AB0125

CXCL17 IN RHEUMATOID ARTHRITIS: INTERFERON-GAMMA INDUCIBLE EXPRESSION AND INHIBITION OF ANGIOGENESIS

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Background: CXCL17 is the latest chemokine discovered and was reported to influence angiogenesis and monocyte trafficking^{1,2}. Its role in rheumatoid arthritis (RA) has not been assessed so far.

Objectives: To assess the role of CXCL17 and its putative receptor G-protein-coupled receptor 35 (GPR35) in RA.

Methods: Synovial tissue from joint replacements of RA and osteoarthritis (OA) patients was used for CXCL17 and GPR35 immunohistochemistry and in-situ-hybridization/immunofluorescence double-stainings. CXCL17 concentration in the synovial fluid of RA and CXCL17 production by synovial fibroblasts and smooth muscle cells after stimulation with TNF- α , INF- γ and IL-1 β was quantified by qPCR and ELISA. Angiogenesis was assessed by a human umbilical vein endothelial cell culture assay.

Results: CXCL17 and GPR35 are widely expressed in the synovial membrane of RA compared to OA (p=0.006). Within the synovial membrane CXCL17-mRNA could be located to vascular smooth muscle cells. INF- γ significantly induced CXCL17-mRNA and protein production in RA synovial fibroblasts (1.88-fold, p=0.019 and 2-fold, p=0.0002 respectively) and rat smooth muscle cells (67-fold, p=0.02 and 3.7-fold, p<0.001). CXCL17 was detected in the synovial fluid of RA (mean 310 pg/ml). In vitro angiogenesis was inhibited by CXCL17. This effect was reversed by specific GPR35 antagonists.

Conclusion: CXCL17 is abundant in RA synovial tissue, localizes to vascular smooth muscle cells and fibroblasts, and is inducible by INF- γ . Antiangiogenic properties are mediated by GPR35. CXCL17 and GPR35 may constitute a hitherto unrecognized regulatory protein in RA pathogenesis and therefore be interesting drug targets.

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AB0126

ANTI-INFLAMMATORY EFFECTS OF SPLEEN TYROSINE KINASE (SYK) INHIBITOR, PICEATANNOL, ON FIBROBLAST-LIKE SYNOVIOCYTE IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation with subsequent cartilage and bone erosion leading to joint destruction. The fibroblast-like synoviocytes (FLS) have a central role in disease pathogenesis and in vitro FLS invasiveness correlates with articular damage in RA patients. Spleen tyrosine kinase (Syk) is an intracellular protein tyrosine kinase in the cells of joint tissues such as bone, cartilage, and synovium, where it is involved in tumor necrosis factor α (TNF- α) receptor signaling. Piceatannol, a naturally occurring hydroxylated analog of resveratrol, was reported to inhibit Syk.

Objectives: The aim of this study is to evaluate the effects of piceatannol on inflammation and the downstream signaling pathway of Syk in RA FLS.

Methods: FLS isolated from synovium of rheumatoid arthritis (RA) patients were cultured with DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. FLS were stimulated with lipopolysaccharide (LPS) for 24 hr after 1 hr treatment of piceatannol. The cytokines were screened using human inflammatory and matrix metalloproteinase (MMP) antibody array kit (Abcam) in the culture supernatant. Also, IL-6, IL-8 and CXCL10 levels were measured by ELISA and the expression levels of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthases (iNOS) were determined by western blotting.

Results: Protein expression level of Syk was suppressed by piceatannol (50 μ M treatment). Piceatannol inhibited the production of cytokines/chemokines such as IL-6, CCL-5, CCL-8 and MMP-1 in RA FLS stimulated with LPS. The levels of IL-6, IL-8 and CXCL10 in RA FLS were dose-dependently decreased. The viability and proliferation of the cells were not affected. Piceatannol significantly suppressed COX-2 and iNOS protein expressions.

Conclusion: Piceatannol had suppressive effects on pro-inflammatory cytokines/chemokines production and had anti-inflammatory effects in RA FLS.

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AB0127

VERY STRONGLY POSITIVE RHEUMATOID FACTOR LEVELS (10-FOLD HIGHER THAN THE UPPER NORMAL RANGE) RATHER THAN "RHEUMATOID FACTOR POSITIVITY" PER SE ARE ASSOCIATED WITH SMOKING IN FEMALE RA PATIENTS WITHOUT A HISTORY OF OCCUPATIONAL DUST AND FUME EXPOSURE

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Background: Rheumatoid factor (RF) forms part of the 2010 EULAR/ACR classification criteria¹.

A weakly positive RF is defined as up to 3-fold higher and a strongly positive RF >3-fold higher than the upper normal range.

However, a longitudinal study of individuals with RF levels at least 4-fold higher than the upper range of normal had an adjusted hazard ratio for RA development of 26 (95% CI 15 to 46) compared to only 6.0 (3.4 to 10) for individuals with levels 2 to 4-fold above the upper normal range².

We have recently reported median RFs of greater than 10-fold higher than the upper normal range in nodular RA which is far more prevalent in smokers than non-nodular RA³. We have arbitrarily defined this level of RF as very strongly positive as it defines a disease subtype.

Additionally, RF levels are strongly associated with additive exposures to both smoking and industrial vapours, gases, dust and fumes (VGDF) in male RA, with only 8% having been exposed to neither VGDF nor cigarette smoke⁴.

Objectives: Accordingly, we have studied female RA in whom VGDF exposures are less common to compare RF levels in an appreciable number of smokers and non-smokers without the significant confounding effect of VGDF exposures to determine if smoking is specifically associated with a very strongly positive RF.

Methods: Medical record analysis yielded 241 female RA patients seen in clinic between January and June 2018 without exposures to industrial VGDF. These patients were stratified for a history of smoking. RF was measured with Tina-quant Rheumatoid Factors II Test System, by Roche Diagnostics Corporation at the time of RA diagnosis. A value of < 14 IU/ml was considered as negative as per manufacturer guidelines, a RF weakly positive (14-42 IU/ml), RF strongly positive (42.1-140 IU/ml) and RF very strongly positive (>140 IU/ml).

Results: There were 109 never smokers and 132 smokers with a median RF of 21 IU/ml and 53 IU/ml respectively, p<0.05. In never-smokers significantly more were seronegative 46/109 (42%) vs. 32/132 smokers (24%), odds ratio (OR)=2.22 (95% confidence interval (CI) 1.28-3.83, P=0.004). Of never smokers 22/109 (20%) were weakly RF positive and very similar to smokers 28/132 (21%). 29/109 (27%) of never smokers were strongly RF positive and a similar proportion of smokers 37/132 (28%) were strongly RF positive. Finally in never smokers, only 12/109 (11%) very strongly RF positive compared to 35/132 (27%) of smokers, OR=2.89 (95% CI 1.41-5.89, p=0.004).

Conclusion: Considering female RA smokers and non-smokers, the difference in RF positivity was exclusively accounted for by an increased prevalence of a very strongly positive RF in female smokers. This suggests that smoking has an impact on very strongly positive RF levels rather than RF positivity per se without the important confounding factor of VGDF exposure.

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AB0128

ANTIOXIDATIVE PROTECTION AND MONOXIGENASE SYSTEM ACTIVITY OF CYTOCHROME P450 (MOG) OF NEUTROPHILIC LEUKOCYTES (NL) FROM SYNOVIAL FLUID (SF) IN RHEUMATOID ARTHRITIS (RA)

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Background: MOG system is present not only in the liver but also in far less quantities in other organs and tissues, including SF.

Objectives: SF in RA patient contains up to 95% of NL that determines the inflammation also by inhibiting MOG.

Methods: SF of knee joints of 8 patients with RA with active synovitis and 4 donors (D) were examined. NL have been isolated by standard methods. After the application of Triton-X-100 0,1% solution, NL has been centrifuged at 25000 g for 30 minutes. The supernatant has been used in the experiment. The activity of MOG has been estimated according to the level of cytochromes P450. P420 and b5 have been estimated by applying the method of differential spectrophotometry. Active forms of oxygen have been registered by applying electron paramagnetic resonance (EPR). The activity of the antioxidant enzymes - Cu-Zn superoxide dismutase (Cu-Zn SOD), Se-glutathione peroxidase (Se-GPO) and catalase (CAT) was estimated by employing classical enzymology methods.

Results: It has been found out that in RA, the level of active oxygen forms increases by 68%. Meanwhile, the enzymes level of antioxidant defense is insufficient. In RA the activity of SOD, GPO and CAT enzymes is inhibited at 52, 66 and 39% respectively. In RA the activity of MOG (P450 and b5) in NL is 4 times lower than normal. The concentration of the inactive form of cytochrome P420, however, doubles. Nevertheless, their ratio P450/P420 (when normal = 5) in case of RA = 5,6 hardly ever changes.

Cytochrome concentration P450, P420 and b5 (nmol/g of protein), level of active oxygen forms (Units/mg of protein) and antioxidant system activity (SOD, GPO - Units/mg of protein; CAT - μ mol/min-mg of protein) in NL in RA

NL	P-450	P-420	b5	Level of active forms	Cu-Zn SOD	Se-GPO	CAT
D	1,25 ± 0,4	0,025 - 0,029 (0,027)	0,43 ± 0,07	15,7 ± 4,1	19,7 - 34,4 (27,1)	1,8 - 2,3 (2,1)	10,8 - 15,3 (13,1)
PA	0,3 ± 0,1	0,054 - 0,063 (0,058)	0,11 ± 0,03	49,4 ± 8,6	10,7 - 15,3 (13,1)	0,6 - 0,8 (0,7)	0,76 - 0,84 (0,8)

Conclusion: A fall in the level of free radical oxidation and antioxidant defense and faster generation of active oxygen forms in RA leads to NL being destroyed and creates favorable conditions for the development of autophagy and apoptosis. The oxidative stress during RA is accompanied by a fall in the activity of MOG and activation of lipid peroxidation and cytokine production in NL as shown earlier. A general fall in the activity of MOG (P-450 and b5) in NL in RA occurs against the background of enhanced conversion of P-450 into the inactive cytochrome P-420. This fact proves that the expression of isoenzymes P450s (CYPs) is reprogrammed whilst the autoimmune pathology is being developed and there is an unbalance of the immune oxygenase homeostasis in RA.

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AB0129

ULTRA-MICROSCOPIC ANALYSIS OF NURSING PHENOMENON IN SYNOVIAL TISSUE OF RHEUMATOID ARTHRITIS

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Background: Applying recent advances in electron microscope technology, cells and cell-cell interactions are observable using virtual slides, three-dimensional (3D) imaging, and serial sections of ultrathin thickness. Inflammation in rheumatoid arthritis (RA) is caused by multiple cell types, including infiltrating inflammatory cells, such as lymphocytes, neutrophils, macrophages, and spindle-shaped fibroblasts. Especially, we are focusing on fibroblast-like synoviocytes (FLSs). These cells have been reported to be positive for multiple markers including CD14, CD68, HLA-DR and vimentin, and we have shown that these cells are dendritic cells in our previous study using electron microscopy. Therefore, FLSs are presumed to function as dendritic cells. and these cells involved in the nursing phenomenon.

Objectives: The objective of this study is to investigate the chronicity mechanism of immunological inflammation in RA by using electron microscopy.

Methods: Synovial tissues collected from RA patients undergoing elbow or knee joint surgery were prepared for this study. Using wide field of view images of the synovial tissue, areas containing the nursing phenomenon were selected by using multiscale electron microscopy. 3D structures of the features were constructed from series of micrographs obtained by using focused ion beam-scanning electron microscopy and those by using transmission electron microscopy with double-axis electron tomography (DA-ET).

Results: From multiscale electron microscopy, many synovial dendritic cells and plasma cells were present in various areas of the lymphnodular infiltration. Serial cross-sectional observation with an FIB-SEM and 3D images revealed that synovial dendritic cells patronized the plasma cells, wrapping around the plasma cells, by the long slender axes. A tomogram of an ultra-thin sliced section created by using DA-ET revealed that synovial dendritic cells formed a membranous fusion with the plasma cells.

Conclusion: The nursing phenomenon involves characteristic histopathological features, which may be a factor in the chronicity of the inflammation mechanism in RA synovial tissue.

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AB0130

THE EFFECTS OF METHOTREXATE ON THE VASCULAR ENDOTHELIUM

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Background: Anti-inflammatory therapy has been recently validated as a treatment option for patients with cardiovascular disease (CVD) [1]. The anti-inflammatory drug methotrexate (MTX) reduces cardiovascular risk in patients with systemic inflammatory rheumatic diseases [2]; possibly by improving endothelial function [3]. However, the molecular basis of the drug's anti-inflammatory and potential anti-atherogenic effects on the vasculature is not well described.

Objectives: Our goal was to characterize the actions of MTX on vascular endothelial cells (EC).

Methods: MTX-treated human umbilical vein (HUVEC) or aortic endothelial cells (HAEC) were analyzed by quantitative real-time PCR (qRT-PCR), immunoblotting and phospho-kinase activity arrays. EC were treated with tumor necrosis factor (TNF)- α to induce pro-inflammatory activation. EC viability and cell cycle were evaluated by flow cytometry using Annexin V and/or propidium iodide staining. For selected experiments, EC were exposed to laminar (LSS; 20dyn/cm²) or oscillatory shear stress (OSS; 0 $\pm$ 5dyn/cm², 2Hz) using a parallel plate model.

Results: Under static conditions, MTX independently increased the activity of multiple kinases in quiescent and TNF α -activated EC. MTX-activated kinases