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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)

Viktor Shishkin, Galina Kudryavtseva, Yuri Malenkov, Viktor V. Shishkin. St. Petersburg State University, St. Petersburg, Russian Federation

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

rie kurose¹, Takahiro Ochi², Takashi Satoh³, Miwa Uzuki⁴, Kenya Murakami³, Akira Kurose¹, Yo-ichi Satoh³, Yasuyuki Ishibashi¹, Kinji Ishida³, Katsutoshi Ogasawara³, Akinari Morikawa⁵, Takashi Sawai⁶. ¹Hirosaki University Graduate School of Medicine, Hirosaki, Japan; ²Osaka Police Hospital, Osaka, Japan; ³Iwate Medical University School of Medicine, Morioka, Japan; ⁴Tohoku Bunka Gakuen University, Sendai, Japan; ⁵Hitachi High-Technologies Corporation Electron Microscope Application Group, Hitachinaka, Japan; ⁶Tohoku University, Sendai, Japan

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

Marie Lang, Charis Pericleous, Robert Maughan, Allan Kiprianos, Justin Mason. Imperial College London, Vascular Sciences, London, United Kingdom

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

included the mitogen-activated protein kinase (MAPK) p38 and Akt. MTX did not inhibit TNF α -induced nuclear factor kappa B (NF κ B) transcriptional activation, signaling or target gene expression. However, MTX induced pro-inflammatory markers such as vascular cell adhesion molecule (VCAM)-1 in an additive manner with TNF α . Functionally, MTX did not induce apoptosis but caused S-phase cell cycle arrest, which, along with p38 and Akt activation, could be abrogated by supplementation with folic acid.

Findings to date in EC subjected to shear stress are somewhat different. MTX had no or a mild inhibitory effect on kinase signaling in EC under LSS and OSS respectively. MTX did not affect cell proliferation nor baseline or OSS-induced VCAM-1 expression in EC under shear stress.

Conclusion: In static EC, low-dose MTX caused cell cycle arrest through folate depletion, which is a known mechanism of action in other cell types. Of note, this response was not seen in EC pre-conditioned by shear stress and emphasizes the impact of biomechanical forces on endothelial phenotype and response to exogenous stimulation. This is the first report to study the effects of MTX on EC under shear stress, which will be crucial in understanding its molecular actions on the vasculature.

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AB0131

THE CORRELATION OF EXPRESSION OF DIFFERENTIAL DRUG-RESISTANT PROTEINS AND INFLAMMATORY CYTOKINES IN COLLAGEN INDUCED ARTHRITIS MODEL

Jie Jiang, Xiangcong Zhao, Ning Yan, Fangyuan Hu, Li Xiaofeng, Caihong Wang. *the Second Hospital of Shanxi Medical University, Tai Yuan, China*

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease. The most characteristic patho-logical changes are chronic synovitis. At present, the combination of traditional disease-difying anti-rheumatic drugs (DMARDs) and biological agents DMARDs has improved the therapeutic effect of RA, but some RA patients still have poor response due to Multidrug resistance (MDR) phenomenon. The main mechanism of MDR is related to the ATP-binding cassette (ABC) transporter superfamily members, which increases drug efflux and reduces intracellular drug concentration. We will study how cytokines regulate the expression of ABC transporters.

Objectives: By comparing the differential expression of ABC transporter family resistance-related proteins P-gp, BCRP, MRP1 and inflammatory cytokines IL-1 β , IL-2, IL-6, IL-10, TNF- α and IL-17 in CIA model mice, to investigate the correlation between Inflammatory cytokines and drug-resistant proteins.

Methods: Fourteen DBA1 mice were successfully induced by collagen and Freund's Adjuvant. According to the scores of synovial pathology, the CIA group was divided into mild, moderate-to-severe groups, another four mice were selected as control. The mRNA expressions of P-gp, BCRP, MRP1 in splenic lymphocyte cells were measured by RT-PCR. The concentrations of IL-1 β , IL-2, IL-6, IL-10, TNF- α , IL-17 in serum were detected by Cytometric Bead Array(CBA). Further analyze the correlation between different inflammatory cytokines and these proteins, then study one of the proteins which is most related with cytokines by immunohistochemical (IHC) in synovium. Two independent samples were analyzed by the Mann-Whitney U test. Non-parametric Kruskal-Wallis H test was performed on multiple independent samples. The correlation between variables was used by Spearman correlation analysis.

Results:

1. Compared with the normal controls, The level of IL-6 and TNF- α in the serum of mild CIA group, moderate-severe CIA group were significantly increased ($Z_1=14.383$, $P_1<0.05$, $Z_2=8.375$, $P_2<0.05$). Compared with the mild CIA group, the level of IL-6 in serum were significantly increased in the moderate-to-severe CIA group ($P<0.05$), but there was no distinct difference in the TNF- α level ($P>0.05$).

2. In the spleen lymphocytes, there was no significant difference in the the mRNA expression level of P-gp and BCRP among the groups, but the mRNA expression level of MRP1 were significantly increased ($P<0.05$). Compared with the mild CIA group, the MRP1 mRNA in the moderate-to-severe CIA group was higher, there was significant difference ($Z=12.634$, $P<0.05$). There was a correlation between mRNA expression of MRP1 and P-gp ($r=0.635$, $P=0.015$).
3. The mRNA expression of MRP1 was positively correlated with IL-6 level ($r=0.711$, $P=0.004$).
4. The expression of MRP1 in normal group, low-level IL-6 group and high-level IL-6 group were respectively as follows: 1.08(0.65,1.30), 1.40(1.13,2.07), 1.90(1.68,2.17).
5. Compared with the controls, the cytoplasm/membrane of the knee and ankle joint synovial tissue in the CIA group was yellowish-brown, which proved that MRP1 expression was positive.

Conclusion: In the CIA arthritis model, synovial tissue lesion is not only related to inflammatory cytokines, but also related to MRP1 expression in the ABC transport resistance protein family, and it is proved that IL-6 is highly correlated to MRP1.

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AB0132

A NOVEL TARGET FOR TREATMENT OF INFLAMMATORY JOINT DISEASES

Susanne Lindquist^{1,2}, Gerd-Marie Alenius¹, Lillemor Berntson¹, Solbritt Rantapää Dahlqvist¹, Lennart Lundberg², Yuhang Wang¹, Olle Hernell^{1,2}. ¹Umeå University, Umeå, Sweden; ²Lipum AB, Umeå, Sweden

Background: The bile salt-stimulated lipase (BSSL) is a hitherto unrecognized player in inflammation. Animals devoid of BSSL (knockout mice) are protected from developing collagen induced arthritis (CIA) and collagen antibody induced arthritis (CAIA), and antibodies directed towards BSSL has been proven to prevent or mitigate arthritis in mouse and rat arthritis models¹. In humans, BSSL is present in blood² and accumulate at sites of inflammation. Patients with acute pancreatitis have significantly increased plasma BSSL levels compared to healthy controls. Whether BSSL in blood originates from pancreas, inflammatory cells, or both remains to be elucidated.

Objectives: To determine BSSL concentration in blood samples from patients with inflammatory joint disorders and to evaluate possible relationships between circulating BSSL levels and disease-activity variables.

Methods: BSSL concentrations in plasma or serum were determined in patients with rheumatoid arthritis (RA), psoriasis arthritis (PsA), and juvenile idiopathic arthritis (JIA) by a sandwich enzyme-linked immunosorbent assay (ELISA). Correlations between BSSL concentrations and disease activity score, erythrocyte sedimentation rate (ESR), blood levels of C-reactive protein (CRP), S100A8/9, leukocyte- and neutrophil counts, proinflammatory cytokines and chemokines were analyzed using Spearman rank-order correlation.

Results: Significant correlations between BSSL concentration in plasma and disease activity score (DAS28, $r_s=0.31$, $p=0.007$), ESR ($r_s=0.58$, $p<0.000$), CRP ($r_s=0.42$, $p=0.012$), leukocytes ($r_s=0.66$, $p<0.000$), and neutrophils ($r_s=0.71$, $p<0.000$) were found in RA. The BSSL plasma concentration decreased with duration of treatment with the TNF α inhibitor infliximab, in parallel with decreasing DAS28 score.

BSSL concentration was significantly higher in sera from PsA patients with both oligo- and polyarthritis compared with healthy controls. Moreover, BSSL concentration in serum correlated significantly with S100A8/9 and CRP concentrations ($r_s=0.54$, $p<0.001$ and $r_s=0.49$, $p<0.001$, respectively). No correlation between levels of BSSL and cytokines or chemokines were found in RA or PsA plasma or serum, respectively.

In JIA, levels of BSSL in serum correlated significantly with JIA disease activity score (JADAS27) ($r_s=0.26$, $p=0.007$), ESR ($r_s=0.47$, $p<0.000$), and leukocytes ($r_s=0.32$, $p<0.000$).

Conclusion: BSSL concentration in serum and plasma correlated with disease activity in patients with inflammatory joint disorders, i.e. RA, PsA and JIA. These data in humans support the relevance of our previous studies in rodents and