Innate immunity in rheumatic diseases

AB0036 BUTYRIC ACID SUPPRESSES MIGRATION OF MONOCYTE-DERIVED DENDRITIC CELLS BY INHIBITING MDIA1-MEDICATED ACTIN POLYMERIZATION

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Background: Butyric acid is known to improve chronic inflammation such as inflammatory bowel disease and arthritis [1, 2]. Dendritic cells activate in inflammatory condition, migrate to regional lymph nodes, and activate naive T cells.
Objectives: In this study, we investigated the effect of butyric acid on the migration ability of monocyte-derived dendritic cells (moDC).
Methods: Human CD14+ Monocytes were purified by positive selection from PBMC using CD14 magnetic beads. Cells were cultured in the presence of GM-CSF (50 ng/ml) and IL-4 (20 ng/ml) for 5 days. After culturing for 5 days, cells were matured with LPS (1ug/ml) for 24 hours. Butyric acid was administered at different dose or period. Surface antigen on moDC was analyzed by flow cytometry (BD FACS VERSE). Migration assay was performed on Boyden chamber CytoSelect 24-Well Cell Migration Assay (5um). Actin was stained with Alexa Fluor 488 Phalloidin before and after migration assay. After administration with butyric acid assigned to each period and concentration, moDC were lysed for western blot analysis for evaluating signaling. Chemiluminescent signals were detected and calculated by Amersham Imager.
Results: We demonstrated that butyric acid decreased the CCR7 expression of moDC, which has a key role in DC homing to the lymph nodes and intestinal Peyer's patches. We also showed that butyric acid decreased the migration ability of moDC. Furthermore, moDCs cultured with butyric acid showed a round shape and poor formation of dendrites and pseudopodia. Then we studied the effect of butyric acid on cytoskeleton, which plays an important role in migration and pseudopodia formation of DCs. Polymerized Actin (F-Actin) staining revealed that butyrate suppressed actin polymerization of moDC in a dose dependent manner. CDC42 works important role of lamellipodia and membrane protrusions. RhoA is upstream of mDia1, and mDia1 was reported to accelerate actin nucleation and elongation. We revealed that butyrate decreased the protein expression of mDia1, RhoA, and CDC42, while beta actin was not downregulated, by Western blot analysis. Our results suggested that butyric acid suppresses migration of moDCs by inhibiting mDia1-medicated actin polymerization.
Conclusion: Butyric acid suppresses migration of moDCs by inhibiting mDia1-medicated actin polymerization.
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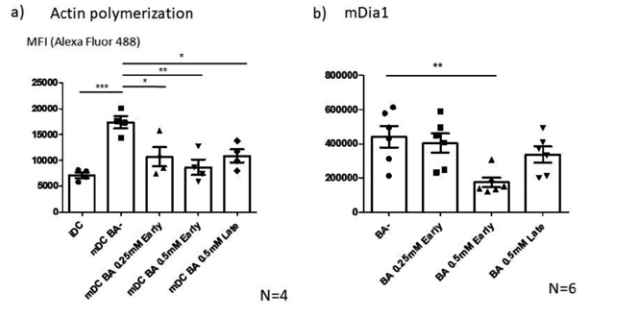

Fig. 1.
a) Butyric acid inhibited actin polymerization of moDC after migraton for FBS. Actin was stained with Alexa Fluor® 488 Phalloidin. MFI was analyzed by FACS.

b) Butyric acid downregurated expression of mDia1. Chemiluminescent signals were detected and calculated by AmershamTM Imager.

All data are presented as mean ± sem
$P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$ Based on Turky

Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2022-eular.959

AB0037 APE1 REGULATES THE MIGRATION OF FIBROBLAST-LIKE SYNOVIOCYTES FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: The level of apurinic/apyrimidinic endonuclease 1 (APE1) is elevated in synovial fluids from patients with rheumatoid arthritis (RA). However, the role of APE1 in RA pathogenesis remains unclear.
Objectives: To explore whether APE1 affects cell migration through reactive oxygen species (ROS) level, fibroblast-like synoviocytes (FLS) from patients with RA were stimulated with human recombinant APE1.
Methods: Synovial tissues were obtained from RA patients who were undergoing synovectomy or joint replacement. The isolated cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and maintained in a 5% CO_2 incubator at 37 °C. FLS were used for experiments after three to six passages. Cells were stimulated with or without recombinant interleukin 17 (IL-17; 10 ng/ml), tumor necrosis factor alpha (TNF-α; 10 ng/ml), and long-lasting recombinant human APE1 (MR201; 1, 10, 100 ng/ml) for 24 h. ROS levels were analyzed using MitoSOX dye. Cell migratory ability was examined using wound migration assay.
Results: RA FLS treated with APE1 showed slightly decreased level of mitochondrial specific ROS. To induce pro-inflammatory conditions, RA FLS were incubated with IL-17 and TNF-α. These cytokines are highly detected in RA synovium and directly stimulate FLS activation. Stimulation with IL-17 and TNF-α upregulated ROS by 30% compared to control. Cytokines-induced increase of ROS was inhibited by 22% in APE1 treatment. When FLS cultures were approximately 90% confluent, FLS monolayers were wounded with pipette tips and treated with IL-17/TNF-α and APE1 for 24 h. Cell migration was increased after treatment with IL-17/TNF-α. Cytokines-induced cell migration was remarkably attenuated by APE1 treatment.
Conclusion: Recombinant APE1 markedly inhibited mitochondrial specific ROS production and IL-17/TNF-α-induced cell migration in RA FLS.
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Acknowledgements: This research was supported by Chungnam National University Hospital Research Fund, 2021.
Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2022-eular.1683

AB0038 DUAL TARGETING PEPTIDE RLS-0071 REDUCES AND INHIBITS MYELOPEROXIDASE (MPO) IN HEALTHY HUMAN VOLUNTEER

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Background: RLS-0071-101 was a first in human clinical trial to evaluate safety of the peptide RLS-0071 in healthy volunteers in a single ascending dose and multiple ascending dose format. RLS-0071, previously referred to as Peptide Inhibitor of Complement C1 (PIC1) is a dual-targeting peptide being developed for clinical use to moderate humoral and cellular inflammation via inhibition of complement activation and neutrophil effectors including myeloperoxidase (MPO) and Neutrophil extracellular trap formation (NETosis).[1,2] Humans that are otherwise asymptomatic are considered at risk for cardiovascular complications if they have a plasma MPO level of > 420 pmol.[3] A post hoc analysis of plasma samples from subjects participating in RLS-0071-101 identified an individual with mildly elevated baseline MPO level (142 pmol/L).
Objectives: Evaluate if RLS-0071 dosing would change MPO level or activity in a subject with elevated baseline MPO.
Methods: Frozen plasma samples prepared from blood collected by venipunture into K2EDTA tubes (BD) was utilized to determine MPO quantity and activity levels. MPO quantity in the plasma was analyzed using a human MPO ELISA kit (BMS2038INST, Invitrogen) and MPO activity within the plasma was analyzed using a fluorescence-based myeloperoxidase assay kit (K745-100, BioVision).
Results: Upon screening 54 subjects from RLS-0071-101 we identified one individual with a mildly elevated MPO level at baseline, a 21-year-old white female with BMI of 21.7. The subject received 9 intravenous infusions of RLS-0071 each at a dose of 10 mg/kg. Her vital signs and body temperature remained normal

throughout the study and the only blood laboratory abnormality were a mildly low plasma protein concentration of Day 2 and Day 4 which was noted both among recipients of the peptide and placebo subjects. Analysis of MPO blood concentrations demonstrated a mildly elevated baseline plasma MPO concentration that decreased after multiple doses of RLS-0071 with partial recovery to baseline 24 hours after cessation of dosing. MPO activity analyzed using a fluorescence-based myeloperoxidase assay kit demonstrated an elevated baseline plasma MPO activity level that decreased after multiple doses of RLS-0071 with partial recovery after 24 hours.

Conclusion: These results suggest promise for RLS-0071 to reversibly moderate plasma MPO activity and potentially affect MPO-mediated diseases including acute coronary syndrome (ACS), atheromatous plaque vulnerability and auto immune conditions.4,5,6,7.

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Disclosure of Interests: Parvathi Kumar Shareholder of: I have stock options available, Consultant of: Served as a consultant for ReAlta life Sciences INC from April- July 2020, Employee of: I am employed by ReAlta Life Sciences, Kenji Cunnion Shareholder of: Shareholder of ReAlta life sciences, Employee of: ReAlta Life Sciences, Neel Krishna Employee of: I am an employee of ReAlta Life Sciences., Ulrich Thienel Shareholder of: Shareholder of ReAlta and JNJ, Employee of: ReAlta Life Sciences, Pamela Hair Employee of: ReAlta Life Sciences, Jessica Goss Employee of: ReAlta Life Sciences, Christos Gabriel: None declared

DOI: 10.1136/annrheumdis-2022-eular.2101

AB0039 MONOCYTE EXTRACELLULAR TRAPS: A NEW POTENTIAL DIAGNOSTIC BIOMARKER IN RHEUMATOID ARTHRITIS?

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Background: Studies demonstrating the important role of neutrophil extracellular traps (NETs) in the pathogenesis of autoimmune diseases, including rheumatoid arthritis (RA), have been published extensively in recent years. The researchers are also discussing the possibility of considering NET as a source of potential diagnostic biomarker in RA. As compared with NETs, the monocyte extracellular traps (METs) formation by mononuclear phagocytes, although they have also been implicated in the development of autoimmune reactions, has been studied insufficiently. No studies have been reported on the MET formation in active RA patients.

Objectives: Assesssment of METs generation by peripheral blood monocytes from RA patients in association with the disease exacerbation.

Methods: The research was carried out in agreement with the WMA Declaration of Helsinki principles after the local ethical board approval. Circulating monocytes were isolated with one-step density gradient centrifugation using double layers of the in-lab-made ficoll-amidotrizoate gradient. Composition of isolated cellular fractions, their viability, and non-specific activation were evaluated by light microscopy using common Romanowsky-Giemsa staining, trypan blue exclusion test as well as NBT test. METs were induced by LPS. Monocyte fractions contained low extents of activated and dead cells. Spontaneous and induced formation of extracellular traps was assessed using fluorescence microscopy [1]. Results were presented as values (95%CI).

Results: 30 healthy volunteers (9 males and 21 females, mean age 37.2 years) were enrolled as a reference group. 37 patients (6 males and 31 females, mean age 42.7 years) with verified RA according to the ACR/EULAR 2010 criteria were included in the study. RA disease activity was assessed using DAS28 score did not exceeded 2.6 in every patient at the inclusion timepoint. In 16 (15.4%) patients, the activity of DAS 28 exceeded 3.2 at subsequent visits (after 3, 8 and 12 months). Spontaneous and induced MET formation by isolated monocytes in RA patients (DAS 28<2.6) was 8.7 (8.3-9.1)% and 26.2 (24.5-27.9)%, respectively. Spontaneous and induced MET formation by isolated monocytes in RA patients (DAS 28>3.2) was 18.1 (17.8-18.4)% and 38.2 (38.0-38.4)%, respectively. Spontaneous and induced MET formation by isolated neutrophils in active RA patients (DAS 28>3.2) was significantly higher than in inactive RA patients and in comparison to the reference group. Induced MET formation was also significantly higher than spontaneous one ($p<0.05$). Monocytes did not demonstrate any difference between ACPA-positive and ACPA-negative RA patients in their MET production. The growth rate of spontaneous MET formation was 114.7%, for induced MET formation – 44.2%. The growth rate of spontaneous MET formation is 2.6 times higher than the induced MET formation.

Conclusion: METs formation by isolated monocytes can be considered as a new potential diagnostic biomarker associated with RA flare. Further study of the mechanisms of MET formation and their composition may improve our understanding of the role of monocytes and METs in the pathogenesis of RA.

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2022-eular.2641

AB0040 STUDYING THE MACROPHAGE ACTIVATION AND THE INTIMA-MEDIA THICKNESS OF THE CAROTID ARTERIES IN UNTREATED PATIENTS WITH RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS (PRELIMINARY DATA)

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Background: Autoimmune Rheumatic Diseases (ARDs) occur with a high risk of atherosclerosis development. The macrophages are at the same time a part of the inflammatory response, and also tightly linked to the foam cell formation, thus taking part in both crucial for atherogenesis processes.

Objectives: To evaluate the macrophage activation, and the intima-media thickness (IMT), subclinical atherosclerosis of the carotid arteries in untreated pts with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

Methods: Thirty six untreated RA pts (30F/6M) and 36 untreated SLE pts (31F/5M) were enrolled.

RA pts had median age of 39 years (range) (33-45), disease duration of 2.5 years (1-5), moderate clinical disease activity DAS of 28 5.3 (3.5;5.8), SDAI of 17 (15;19), HAQ of 1 (0.75;1).

SLE pts had median age of 37 years (range) (32-41), disease duration of 3.5 years (1-11), SLEDAI 2K of 7 (4-8), BILAG of 14,5 (10,2-23).

Isolation of monocytes was carried out according to the standard procedure for obtaining a leukocyte fraction in a Ficoll gradient and subsequent selection of CD14 + cells using magnetic separation. After isolation, the cells were cultured in X-Vivo medium. To assess the degree of monocyte activation, cells were stimulated by the addition of LPS. The value of monocyte activation was expressed as a ratio of the level of secretion of proinflammatory cytokines by monocytes cultured with and without LPS addition. Secretion levels were determined by ELISA. The belonging of the isolated cells to CD14 + monocytes was additionally confirmed by flow cytometry. All pts undertook carotid duplex ultrasonography.

Results: Macrophage activation was 8.9 (range) (2.7;40.8) in RA pts and 4.8 (2.3;5.6) in SLE pts ($p=0.09$). In RA and SLE pts macrophage activation was independent of age, sex, body mass index, cardiovascular risk (CVR) factors (arterial hypertension, overweight, smoking, family history of cardiovascular diseases), activity scores (DAS28, SDAI, SLADAI 2K), and disease-specific autoantibodies levels.

In RA pts and SLE pts, the carotid mean IMT (m-IMT) was 0.66 ± 0.12mm and 0.61 ± 0.10mm, the maximum IMT (M-IMT) was 0.76 ± 0.16mm and 0.69 ± 0.14mm, respectively, $p>0.05$ in both cases. The prevalence of raised lesions (IMT>0.9 mm) were observed in 5 RA pts (14%) and 2 SLE pts (5%); the atherosclerotic plaques (ASP) (IMT ≥ 1.2 mm) – in 7 RA pts (20%) and 6 SLE pts