AB0093 BLYS AND APRIL OVEREXPRESSION IN EARLY RHEUMATOID ARTHRITIS: ASSOCIATION WITH B CELLS AND MYELOID SUBSETS

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Background: studies on B cell-mediated autoimmune diseases highlight the relevance of the B Lymphocyte Stimulator (BLyS) and A Proliferation-Inducing Ligand (APRIL), but emerging evidence points to an interaction with cell lineages other than B subsets. Although disturbances in the B cell compartment underlie the early stages of rheumatoid arthritis (RA), this phenomenon is poorly understood.
Objectives: to investigate the cellular populations responsible of BLyS expression and their association with the soluble forms of BLyS, APRIL and its receptor TACI (Transmembrane Activator and CALM Interactor) in early RA and to evaluate the changes in these parameters upon TNFα-blockade.
Methods: membrane BLyS (mBLyS) expression was assessed on B cells, monocytes (MØ), myeloid (mDC) and plasmacytoid (pDC) dendritic cells and neutrophils (NØ) by flow cytometry in fresh blood samples from 37 RA patients [DAS28 score (mean±SD): 4.84±1.44, disease duration (mean (range)): 1.26 (0–11) years, 23 (62.1%) RF+, 19 (51.3%) ACPA+, 19 untreated] and 31 healthy controls (HC). A subgroup of 13 biologic-naïve RA patients was prospectively followed for three months upon TNFα-blockade. Serum levels of soluble BLyS (sBLyS), APRIL (sAPRIL) and TACI (sTACI) were quantified by immunoassays.
Results: mBLyS expression was increased on B cells ($p=0.002$), MØ ($p<0.001$), mDC ($p<0.001$) and NØ ($p=0.014$) in RA patients. Higher sBLyS ($p=0.018$) and sAPRIL ($p<0.001$) serum levels were found in RA, whereas those of sTACI were not different compared to HC ($p=0.460$). Serum sAPRIL levels paralleled those of sTACI ($r=0.325$, $p=0.040$), and mBLyS expression on B cells ($r=0.463$, $p=0.009$), MØ ($r=0.521$, $p=0.003$), mDC ($r=0.438$, $p=0.014$) and NØ ($r=0.509$, $p=0.009$) in HC but not in RA patients. Serum levels of sTACI were negatively associated with DAS28 score ($r=-0.272$, $p=0.006$) in RA. However, sAPRIL was associated with mBLyS expression on mDC in patients with longer disease duration (>3 months) ($r=0.779$, $p<0.001$), but not in those recruited at onset ($r=0.245$, $p=0.361$). In the whole RA group, TNFα serum levels were found to be correlated with sAPRIL ($r=0.499$, $p<0.001$) and sBLyS ($r=0.362$, $p=0.013$). Similarly, IFNα and sAPRIL were positively associated ($r=0.423$, $p<0.001$). TNFα-blockade was associated with decreasing mBLyS expression on B cells, MØ, mDC and NØ (all $p<0.050$) and a slight increase in sTACI ($p=0.064$). Higher levels of mBLyS on MØ and mDC at baseline were associated with a poor clinical response upon TNFα-blockade ($n=8$; $p=0.006$ and $p=0.010$ compared to HC, respectively).
Conclusions: a role for B cell-activating factors in the pathogenesis of early RA is supported. B cells and myeloid populations (MØ, mDC and NØ) can account for the BLyS overexpression in RA, although important differences arise in their involvement. TNFα and IFNα are related to sBLyS and sAPRIL levels. An increased production of the soluble forms of BLyS and APRIL in addition to a less efficient feedback from their decoy receptors may delineate its detrimental effect.
Disclosure of Interest: None declared
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AB0094 MIGRATION, COLONIZATION AND DISTRIBUTION OF BONE MARROW MESENCHYMAL STEM CELLS TRANSPLANTED IN CIA RATS WERE TRACED BY GREEN FLUORESCENT PROTEIN

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Background: The mechanism of mesenchymal stem cells involved in injury and repair in vivo is not fully understood, so it is difficult to evaluate the effect of stem cells on rheumatoid arthritis.
Objectives: To investigate the migration, colonization and distribution of bone marrow mesenchymal stem cells (BM-MSCs) traced by green fluorescent protein (GFP) in the immune organs and joints of CIA rats, and study the mechanism of MSCs in repairing damage.
Methods: 1.MSCs labeled with GFP was cultured, amplified and identified in vitro.
2.Injected with mixture of II type collagen and complete Freund's adjuvant into Wistar rats space at 14 days: It was randomly divided into five groups, including early intervention group (n=20), late intervention group (n=20), CIA early control group (n=12), late control group (n=12) and normal control group (n=12).(2) MSCs were injected from tail veins according to the number of 1×10^7/kg,and the control groups were given equal volume of normal saline. (3) Observe the changes of arthritis index, arthritis swelling degree,and appearance of imageology and pathology.
3.The spleen, thymus, lymph nodes and joint tissues of the rats were made into pathological sections when transplanted at 3, 11, 30 and 42 days.The migration and distribution of the transplanted cells in the immune organs and the inflammatory joints were detected by immunohistochemistry.
Results: 1.(1)The arthritis index and degree of joint swelling in early and later intervention groups were decreased significantly than that of CIA control groups ($P<0.05$). (2)The early intervention group had lower arthritis index and the degree of joint swelling than the later intervention group ($P<0.05$).
2.The positive results of GFP was successfully detected by immunohistochemical method in the immune organs and joints of CIA rats, and sustainable for at least 42 days.
Conclusions: 1.MSCs transplanted through tail vein can migrate to the spleen, lymph nodes and thymus and joints,and can be long-term (42 days) colonization in these organizations.
2.The intervention of MSCs for CIA rats was effective, and early intervention effect was better than advanced intervention.
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AB0095 BY INTRAVENOUS INFUSION MARKED BY GREEN FLUORESCENT PROTEIN TO REVEAL BONE MARROW MESENCHYMAL STEM CELLS' DISTRIBUTION AND DIFFERENTIATION OF COLLAGEN INDUCED ARTHRITIS RATS

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Background: Rheumatoid arthritis (RA) is a autoimmune disease,which is characterized by the osteoclasia or the high deformity rate of cartilage and bone. According to some materials, Mesenchymal stem cells (MSCs) were definited as the cell full of proliferation, differentiation capacity, and potential immune regulation. MSCs transplantation could be a appropriate-designed pattern to the joint damaging from rheumatoid arthritis (RA).However, the repairing mechanism against osteoclasia of cartilage and bone is still unclear.
Objectives: This study used collagen induced arthritis (CIA) rats as animal model to explore MSCs' tissue repairing mechanism.
Methods: We observed the ability of BMSCs differentiating into cartilage cells by toluidine blue staining in vitro;Then,BMSCs were labeled by the green fluorescent protein (GFP), infused into CIA through rats' tail vein infusion. In different point time, the rat's joints were made paraffin section,we observed the differentation of GFP positive cells and the distribution of GFP-positive cells differentiated chondrocytes by immunohistochemicale method.
Results: First,we found BMSCs in vitro can differentiate into cartilage cells under a certain-culture condition. Then, BMSCs were labeled by the green fluorescent protein (GFP), infused into CIA through rats' tail vein infusion. In different point time, the rat's joints were made paraffin section,which the GFP positive cells were observed in synovium and bone marrow tissues after transplantation on the 3th day, and in cartilage tissues on the 11th day, then increased in cartilage tissues on the 30th day,42th day, by laser scanning confocal microscope. Anti-type II collagen, GFP double positive cells were found in articular cartilages (especially damaged part)by Anti-II collagen immunofluorescence technology.

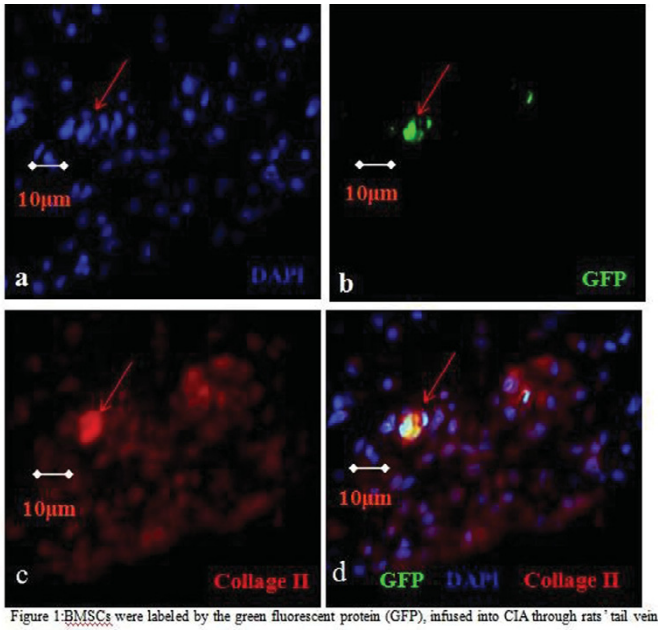

Figure 1:BMSCs were labeled by the green fluorescent protein (GFP), infused into CIA through rats' tail vein infusion. Fluorescence microscopic observation of joint paraffin section. Chondrocyte were stained with an antibody specific to type II collagen and observed by fluorescence microscopy (red fluorescence). Nucles were stained with DAPI (blue fluorescence).GFP-MSCs were observed by fluorescence microscopy (green fluorescence).Anti-type II collagen and GFP double positive cells were found in articular cartilages.

Conclusions: BMSCs were restricted to the joint injury or inflammatory site,differentiated into chondrocytes, and then participated in the cartilage repairing.

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AB0096 EFFICACY AND SAFETY OF ORAL ADMINISTRATION OF PURE CELASTROL IN AIA RATS

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Background: Celastrol, a pentacyclic-triterpene isolated from *Tripterigium wilfordii* roots, has shown great therapeutic potential for the treatment of several inflammatory diseases, including rheumatoid arthritis (RA). We have previously demonstrated that celastrol has significant anti-inflammatory and bone protective effects in the adjuvant-induced rat model of arthritis (AIA), when administered via intraperitoneal route. For further preclinical evaluation of celastrol as a candidate compound for RA treatment, an effective and safe oral administration is crucial.

Objectives: In this work we aimed to study the dose range for both therapeutic and toxic effects for oral administration of pure celastrol using the AIA rat model.

Methods: Celastrol (1, 2.5, 5, 7.5, 12.5 and 25μg/g/day, N=5/group) was administrated orally in female AIA rats after 8 days of disease induction (therapeutic model) for a period of 14-days. A group of healthy (N=8) and untreated arthritic (vehicle, N=15) gender and age-matched Wistar rats were used as control. During the period of treatment, the inflammatory score, ankle perimeter and body weight were measured. At the end of the treatment, animals were sacrificed, blood was collected for clinical pathology, and necropsy was performed, with collection of internal organs for histopathological analysis and of paw samples for disease scoring.

Results: Oral administration of pure celastrol at 2.5, 5 and 7.5μ g/g/day reduced the inflammatory score and ankle swelling, preserved articular joint structure with a reduction in synovial inflammatory infiltrates and proliferation, halted articular bone destruction, and diminished the number of synovial CD68+ macrophages (a biomarker of response to anti-arthritic treatment). This compound also reduced the number of osteoclasts and osteoblasts present in joints. Bone resorption and turnover was also reduced at both 5 and 7.5μg/g/day, with a significant decrease in serum levels of TRACP-5b, P1NP and CTX-I. Of note, no significant variation in body weight, evidence of nephro-, hepato- or cardiotoxic effects, nor alterations in blood cell counts were observed at these concentrations. However, the dose of 7.5μ g/g/day was already associated with thymic and hepatotoxic changes, and higher doses showed toxicity signs. The lethal dose (LD) and LD_{50} were defined as 25μg/g/day and 12.5μg/g/day, respectively. Of note, oral celastrol at 1μg/g/day had no effect in arthritis progression.

Conclusions: Our results clearly show that 2.5μg/g/day is the lowest and 5μg/g/day is the highest effective and safe oral doses of celastrol in the setting of AIA rat model. These findings suggest that while celastrol is potentially very effective to treat RA, it has a narrow therapeutic window.

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AB0097 METHOTREXATE AND LOW DOSE PREDNISOLONE DOWNREGULATE OSTEOCLAST FUNCTION IN MONOCYTES FROM EARLY RHEUMATOID ARTHRITIS PATIENTS

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Background: Rheumatoid arthritis (RA) is a systemic, immune mediated inflammatory disease that is associated with bone erosions and joint destruction. Methotrexate (MTX) slows bone damage but the mechanism by which it acts is still unknown.

Objectives: In this study we aimed to assess the effect of MTX and low dose prednisolone (MTX+PDN) on circulating osteoclast (OC) precursors and OC differentiation in RA patients.

Methods: RA patients before and at least 6 months after MTX therapy were analyzed and compared with healthy donors. A blood sample was collected in order to assess receptor activator of NF-κB (RANK) ligand (RANKL) surface expression on circulating leukocytes and frequency and phenotype of monocyte subpopulations. Serum quantification of bone turnover markers and cytokines and *in vitro* OC differentiation assays were performed.

Results: The number or $RANKL^+$ neutrophils increased in RA patients when compared to healthy donors (p=0.006) and after treatment with MTX+PDN their count was reduced to healthy control numbers (p=0.0155). Classical activation markers of monocytes such as HLA-DR, CD86, CCR2 and CD11b, and also RANK were increased in RA patients at baseline, comparing to control healthy donors. After MTX+PDN exposure, expression decreased to healthy control levels. Serum RANKL levels were increased at baseline comparing to healthy donors (p=0.0164) and normalized after therapy.

Although the number of OC was not different between groups, resorbed area and resorbed area/pit were elevated when compared to controls (p=0.0436 and 0.0249, respectively) and reduced after treatment ($p<0.0001$).

Conclusions: Our results suggest that MTX+PDN play an important role in downregulating OC function, which we believe occurs through a decrease in RANK surface expression in monocytes.

Disclosure of Interest: None declared

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AB0098 EFFECTS OF TOFACITINIB IN EARLY ARTHRITIS BONE LOSS

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Background: Rheumatoid arthritis (RA) causes immune mediated local and systemic bone damage.

Objectives: The main goal of this work was to analyze, how treatment intervention with tofacitinib prevents the early disturbances on bone structure and mechanics in adjuvant induced arthritis rat model. This is the first study to access the impact of tofacitinib on the systemic bone effects of inflammation.

Methods: Fifty Wistar adjuvant-induced arthritis (AIA) rats were randomly housed in experimental groups, as follows: non-arthritic healthy group (N=20), arthritic non-treated (N=20) and 10 animals under tofacitinib treatment. Rats were monitored during 22 days after disease induction for the inflammatory score, ankle perimeter and body weight. Healthy non-arthritic rats were used as controls for comparison. After 22 days of disease progression rats were sacrificed and bone samples were collected for histology, micro-CT, 3-point bending and nanoindentation analysis. Blood samples were also collected for bone turnover markers and systemic cytokine quantification.

Results: At tissue level, measured by nanoindentation, tofacitinib increased bone cortical and trabecular hardness. However, micro-CT and 3-point bending tests revealed that tofacitinib did not revert the effects of arthritis on cortical and trabecular bone structure and on mechanical properties.

Conclusions: Possible reasons for these observations might be related with the mechanism of action of tofacitinib, which leads to direct interactions with bone metabolism, and/or with kinetics of its bone effects that might need longer exposure.

Disclosure of Interest: None declared

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