GSK, Mepha, MSD, Pfizer and UCB in the field of arthritides and related disorders, Caroline Ospelt: None declared, Mojca Frank-Bertoncelj : None declared

DOI: 10.1136/annrheumdis-2019-eular.5553

THU0007 IL-26 PROMOTES OSTEOCLASTOGENESIS IN RHEUMATOID ARTHRITIS

Sang-Heon Lee¹, Kyung Ann Lee², Hae-Rim Kim¹. ¹Konkuk University Medical Center, Seoul, Korea, Rep. of (South Korea); ²Soonchunhyang University Seoul Hospital, Internal Medicine, Seoul, Korea, Rep. of (South Korea)

Background: IL-26 is a 171-amino acid protein, which is classified as a member of the Th17 cytokine family. The role of IL-26 in osteoclastogenesis in RA is needed to be clarified to understand the pathogenesis of RA.

Objectives: To examine the functional role of interleukin-26 (IL-26) in the expression of RANKL and induction of osteoclastogenesis in rheumatoid arthritis (RA).

Methods: The expression of IL-20R α , CD55 and RANKL in RA synovial fibroblasts was analyzed using confocal microscopy. RA Fibroblasts like synoviocytes (RA-FLS) were treated with recombinant human IL-26, and the expression of RANKL messenger RNA (mRNA) and protein was measured using real-time polymerase chain reaction and ELISA. Human peripheral blood monocytes were cultured with macrophage colony-stimulating factor and IL-26, after which osteoclastogenesis was evaluated by counting the number of tartrate-resistant acid phosphatase-positive multinucleated cells. Osteoclastogenesis was also evaluated after monocytes were co-cultured with IL-26-prestimulated FLS.

Results: The IL-26 concentration in the FLS was higher in RA patients than in patients with osteoarthritis (OA). In RA-FLS treated with rhIL-26, the expression of RANKL mRNA and protein was increased in a dose-dependent manner. IL-26 increased the expression of RANKL in RA-FLS, and the IL-26-induced RANKL expression decreased by the inhibition of IL-20R α . IL-26-induced RANKL expression was down-regulated significantly by the inhibition of SHP-1, ERK, JNK, stat1, c-jun or p38 MAPK/NF- κ B signaling. When monocytes isolated from human peripheral blood were cultured with IL-26, they were differentiated into osteoclasts in the absence of RANKL. Monocytes were also differentiated into osteoclasts when they were cocultured with IL-26-pre-treated RA-FLS.

Conclusion: IL-26 has a dual effect on osteoclastogenesis in RA: 1) direct induction of osteoclastogenesis from monocytes and 2) up-regulation of RANKL production in RA-FLS. This IL-26/RANKL axis could be a potential therapeutic target for bone destruction in RA.

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Acknowledgement: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT and Future Planning (NRF-2017R1A2B4006015, NRF-2018R1D1A1A02050982 and NRF-2018R1A2B2006820)

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.6724

THU0008 GM-CSF PATHWAY SIGNATURE IDENTIFIED IN TEMPORAL ARTERY BIOPSIES OF PATIENTS WITH GIANT CELL ARTERITIS

Maria C. Cid¹, Rohan Gandhi², Marc Corbera-Bellalta¹, Sujatha Muralidharan², John F. Paolini². ¹Hospital Clinic, University of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Vasculitis Research Unit, Department of Autoimmune Diseases, Barcelona, Spain; ²Kiniksa Pharmaceuticals, Lexington, United States of America

Background: Giant Cell Arteritis (GCA) is a type of large vessel vasculitis that can cause blindness and aortic aneurysms. A significant unmet medical need remains in GCA, as current treatment options are limited, and relapse increases corticosteroid (CS) exposure and toxicity.

The primary role of macrophages/dendritic cells (DCs) and T_H1/T_H17 lymphocytes in GCA pathogenesis has been highlighted previously. Granulocyte-macrophage colony stimulating factor (GM-CSF) may contribute to GCA pathogenesis by stimulating giant cell formation.¹ GM-CSF produced by CD4⁺ T helper T_H1 and T_H17 cells can stimulate conventional DCs

and promote differentiation of monocyte-derived DCs.² GM-CSF may drive DCs to program naïve CD4⁺ cells to T_H1, T_H17, and T follicular helper phenotypes (IFN γ /IL-17/IL-21). Notably GM-CSF RNA has been reported in GCA lesions³ and in peripheral blood mononuclear cells of symptomatic patients.⁴

Objectives: We hypothesized elevation of the GM-CSF pathway signature in GCA vessels versus controls.

Methods: Two independent sources of temporal artery biopsies were utilized. First, GCA (n=17) and control (symptomatic patients suspected for GCA, but with a normal temporal artery biopsy; n=5) biopsies were analyzed for 15 mRNA transcripts representing T_H1, T_H17, and GM-CSF signaling (RNAscope; RS) and for mRNA transcripts representing the autoimmune panel (Nanostring; NS). Semi-quantitative scoring was performed on RS images, and fold-change of representative T_H1, T_H17 and GM-CSF related mRNA transcripts were calculated via NS nCounter analysis. Additional GCA and control biopsies were obtained and analyzed by RT-PCR for a subset of transcripts (n=10 each) and by confocal microscopy for GM-CSF and GM-CSF-R α protein (n=2 each).

Results: The GM-CSF signaling pathway molecular signature was confirmed to be upregulated by 4 independent analyses.

GM-CSF-associated and T_H1-associated genes were upregulated in GCA biopsies versus control (GM-CSF: 3-4x RS; GM-CSF-R α : 6.7x NS, 6x RS; and CD83: 3.9x NS, 6x RS; TNF α : 2x NS, 3x RS; IFN γ : 2x RS; IL-1 β : 6x RS). T_H17 associated genes were not elevated, potentially due to concomitant CS treatment.

Upregulation of both GM-CSF (12x) and GM-CSF-R α (3x) mRNA was confirmed in a separate cohort of biopsies from GCA patients vs. controls by RT-PCR (Figure). GM-CSF and GM-CSF-R α proteins were detected in the luminal endothelium, neovessels and inflammatory cells of GCA patients. In normal temporal arteries, GM-CSF protein was not detected, and some GM-CSF-R α expression was observed in the luminal endothelium.

Pu.1, a transcription factor downstream of GM-CSF signaling, was increased 8x in GCA vs. controls (RS, NS) (Figure).

Conclusion: GM-CSF and T_H1 pathway signatures were demonstrated in GCA patient temporal arteries by independent analytical techniques. Active GM-CSF signaling in diseased tissue is evidenced by increased expression of Pu.1 in the vessel wall. These data implicate the GM-CSF pathway in GCA pathophysiology and increase confidence in rationale for targeting GM-CSF in GCA.

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Abstract THU0008 Figure 1.

Disclosure of Interests: Maria C. Cid Grant/research support from: Kiniksa Pharmaceuticals, Consultant for: Roche, GSK, Janssen, Abbvie, Speakers bureau: Boehringer-Ingelheim, Vifor, Rohan Gandhi Shareholder of: Kiniksa Pharmaceuticals (clinical development stage biopharmaceuticals company), Employee of: Currently employed at Kiniksa Pharmaceuticals, Marc Corbera-Bellalta: None declared, Sujatha Muralidharan Shareholder of: Kiniksa Pharmaceuticals (clinical development stage biopharmaceutical company), Employee of: Currently employed at Kiniksa Pharmaceuticals, John F. Paolini Shareholder of: Kiniksa Pharmaceuticals (clinical development stage biopharmaceutical company), Employee of: Currently employed at Kiniksa Pharmaceuticals

DOI: 10.1136/annrheumdis-2019-eular.2694

THU0009 ASSOCIATIONS BETWEEN CYTOKINE LEVELS AND TH17/TREG IMMUNE BALANCE IN PATIENTS WITH BEHCET'S DISEASE

Yue Liu¹, Yan Yang², Rui Su³, Xinyu Zheng³, Li Xiaofeng³, Caihong Wang³. ¹The Second Hospital of Shanxi Medical University, The Department of Rheumatic Immunology, Taiyuan, China; ²The Second Hospital Of Shanxi Medical University, The Department of Rheumatic Immunology, Taiyuan, China; ³The Second Hospital of Shanxi Medical University, The Department of Rheumatic Immunology, Taiyuan, China

Background: Behcet's disease (BD) is a chronic systemic vascular inflammatory disease. Autoimmune imbalance associated with genetic and infectious factors promotes the immune response of neutrophils and T cells, which is BD's important pathogenesis. Several studies indicate the Th17/Treg immune imbalance may play an important role in BD pathogenesis^[1-2]. Although the proportion of Th17 cells was notably increased, which was accompanied by an increased levels of IL-17, IL-23^[1], whether other cytokines are associated with Th17/Treg immune balance is unclear.

Objectives: The aim of this study was to examine associations between levels of a broad selection of cytokines and Th17/Treg immune balance in patients with BD.

Methods: The study included 66 BD patients and 66 healthy controls. The absolute counts of lymphocyte subsets and CD4+T cell subsets were detected by flow cytometry for all participants. Serum levels of the following 6 cytokines were determined in the same samples using a cytometric beads array: Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Interferon- γ (INF- γ) and Tumor necrosis factor- α (TNF- α) for all BD patients. T tests was used to compare continuous measures. Correlations between levels of cytokines and Th17/Treg were assessed by Spearman's rank correlation tests.

Results: (1) Compared to healthy controls, the absolute counts of T cells were increased ($P=0.048$), the absolute counts of CD8+T cells were also increased ($P=0.031$) in BD group, the absolute counts of Th1 and Th17 cells were significantly increased ($P<0.05$) in BD group, the absolute counts of Th2 cells were decreased ($P<0.05$) in BD group, the ratio of Th1/Th2 was increased ($P<0.001$), and the ratio of Th17/Treg were increased ($P<0.001$) in BD group. There were no differences between the absolute counts of the two groups of B, NK, T+B+NK, CD4+T and Treg cells. (2) Among the investigated cytokines, the differences between the levels of IL-2, IL-6, INF- γ and normal range have statistical significance ($P<0.001$). Finally, two cytokines were positively correlated with Th17/Treg: i.e., IL-2 ($r=0.279, P=0.023$) and IL-4 ($r=0.260, P=0.035$) ($P\geq 0.2$ for all other cytokines).

Conclusion: Our research shows that T cell homeostasis perturbation, especially Th1 and Th17 expansion and Th17/Treg immune imbalance, is the cornerstone of BD pathogenesis. These findings suggest that immune responses associated with increased levels of IL-2 and IL-4 may promote Th17/Treg immune imbalance. This also highlights the role of IL-2 and IL-4 in maintaining a balance in the Th17/Treg ratio. Further studies are required to evaluate these preliminary findings in different patient populations and also examine the possible molecular mechanisms behind our observations.

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Abstract THU0009 Figure 1. Figure: The absolute counts of lymphocyte subsets in RA patients and healthy controls. Data are expressed as means $\pm$ SD (ABCDE). Correlation coefficient and regression line of Th17/Treg with IL-2 and IL-4 serum levels were represented as scatter plots (FG).

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.1791

THU0010 THE ANTI-INFLAMMATORY CYTOKINE INTERLEUKIN 37 IS AN ENDOGENOUS INHIBITOR OF TRAINED IMMUNITY

Giulio Cavalli^{1,2,3}, Mark Gresnic⁴, Rob Arts², Travis Nemkov³, Angelo D'alessandro³, Silvia Giugliano⁵, Elan Eisenmesser², Lorenzo Dagna¹, Leo Joosten², Mihai Netea², Charles Dinarello^{2,3}. ¹Vita-Salute San Raffaele University, Milan, Italy; ²Radboud University Medical Centre, Nijmegen, Netherlands; ³University of Colorado Denver, Aurora, CO, United States of America; ⁴Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany; ⁵Humanitas University, Milan, Italy

Background: Trained immunity (TI) is a *de-facto* innate immune memory program induced in monocytes/macrophages by exposure to pathogens or vaccines, which evolved as a protective mechanism against infections. TI is characterized by rewiring of functional, epigenetic and metabolic programs of innate immune cells such as monocytes and macrophages, which sustain enhanced production of pro-inflammatory cytokines. Since aberrant activation of TI is implicated in inflammatory diseases, tight regulatory mechanisms are likely in place, but the mechanisms responsible for this modulation remain elusive.

Objectives: Scope of this study was to evaluate the role of IL-37, an anti-inflammatory cytokine that curbs inflammation as well as modulates metabolic pathways, as an endogenous regulator of trained immunity.

Methods: The effects of recombinant IL-37 were evaluated in a mouse model of TI induced by the administration of beta-glucan *in vivo* (survival to a lethal inoculum of infectious agents, production of inflammatory cytokines, recruitment of inflammatory cells at the sites of infection). Subsequently, the effects of IL-37 were evaluated *ex vivo* on bone marrow monocytes (production of inflammatory cytokines, metabolomic analysis of the activation status of the main pathways of cellular energy metabolism).