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FRI0017 A NOVEL CONCEPT OF M1 AND M2 MONOCYTES IN RHEUMATOID ARTHRITIS: PRO-INFLAMMATORY MONOCYTE POLARIZATION IMBALANCE, ANTI-CITRULLINATED PROTEIN ANTIBODY AND OSTEOCLASTOGENESIS

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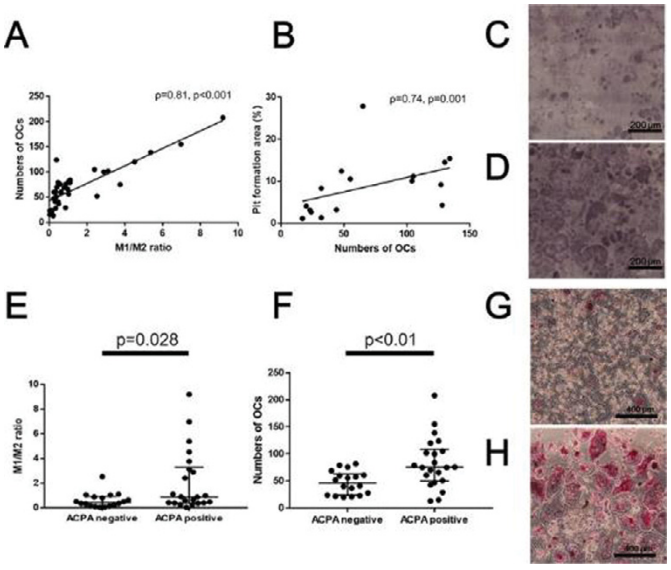
Background: Monocytes can differentiate into either proinflammatory, microbicidal M1 macrophage or anti-inflammatory M2 macrophage subtypes. In addition to macrophages, regarding monocyte subsets, M1 monocytes and M2 monocytes mirroring the M1/M2 macrophage polarization concept were suggested.
Little is known regarding the relationships between osteoclastogenesis and M1/M2 monocyte subsets.
Objectives: We investigated the relationships among M1 monocytes, M2 monocytes, osteoclast differentiation ability and clinical characteristics in patients with rheumatoid arthritis (RA).
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from RA patients and healthy donors, and we then investigated the number of M1 monocytes or M2 monocytes by fluorescence-activated cell sorting. We defined positive CD14, CD68 and CCR2 monocytes as M1 monocytes, and in separate tubes, we defined positive CD14, CX3CR1 and CD163 or CD206 monocytes as M2 monocytes.
We also obtained and cultured CD14-positive cells from PBMCs from RA patients and healthy donors to investigate osteoclast differentiation in vitro. We defined osteoclasts as tartrate-resistant acid phosphatase (TRAP) staining-positive cells with >3 nuclei. We counted the osteoclasts in the whole wells of a 96-well dish. Pit formation assays were performed to evaluate function of osteoclasts.
Results: Forty RA patients and 20 healthy donors were included. Twenty-two patients (55%) were ACPA-positive.
The median M1/M2 ratio was 0.59 (0.31–1.11, IQR). There were no significant differences between the RA patients and healthy donors.
There was a positive correlation between the M1/M2 ratio and the differentiated osteoclast number in vitro in RA patients (ρ=0.81, p<0.01) (A). The numbers of osteoclasts in vitro were significantly correlated with the area percentage of the pit formation area (ρ=0.74, p=0.001) (B).We demonstrated that the RA patients who had lower M1/M2 ratio and fewer osteoclasts (C) had smaller resorbed areas compared to the RA patients who had higher M1/2 ratio and greater numbers of osteoclasts (D).
The ACPA-positive patients had significantly higher M1/M2 ratio in vivo (p=0.028) (E) and significantly greater numbers of osteoclasts in vitro (p<0.01) (F) than the ACPA-negative patients. We show an ACPA-negative patient's osteoclasts in vitro (G) and those of an ACPA-positive patient (H).



Multivariable regression analysis revealed that the M1/M2 ratio was the sole significant contribution factor to in vitro osteoclastogenesis (β-coefficient 16.3, p<0.0001).
RA patients with M1/M2 ratio >1 (having relatively more M1 monocytes) had higher erythrocyte sedimentation rates (p=0.011) and C-reactive protein (p=0.032) than RA patients with M1/M2 ratio ≤1.
M1-dominant monocytes in vitro produced higher concentrations of IL-6 upon stimulation with lipopolysaccharide than M2 monocytes (p=0.032).
Conclusions: The M1/M2 ratio is strongly correlated with the in vitro differentiation of osteoclasts in patients with RA. The RA patients with positive ACPA had higher M1/M2 ratio and higher numbers of osteoclasts.
M1 and M2 monocyte subsets may become a new target of treatments for RA.
Disclosure of Interest: None declared
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FRI0018 TARGETED INHIBITION OF JANUS KINASES ABATES IFN-GAMMA-INDUCED INVASIVE BEHAVIOR OF FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Emerging evidence suggests that fibroblast-like synoviocytes (FLS) are key effector cells in rheumatoid arthritis (RA) and research into the mechanisms defining FLS activity in RA indicate that cytokines secreted by leukocytes play a crucial role. Nevertheless, the contribution of IFNγ, which is increased in rheumatoid synovitis, to the inflammatory synovial tissue reaction is not known.
Objectives: To explore the function of the T-cell cytokine IFNγ for mesenchymal tissue remodeling in RA, and to determine whether IFNγ-signaling controls the invasive potential of FLS.
Methods: To assess architectural responses, FLS were cultured in three-dimensional micromasses. FLS motility was analyzed in migration-, spreading- and invasion assays. Signaling events relevant to cellular motility were defined by western blots. Baricitinb and siRNA pools were used to suppress Janus Kinase (JAK) functions.
Results: Histological analyses of micromasses revealed unique effects of IFNγ on FLS shape and tissue organization. This was consistent with accelerated migration, pronounced actin and focal adhesion (FA) re-organization upon IFNγ stimulation. Since actin and FA dynamics and, thus, cell motility are integrated by the focal adhesion kinase (FAK), we next analyzed its activity. Indeed, IFNγ stimulation induced the phosphorylation of FAK-Y925, a phosphosite implicated in FAK-mediated cell migration. siRNA knockdown of JAK2, but not JAK1, abrogated FAK activation by IFNγ. Correspondingly, IFNγ-inudced FAK activation and invasion of FLS was abrogated by the JAK-inhibitor baricitinib.
Conclusions: Our study contributes insight into the synovial response to IFNγ and reveals JAK2 as a potential therapeutic target for FLS-mediated joint destruction in arthritis, especially in RA.
Disclosure of Interest: None declared
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FRI0019 HUMAN MESENCHYMAL STROMAL CELLS REDUCE TNFα SECRETION OF ACTIVATED PBMC VIA CTLA-4

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Background: The inhibitory costimulatory molecule Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) plays a crucial role in conveying immune tolerance in both inflammatory disorders and regenerative processes such as bone healing. During regenerative processes, mesenchymal stromal cells (MSC) provide the building bricks for reestablishing structural integrity but do also control inflammation by their immunomodulatory activities under restrictive microenvironmental conditions such as hypoxia. Here, we hypothesize these cells to support the control of inflammation via CTLA-4 in order to facilitate tissue regeneration such as bone fracture healing.
Objectives: Therefore, we analyzed expression of CTLA-4 by human MSC and their ability to convey immune suppression.
Methods: MSC were isolated from bone marrow of patients undergoing total hip replacement and characterized (i) by surface marker staining using flow cytometry and (ii) via assessing their osteogenic and adipogenic differentiation potential. MSC were cultured under normoxic (~18% O_2) and hypoxic (<1.5% O_2) con-