AB0133

EXPRESSION OF PRO-RESOLVING SPECIALISED MEDIATORS' RECEPTORS IN RHEUMATOID ARTHRITIS

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Background: Inflammation is the physiologic response against noxious stimuli to restore homeostasis and tissue repair. At the initial phase, characterised by an increase in pro-inflammatory cytokines aiming to neutralise the tissue injury, the resolution process must follow to down-regulate the inflammation and to promote the tissue repair. That latter phase is driven by the so called Pro-Resolving Specialised Mediators (SPMs), such as Resolvin (RvD and RvE), Protectins, Maresins and Lipoxin A4 (LXA4), bioactive metabolites of omega-3 fatty acids that act by interacting with specific cellular receptors: CMKLR1 and BLT1 for RvE1, FPR2 and GPR32 for RvD1 and FPR2 for LXA4. In rheumatoid arthritis (RA) the reactive inflammation becomes persistent and the innate immune response turns into the adaptive immune activation. Nowadays there is no evidence whether SPMs are involved in RA pathogenesis.

Objectives: Purpose of this study was to evaluate the expression of CMKLR1, FPR2 and BLT1 in RA patients and to correlate it to the disease activity.

Methods: Patients affected with RA, according to the 2010 EULAR/ACR classification criteria, were enrolled in this study. Exclusion criteria were: minority age, status of pregnancy or breastfeeding, concomitant any other autoimmune disease. At entry, ESR, CRP, DAS28-ESR, CDAI, Health Assessment Questionnaire Disability Index (HAQ) and peripheral venous blood sample were collected. Based on DAS28-ESR, patients were divided into high-moderate (H-Mo/RA if DAS28-ESR ≥ 3.2) and low-remission (L-Rem/RA if DAS28 < 3.2) disease activity group. The expression of CMKLR1, FPR2 and BLT1 in peripheral T cells (CD3) and monocytes (CD14) was evaluated by flow-cytometry assay. Differences for continuous variables were evaluated using the Mann-Whitney test, while for categorical data the Fisher's probability test. Correlations were assessed using the Spearman test.

Results: Thirty RA patients, 21 H-Mo/RA and 9 L-Rem/RA, were studied. While no difference in the expression of CMKLR1, FPR2 and BLT1 in CD3-T cells between the 2 groups was found, SPMs receptors were differently expressed on CD14-monocytes. BLT1⁺CD14⁺ cells were significantly higher in L-Rem/RA (90,96%) than in H-Mo/RA/RA (56,70%) ($p: 0.0001$). Likewise, FPR2⁺CD14⁺ cells were significantly higher in L-Rem/RA (92,29%) than in H-Mo/RA/RA (79,94%) ($p: 0.01$). CMKLR1 expression in monocytes was not regulated by disease activity. We also demonstrated an inverse correlation between BLT1 level in monocytes and ESR ($p: 0.01$), CRP levels ($p: 0.008$), DAS28-ESR ($p: 0.03$), CDAI ($p: 0.0076$) and HAQ ($p: 0.0138$) and a weak correlation between FPR2 expression and HAQ ($p: 0.05$) (figure 1).

Abstract AB0133 – Figure 1. Statistically significant correlation between the expression of FPR2 or BLT1 in monocytes and different parameters such as ESR ($p: 0.0166$; $r: -0.434$), CRP ($p: 0.0084$; $r: -0.4726$), DAS28-ESR (0.0318 ; $r: -0.3927$), CDAI ($p: 0.0076$; $r: -0.4936$) and HAQ (FPR2: $p: 0.05$; $r: -0.4438$; BLT1: $p: 0.0138$; $r: -0.5169$).

ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; DAS28-ESR: Disease Activity Score of 28 joints; CDAI: Clinical Disease Activity Index; HAQ: Health Assessment Questionnaire Disability Index. P -value < 0.05 was considered statistically significant.

Conclusions: In this study, FPR2 and BLT1 expression seem to be regulated by the activity of RA disease. As FPR2 and BLT1 should be involved in down-regulating the inflammation by monocytes, it might be hypothesised that a defective signalling through these SPMs receptors may contribute to sustain chronic inflammation in active RA. However, further studies are needed to explore the intricate mechanisms beyond inflammation and its resolution.

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AB0134

PHOSPHORELATED STAT3 EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN RHEUMATOID ARTHRITIS

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Background: The JAK-STAT pathway is mainly involved in the regulation of the expression of cytokines and growth factors; so it controls the proliferation, differentiation, migration and death of the cells of the immune system. In patients with RA, studies have shown increased activation of STAT3 with undergoes phosphorylated (pSTAT3), probably due to increased levels of cytokines such as IL-6, IL-11, IL-12.

Objectives: This study aims at investigating the level of expression of STAT3 and pSTAT3 in peripheral blood lymphomonocytes cells of patients with Rheumatoid Arthritis (RA), correlating them with disease activity (ESR, CRP, clinimetric tests).

Methods: Patients affected with RA, according to the 2010 EULAR/ACR classification criteria, were enrolled in this study. Exclusion criteria were: minority age, status of pregnancy or breastfeeding, concomitant any other autoimmune disease. At entry (new treatment with csDMARDs or biological drugs) ESR, CRP, DAS28-ESR, CDAI and peripheral venous blood sample were collected. The expression of STAT3 and pSTAT3 in lymphomonocytes cells, CD4 and CD14 cells were evaluated by flow-cytometry assay. The level of STAT3 gene was studied by qPCR. Differences for continuous variables were evaluated using the Mann-Whitney test, while for categorical data the Kolmogorov-Smirnov test. Correlations were assessed using the Spearman test.

Abstract AB0134 – Figure 1

Results: Twenty RA patients were studied, comparing STAT3 and pSTAT3 levels with 9 seronegative arthritis patients (SA) and 8 healthy control (HC). While no difference in the expression of STAT3 levels between the 3 groups (in flow-cytometry and qPCR assays), pSTAT3 was higher in RA (9,5%) than in SA (2,74%) ($p:$

0.005) or HC (4.81%) (p : 0.04); between the 3 groups, pSTAT3 was differently expressed in CD4-lymphocytes and CD14-monocytes (figure 1). We also demonstrated a correlation between pSTAT3 level in CD14 +cells and ESR (p : 0.026; r : 0.4625). We found no correlation between pSTAT3 expression and Body Mass Index or disease duration.

Conclusions: In this study, STAT3 seems to be activated more in RA than SA or HC. Monocytes (as macrophage) and T-cells have important role in RA. Isomaki et al. demonstrated a correlation between IL-6 level and STAT3 activation so it might be suggested a possible role of pSTAT3 as a biomarker of disease activity. However, further studies are needed to explore the important role of JAK-STAT pathway in RA.

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AB0135 CIRCADIAN RHYTHMS OF IMMUNE CELLS IN HEALTHY INDIVIDUALS AND PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Clinical symptoms of rheumatoid arthritis (RA), such as joint stiffness, swelling and pain, manifest in circadian pattern with the highest intensity occurs at early dawn. This is known to correlate with the circadian expression of IL-6, which peaks before the onset of the symptom. Despite this finding, the circadian behaviour of immune system in cellular and molecular level in RA patients has not yet been extensively investigated.

Objectives: Our previous study suggested that immunological circadian rhythms in patients with RA were altered when compared to the healthy individuals. Currently, we are performing 24 hours study involving RA patients and healthy individuals to further monitor the dynamic occurrence of diverse immune cells in the periphery.

Methods: Up to this time five eligible RA patients aged 45–75 years and twelve eligible healthy controls were recruited to join the study. On the study day, the blood was drawn in two hours interval throughout 24 hours. The participants were provided with regular meal, allowed to eat snacks ad libitum and carry passive activities. The absolute number of circulating immune cells was determined using TruCount. RNA were isolated from CD14⁺ monocytes and analysed by real-time PCR.

Results: The major populations of immune cells in the periphery of healthy controls, including CD4 T cells, CD8 T cells, regulatory T cells, B cells and monocytes, displayed circadian rhythm that peaks during the rest phase. The rhythms are in general shifted a few hours later in the RA patients. Noteworthy, CD14 monocyte, which is one of the major sources of IL-6 in RA, showed a more pronounced rhythm with higher amplitude in RA patients compared to healthy individuals. Furthermore, the following clock genes are rhythmically expressed in CD14 monocytes of both groups: *Rorα*, *Per1*, *Per2*, *Per3* and *DBP*. The peak of *Rorα*, *Per1*, *Per3* *DBP* and *CRY1* is shifted a few hours later in RA patients. Interestingly, circadian variation is not observed in the expression of *RevErbα* in healthy individuals, while in the RA patients a rhythm is established.

Conclusions: In general, circadian rhythm of immune system in cellular and molecular level in RA patients appears to undergo phase shift and peaks a few hours later in comparison to healthy individuals. New established rhythms were also observed in cellular and molecular level. Another round of study involving seven RA patients is planned this spring to complete the project. Considering our data, we will continue to investigate circadian rhythms in expanded immune cell population using mass cytometry, immunoassay and microarray. Identification of immunological circadian rhythms in patients with RA and healthy individuals will help us to expand our knowledge in autoimmunity and provide an outlook on potential future implications.

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AB0136 THE EFFECT OF IL-6 RECEPTOR ANTIBODY FOR THE TREATMENT OF MCH-LPR/LPR-RA1 MICE THAT SPONTANEOUSLY DEVELOPED ANKYLOSING ARTHRITIS

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Background: Mch-lpr/lpr-RA1 (RA1) mice are new strain mice which spontaneously developed arthritis in ankle and finally leads to ankylosis. There is no report that drug treatment has been applied to these mice.

Objectives: To examine the effect of mouse IL-6 receptor antibody MR16-1 for the treatment of RA-1 mice.

Methods: Male RA1 mice were randomly divided into treated and control groups. MR 16-1 was applied from 10 weeks of age for the treatment group. Saline was applied for the control group. The drug was administered every two weeks with the initial dose of 2 mg, then 0.5 mg. The effects were evaluated by histological synovitis score, *in vivo* imaging using ICG-encapsulated liposomes and the expression of serum SAA and IL-6.

Results: The tissue evaluation was carried out at 14 weeks, 17 weeks and 20 weeks of age. The histological score of treated groups were significantly improved compared with control group at every age. The interclass correlation coefficient was 0.771. *In vivo* imaging using ICG-encapsulated liposomes showed that significant signal decrease in treated groups at 14 weeks, but no significant difference was observed after 16 weeks. Blood SAA was significantly improved at 17 weeks of age.

Abstract AB0136 – Figure 1

Conclusions: IL-6 receptor antibody is effective for the treatment of ankylosing arthritis of RA1 mice. IL-6 might be a new potential target of treatment for ankylosing arthritis.

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AB0137 ARTHRITIS-ASSOCIATED EGGERTHELLA LENTA MODULATE DISEASE VIA METABOLIC AND MICROBIAL ALTERATIONS

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Background: Role of environmental factors in predisposition to develop rheumatoid arthritis (RA) has gained interest due, in part, to the studies showing an association of gut microbiota with immune homeostasis. Although the etiology of RA is unknown, recent studies on the role of gut microbiota in inflammatory adaptive immune response have led to the concept that interaction between the host microbiome and genetic factors influences autoimmunity. We have shown an association of rare lineage commensals, *Eggerthella lenta*, with RA.

Objectives: In this study, we aimed to determine how human gut commensal *E. lenta* modulates gut epithelial integrity and inflammation via metabolites in humanised mice expressing RA-susceptible HLA-DQ8.

Methods: DQ8 mice following immunisation with type II collagen develop arthritis and antigen-specific cellular and humoral response. DQ8 mice orally gavaged