Myeloid-specific molecular mediators of subchondral bone damage in antigen-induced arthritis

P.C1.08.11

N. Lukač[1,2], M. Fadljević[1], I. Radanović[1], E. Lazić Mosler[4,5], A. Šućur[1,3], D. Flegar[1,3], T. Kelava[1,3], V. Katavić[1,2], D. Grčević[1,3], N. Kovačić[1,2]

[1]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia, [2]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia, [3]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia , [4]General Hospital Dr. Ivo Pedišić, Sisak, Croatia, [5]Catholic University of Croatia, Zagreb, Croatia

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune joint inflammation marked by cartilage and bone destruction, and subsequent permanent disability. Currently available therapeutics improved the prognosis, but still have limited effect on the attenuation and reversal of bone destruction.

Using antigen-induced arthritis (AIA), animal model of RA, we found that mice deficient for Fas gene (Fas -/-) develop non-destructive arthritis, accompanied by lower frequency of myeloid ($CD11b^+Gr1^+$) cells in the synovial compartment.

AIM OF THE STUDY

We aim to identify myeloid-specific molecular mediators of bone-resorption in AIA, by analyzing differentially expressed genes in myeloid population from wild-type (B6) and Fas -/- mice with AIA.

SPECIFIC AIMS:

1. To compare the transcriptome of sorted synovial $CD11b^+Gr1^+$ cells in mice with destructive arthritis (B6 AIA) with their transcriptome in mice with non-destructive arthritis (Fas -/- AIA)
2. To determine differentially expressed genes and confirm changes in expression of chosen genes
3. To functionally evaluate selected differentially expressed genes

MATERIALS AND METHODS

12 wk C57BL6 (B6) and Fas-/- mice

day 0

200 μg mBSA in CFA, subcutaneously (s.c.) (**AIA & ctrl**)

PBS, s.c. (**NI**)

day 7

100 μg mBSA in CFA, s.c. (**AIA & ctrl**)

PBS, s.c.(**NI**)

day 21

50 μg mBSA in 10 μL PBS, intra-articularly (i.a., **AIA**)

10 μLPBS i.a. (**ctrl, NI**)

day 31

μCT
Epiphyseal trabecular bone (BV/TV, Tb.Th., Tb.Sep., Tb.N.)

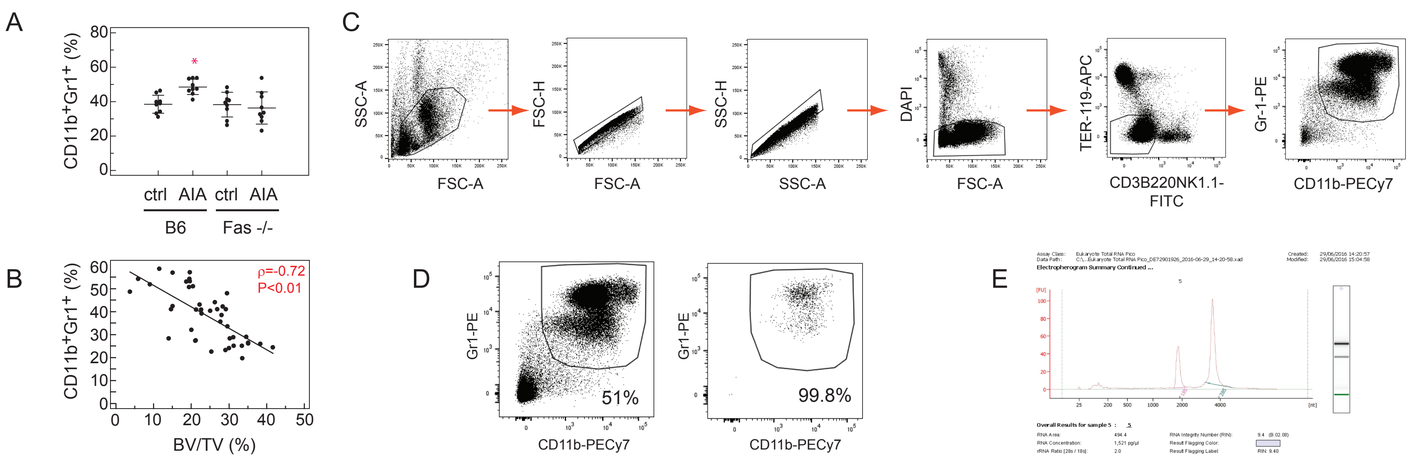
Flow cytomery and sorting
Anti-mouse CD45-FITC, CD11b-PE, Gr1-PECy7, B220/CD3/NK1.1/CD31/TER119-APC, CD51-APCeF780

Affymetrix GeneChip Mouse Gene ST 2.0 Array - synovial myeloid ($CD11b^+Gr1^+$) cells
Bioinformatic analysis: Bioconductor (*oligo, limma* package), ToppGene (*ToppFun* tool)

qPCR
$CD11b^+Gr1^+$, bulk joint tissue

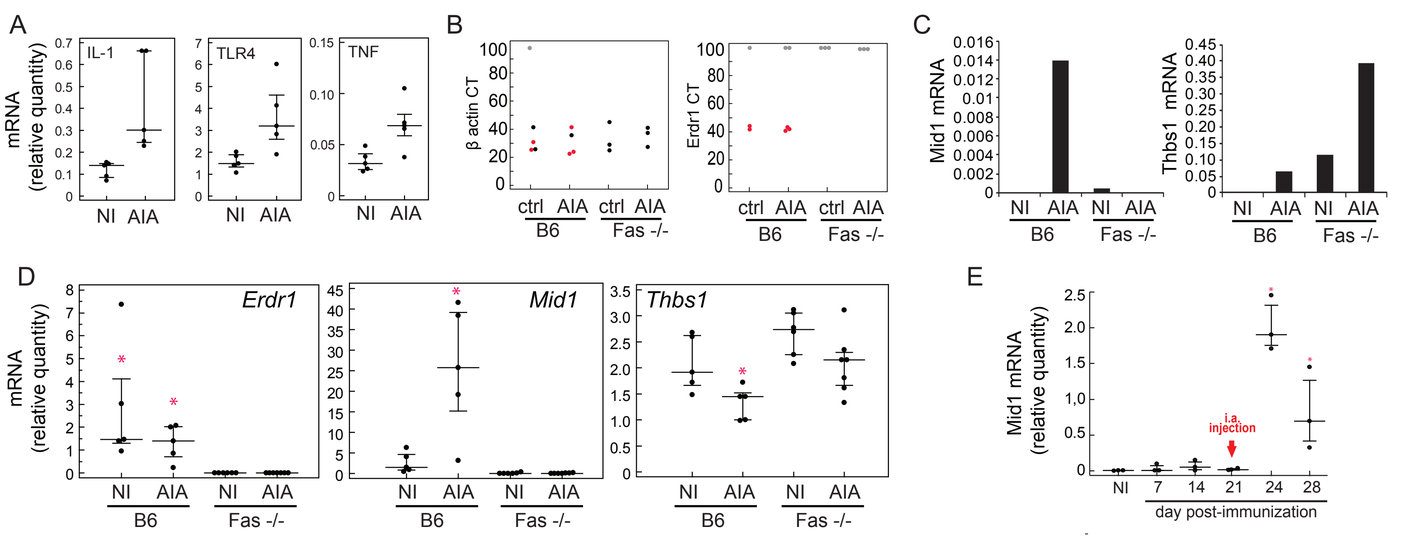
RESULTS

Analysis and sorting of synovial myeloid cells



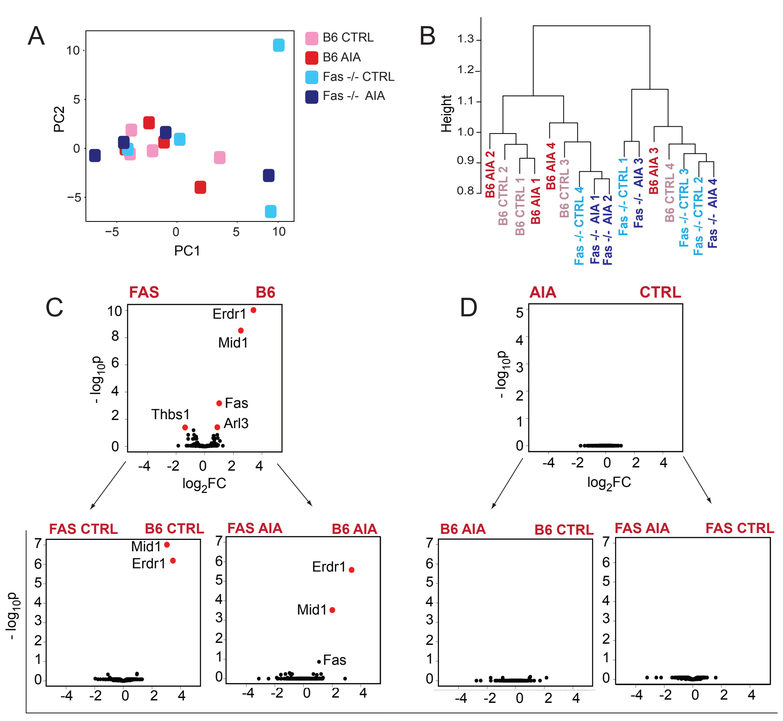
Proportion of myeloid ($CD11b^+Gr1^+$) cells (**A**) was upregulated in the synovial compartment of arthritic (AIA) wild-type (B6) mice in comparison to control (ctrl) mice, while this upregulation was absent in Fas deficient (Fas -/-) mice with arthritis (AIA). Proportion of synovial myeloid cells was negatively associated with femoral epiphyseal trabecular bone volume (BV/TV,%), rank correlation (**B**). For flow cytometry analysis, cells were released by collagenase, flushed from the synovial cavity and analyzed by flow cytometry. Pure population of synovial myeloid cells was prepared by fluorescence-activated cell sorting (FACS) of single, live ($DAPI^-$), non-erythroid ($TER119^+$), non-lymphoid (CD3-B220-NK1.1-) $CD11b^+Gr1^+$ cells (**C**). Purity of sorted population was confirmed by re-analysis of sorted sample (**D**). Sorted cells were further used for RNA extraction and gene expression analysis. RNA integrity was estimated by RNA Pico Chip, and samples with satisfactory integrity (**E**) were selected for gene expression analysis. * $p<0.05$, Kruskal-Wallis test.

Validation of differentially expressed genes by PCR (*Mid1, Thbs1* and *Erdr1*)

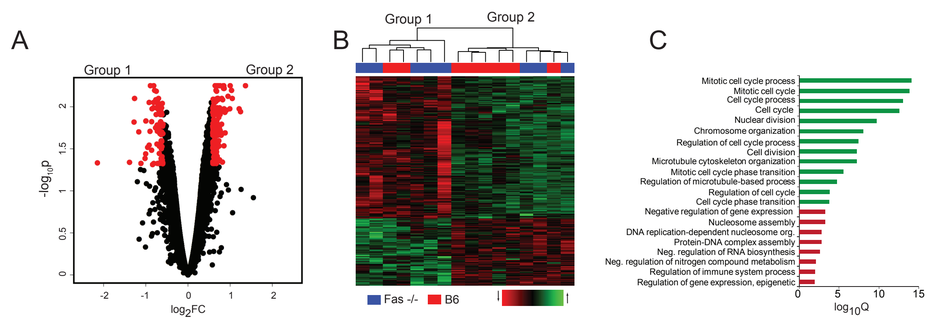


Differential gene expression analysis revealed similar expression patterns of immunised control mice and mice with arthritis (AIA), suggesting that myeloid cells are stimulated by immunization. We could confirm clear upregulation of inflammation-related genes in AIA vs. NI joint tissue (**A**), so we further used non-immunized (NI) mice as validation control. First we attempted to validate differentially expressed genes in the individual samples from sorted cells which were not used for arrays (**B**). In this set of samples, housekeeping gene (*β-actin*) was detected in all but one B6 AIA sample, whereas *Erdr1* was detected in 6 out of 8 B6 samples, and none of Fas -/- samples. Due to requirement of higher amount of total RNA for PCR analysis, in the second set, we pooled samples of minimum 3 mice for myeloid cell isolation (**C**) and confirmed substantial expression of *Mid1* gene in samples from B6 AIA mice, with traces or non-detectable transcripts in myeloid cells from B6 NI mice, and both groups of Fas -/- mice. Thrombospondin 1 (*Thbs1*) gene was upregulated in synovial myeloid cells from Fas -/- AIA mice. Since myeloid cells are the most abundant cell population detected in arthritic joints we aimed to establish how it alters the overall expression in joint tissue (**D**). We established clear upregulation of *Mid1*, and downregulation of *Thbs1* in B6 AIA mice. Over different time points during arthritis induction appearence of *Mid1* was detected already during immunisation, with significant upregulation after induction of arthritis (**E**). Gene expression was analyzed by qPCR, values are calculated according to the standard curve of gene expression in the callibrator sample and normalized to expression value of *β-actin*. In **A**, amplification is indicated by a cycle treshold (CT) values. * statistically significant difference, $p<0.05$, Kruskal-Wallis test

Gene expresssion pattern of synovial myeloid population in resorptive and non-resorptive arthritis



Differential gene expression in synovial myeloid cells. Principal cmponent analysis (PCA) did not reveal grouping according to expression pattern of all genes (**A**). Hierarchical clustering assigned samples into B6 and Fas-/- dominant clusters, although 3 samples from Fas-/- mice clustered with B6 and 2 samples from B6 mice clustered with Fas-/- (**B**). Consistent and significant upregulation of midline 1 (*Mid1)* and erythroid differentiation regulator (*Erdr1)* genes was detected in B6 samples (**C**).There were no differentially expressed genes between control immuized (CTRL) group of both B6 and Fas -/- mice and mice with arthritis (AIA, **D**), pointing to potential changes in expression pattern induced by immunisation. Analysis was performed after hybridizing total RNA extracted from isolated myeloid cells to Affymetrix Mouse Gene ST 2.0 Arrays, and measuring signal intensities representing gene expression magnitude in Bioconductor. Differentially expressed genes are depicted on volcano plots, showing the logarithmic value of fold change in gene expression (log_2FC) relative to the negative logarithm of p value ($-log_{10}p$). Black dots represent a single gene, and differentially expressed genes according to their fold change and p value (<0.05, Benjamini-Hochberg adjustment for multiple hypothesis testig) are marked red.



Differential gene expression analysis in two hierarchical clusters. Between Fas-/- (group 1) and B6 (group 2) dominant groups (FC >1.5, p <0.05 (Benjamini-Hochberg)) we identified 181 upregulated and 81 downregulated genes marked red in the volcano plot (A), and presented by the heatmap (B). According to gene set enrichment analyses (GSEA), B6-dominant group is characterized by higher expression of genes involved in cell cycle progression and mitotic activity (**C**).

CONCLUSIONS

- Resorptive AIA is characterised by increased frequency of synovial myeloid ($CD11b^+Gr1^+$) cells.
- Synovial $CD11b^+Gr1^+$ cells from mice with resorptive arthritis express more *Mid1* and *Erdr1* genes, and less *Thbs1* gene.
- Inflammatory response in resorptive AIA is marked by higher myeloid proliferation potential.
- Mid1 gene is a potential novel mediator for inflammation-mediated joint destruction in arthritis since it is clearly upregulated by induction of arthritis.
- Activation of Mid1 gene has already been reported in allergic airway inflammation, and dependent on death receptor TRAIL.

This work is supported by Croatian science foundation grant #7406

ECI 2018 attendance was supported by EFIS