IL-26 PROMOTES OSTEOCLASTOGENESIS IN RHEUMATOID ARTHRITIS

Sang-Heon Lee, Kyung Ann Lee, Hae-Rim Kim, Konkuk University Medical Center, Seoul, Korea, Rep. of (South Korea); Soonchunhyang 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 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-pretreated FLS.

Results: The IL-26-mRNA concentration in the FLS was higher in RA patients than in patients with osteoarthritis (OA). In RA-FLS treated with IL-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. IL-26–induced RANKL expression was down-regulated significantly by the inhibition of Shp-1, Erk, Jnk, Stat1, c-jun or p38 MAPK/ NF-kB 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–pretreated 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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GM-CSF PATHWAY SIGNATURE IDENTIFIED IN TEMPORAL ARTERY BIOPSIES OF PATIENTS WITH GIANT CELL ARTERITIS

Maria C. Cid, Rohan Gandhi, Marc Corbera-Bellalta, Sujatha Muralidharan, John F. Paolini, Hospital clinic, University of Barcelona, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Vasculitis research unit, Department of Autoimmune Diseases, Barcelona, Spain; *Kliniksa Pharmaceuticals, Lexington, United States of America

Background: Giant Cell Arteritis (GCA) is a type of large vessel vasculitis that can cause blindness and 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_{H}1/T_{H}17 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. GM-CSF produced by CD4+ T helper T_{H}1 and T_{H}17 cells can stimulate conventional DCs and promote differentiation of monocyte-derived DCs. GM-CSF may drive DCs to program naïve CD4+ T cells to T_{H}1, T_{H}17, and T follicular helper phenotypes (IFN_{gamma}/IL-17/L-21). Notably GM-CSF RNA has been reported in GCA lesions and in peripheral blood mononuclear cells of symptomatic patients.

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 temporary artery biopsy; n=5) biopsies were analyzed for 15 mRNA transcripts representing T_{H}1, T_{H}17, and GM-CSF signaling (RNAseq; 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_{H}1, T_{H}17 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-Rx 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_{H}1-associated genes were upregulated in GCA biopsies versus control (GMCSF: 3x-4x RS; GM-CSF-Rx: 6x NS, 6x RS; and CD83: 3.9x NS, 6x RS; TNF_{alpha}: 2x NS, 3x RS; IFN_{gamma}: 2x RS; IL-1b: 6x RS). T_{H}17 associated genes were not elevated, potentially due to concomitant CS treatment.

Upregulation of both GM-CSF (12x) and GM-CSF-Rx (3x) mRNA was confirmed in a separate cohort of biopsies from GCA patients vs. controls by RT-PCR (Figure). GM-CSF and GM-CSF-Rx 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-Rx 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_{H}1 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.

REFERENCES:
ASSOCIATIONS BETWEEN CYTOKINE LEVELS AND TH17/TREG IMMUNE BALANCE IN PATIENTS WITH BEHÇET’S DISEASE

Yue Liu1, Yan Yang2, Rui Su3, Xinyu Zheng3, Li Xiaofeng2, Caihong Wang3. 1The Second Hospital of Shandong University, The Department of Rheumatology and Immunology, Taiyuan, China; 2The Department of Rheumatic Immunology, Taiyuan, China; 3The Department of Rheumatic Immunology, Taiyuan, China

Background: Behçet’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. Although the proportion of Th17 cells was notably increased, which was accompanied by an increased levels of IL-17, IL-23α, 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 significantly increased in BD group, the absolute counts of Th1 and Th17 cells were significantly increased (P<0.035) 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. 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= 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.

Disclosures of Interests: None declared


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

Giulio Cavali1,2,3, Mark Gresnigt4, Rob Arts2, Travis Nemikov5, Angelo D’Alessandro6, Silvia Giugliano5, Eian Eisenmesser7, Lorenzo Dagna8, Leo Joosten3, Mihai Nelea5, Charles Dinarello5,6, Vito Salute San Raffaele University, Milan, Italy; 5Radboud University Medical Centre, Nijmegen, Netherlands; 2University of Colorado Denver, Aurora, CO, United States of America; 4Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany; 2Humanitas 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 re-wiring 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 regulatory 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, metabolic analysis of the activation status of the main pathways of cellular energy metabolism).

Disclosure of Interests: None declared