMMASOME IN ANKYLOSING SPONDYLITIS

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Background: Inflammasomes are cytoplasmic multiprotein complexes that recognise various exogenous and endogenous danger signals in myeloid cells, particularly macrophages. Inflammasome activation eventually induces inflammatory responses in macrophages by activating gasdermin-d-mediated pyroptosis and the secretion of pro-inflammatory cytokines including interleukin (IL)-1 β and IL-18 in a caspase-1-dependent manner. The production of IL-1 has been found to be highly induced in AS¹ and caspase-1 level was significantly elevated in spondyloarthritis patients than in those with other arthritic diseases,² suggesting the possibility that inflammasomes might be involved in pathogenesis of ankylosing spondylitis (AS). However, few studies have addressed the roles of inflammasomes in AS.

Objectives: This study was performed to investigate the role of NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome in the peripheral blood mononuclear cells (PBMCs) from AS patients.

Methods: PBMCs from 11 patients and 9 healthy controls were isolated and mRNA expression levels of NLRP3 inflammasome, pro-IL-1 β , IL-1 β , pro-caspase-1, and caspase-1 were determined using quantitative real-time PCR. IL-17A (100 ng/mL) was added to the cell cultures, then, cytokine expression, signalling pathways, and inflammasome machinery are determined using real-time RT-PCR and Western blot. After transfection of siNLRP3 to AS PBMCs, mRNA and proteins levels of IL-1 β and caspase-1 were determined using real time PCR and western blot.

Results: Expressions of NLRP3, IL-1 β , and caspase-1 mRNAs, but not IL-18, were increased in PBMCs from AS patients compared to controls. Incubation of AS PBMCs with IL-17A significantly increased NLRP3, IL-1 β , IL-18, and caspase-1 expressions in AS PBMCs. Western blot showed IL-17A treatment induced the phosphorylation of Akt, p38 MAPK, and NF- κ B p65 in AS PBMCs. Down-regulation of NLRP3 by transfection of siRNA decreased mRNA expressions and productions of IL-1 β and caspase-1 in AS PBMCs.

Conclusions: Activity of NLRP3 inflammasome was increased in AS patients and IL-17A potentiated the activation of NLRP3 inflammasome. Our data suggest the possible role of NLRP3 inflammasome in inflammatory response in AS.

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FRI0161 ROLE OF GITR/GITRL IN MODULATING TH9 RESPONSE IN PSORIATIC ARTHRITIS

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Background: GITR/GITRL interaction is pivotal in regulating the activity of Treg and of Th9 effector cells.¹ Psoriatic arthritis (PsA) is a chronic inflammatory disease characterised by a strong expression of IL-9 and Th9 polarisation in the inflamed gut, synovial tissues and peripheral blood.² Factors involved in the regulation of Th9 responses in PsA have been not yet investigated.

Objectives: Aim of the study: to investigate the role of GITR/GITRL in PsA.

Methods: GITR, GITRL expression were assessed by rt-PCR and immunohistochemistry in synovial biopsies, gut specimens and bone marrow of PsA patients and healthy controls (HC). The co-localization of CD4 and GITR was analysed by immunofluorescence. The expression of GITR was also assessed by flow cytometry analysis in isolated PBMC of patients and controls (Tregs, CD4 and CD8).

Peripheral and intestinal IL-9 producing cell (Th9) percentages and cytotoxic activity of T lymphocytes were also studied by flow cytometry analysis *ex vivo* and after *in vitro* stimulation with GITRL. A mouse model of induced arthritis (collagen induced arthritis, CIA) was used to study GITR/GITRL axis and the effect of anti-GITRL blocking agent.

Results: Increased GITR and GITRL expression was observed in the inflamed gut and synovial samples of PsA patients. Analysis of GITR expression among PBMC and LPMC from PsA patients demonstrated its down-regulation among Tregs and upregulation on effector CD4⁺ and CD8⁺T cells. In *in vitro* studies, GITR co-stimulation potently induced Th9 activation and IL-9 production. In particular, GITR ligation subverted the induction of Foxp3(+) Tregs, directing the activated CD4(+) T cells to a Th9 phenotype and enhancing the function of DCs and cytotoxic T lymphocytes. Moreover, in a murine model of CIA massive expression of IL-9 and GITR was observed in the synovial tissues and anti-GITR therapy significantly ameliorated arthritis score.

Conclusions: We demonstrated that GITR/GITRL axis modulates IL-9/Th9 responses in PsA, representing GITR activation a relevant upstream pathway involved in Th9 polarisation. A novel mechanism by which GITR agonist exert an inflammatory response was also demonstrated in PsA indicating GITR blocking agents as possible therapy in PsA.

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FRI0162 THE LINK BETWEEN ANGIOGENESIS AND OSTEOGENESIS IN SPONDYLOARTHRITIS

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Background: Spondyloarthritis is characterised by inflammation, extensive angiogenesis and pathological osteogenesis. Transmembrane (tm)TNF transgenic (tg) mice¹ that overexpress tmTNF exhibit features of SpA, including chronic inflammation and pathological osteogenesis. tmTNF ligation to TNF receptor 2 in endothelial cells (ECs) can induce signal transduction pathways, that may promote these processes. Of note, angiogenesis and osteogenesis are coupled by EC differentiation towards a type H (CD31^{hi}endomucin^{hi}) phenotype.²

Objectives: To investigate the link between pathological angiogenesis and osteogenesis in tmTNF tg mice.

Methods: Vertebrae and ankles from 6 and 12 weeks and 8 months old tmTNF tg mice or sex-matched non-tg littermates (n=18) were prepared by cutting 60 μ m thick cryosections for confocal imaging.

Results: tmTNF tg mice exhibited ectopic osteogenesis which was not observed in non-tg littermates. The provided image demonstrates an ectopic lesion at the white arrow. Immunostainings showed that type H vessels are in the vicinity of the ectopic osteogenesis and osterix⁺ osteoprogenitors. At six weeks of age, osterix⁺ cells are located throughout the ectopic lesion, while at eight months, osterix⁺ cells are only present at the border of the lesion. Furthermore, there is increased osteogenesis and a different vessel architecture within the vertebrae of tmTNF tg mice compared to non-tg littermates that progresses with age. Non-tg littermate vertebrae only have physiological osteogenesis, which is in the metaphysis and periosteum. In addition, tmTNF tg mice also exhibit altered bone marrow (BM) architecture containing extensive lymphoid aggregates, which predominantly consisted of B220⁺ B cells.