Center of Experimental Rheumatology

Modic change bone marrow neutrophils are activated and activated neutrophils degrade cartilage endplates

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Background

What are Modic type 1 changes (MC1)?

- Painful vertebral bone marrow lesions
- Visualized as signal intensity changes on magnetic resonance images
- MC1 occur adjacent to degenerated intervertebral discs at locations where cartilage endplates (CEPs) are damaged

What we know about the MC1 pathobiology:

- Key hallmark: Bone marrow inflammation
- Neutrophilic infiltrates and dysregulated granulopoiesis are indicative for neutrophil involvement in MC1

What we do not know:

1. If MC1 derived bone marrow neutrophils
   - are activated
   - release enzymes that potentially damage CEPs
2. If releases of activated blood neutrophils are able to degrade CEPs

Relevance to clarify the role of neutrophils in inflammatory MC1 processes

- Treatments that target MC1 pathomechanisms are not available
- MC1 bone marrow neutrophils are potential treatment targets

Objectives

1) To investigate if MC1 bone marrow neutrophils are activated
2) To measure neutrophil elastase activity – an enzyme able to degrade articular cartilage - in the supernatant of MC1 bone marrow neutrophils
3) To discover if the supernatant of activated blood neutrophils degrades CEPs

MC1 bone marrow neutrophil characterization

Bone marrow neutrophil isolation:

- Low back pain patients with MC1 undergoing lumbar spondylodesis
- From each patient: One MC1 and one intra-patient control aspirate was collected (**Figure 1**)

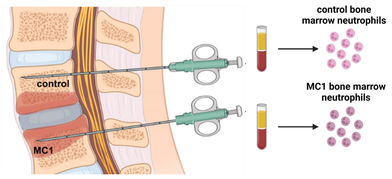

Figure 1: Sample collection and neutrophil isolation

1. Transcriptomic analysis (RNA sequencing)

- Isolation of CD45^{+}CD66b^{+} bone marrow neutrophils by cell sorting
- Bulk RNA sequencing (n=7+7)
- Identification of differentially expressed genes (DEGs) (p<0.01)
- Overrepresentation analysis (ORA) (significant if false discover rate (FDR) <0.05)

2. Neutrophil activation/maturation analysis (flow cytometry)

- CD66b intensity (activation marker) (n=9+9)
- Fractions of immature band neutrophils (CD66b+CD10-CD11b^{high}) (n=7+7)
- Paired t-test to compare median fluorescence intensity (MFI) and fractions of immature neutrophils between MC1 and controls

3. Neutrophil elastase activity

- MC1 and control bone marrow neutrophil isolation (n=5+5): EasySep™ Human Neutrophil Isolation Kit (STEMCELL Technologies)
- 3h cultivation (25 mio neutrophils / ml) at 37°C
- Neutrophil elastase activity assessment: Fluorogenic neutrophil elastase substrate
- Relative activity (MC1 vs. control): Tested against null hypothesis (μ0=100%) using a one sample t-test.
- P-values<0.05 were considered as statistically significant.

CEP damage model with activated blood neutrophils

- Collection of three lumbar circular CEP biopsies per patient (patients n=6)
- CEP biopsies were halved and exposed to activated (100nM PMA, 3h) and non-activated neutrophil supernatant of different concentrations (25 mio/ml vs. 12.5 mio/ml) of one blood donor
- CEP tissue released sulphated glycosaminoglycan (sGAG) was assayed using dimethylmethylene blue assay
- Release from half-biopsy specific control was set to 100 % and tested against null hypothesis (μ=100%) (**Figure 2**)

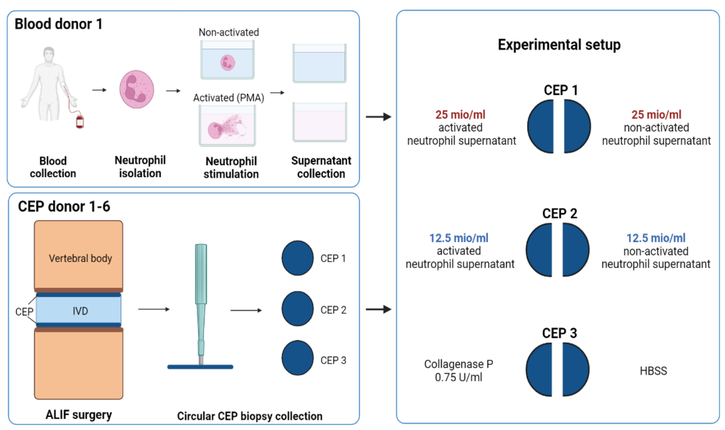

Figure 2. Neutrophil-mediated CEP damage model.

MC1 bone marrow neutrophils have higher CD66b activation marker expression and increased fractions of pro-inflammatory band neutrophils

MC1 bone marrow neutrophils:

- Significantly higher CD66b MFI on MC bone marrow neutrophils (ΔMC1-control = 1475 MFI ± 1801, p=0.03) (**Figure 4A**)

→ supporting neutrophil activation in MC1

- In six out of seven patients: Increased proportions of immature pro-inflammatory band neutrophils: (ΔMC1-control = 6.69 % ± 8.00%, p=0.07) (**Figure 4B**)

→ Immature band neutrophils expand under inflammatory conditions

→ Indicates an increased demand for neutrophils in the MC1 bone marrow

→ Suggests a role in inflammatory MC1 pathomechanisms

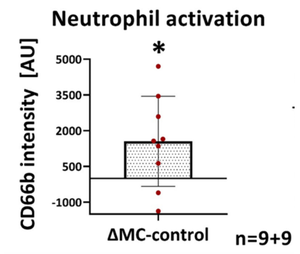

Figure 4: CD66b intensity as measure of neutrophil activation (left) and fractions of immature band neutrophils (right) in MC bone marrow identified by flow cytometry.

MC1 bone marrow neutrophils release more neutrophil elastase

MC1 bone marrow neutrophils:

- Neutrophils expand in joints of patients with rheumatoid arthritis and promote articular cartilage damage mediated by neutrophil elastase
- Higher neutrophil elastase activity in four out of five MC1 bone marrow neutrophil supernatants 212.9%±148.0, p=0.16) (**Figure 5**),

→ indicating increased production of an enzyme that may damages CEPs

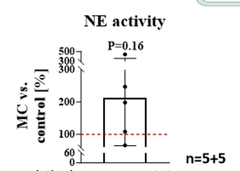

Figure 5: Neutrophil elastase activity measured with fluorgenic neutrophil elastase substrate.

Activated blood neutrophils degrade human cartilage endplates

Significant release of sGAG from the CEP tissues in a dose-dependent manner:

- 25 mio neutrophils/ml: 380.1% ± 177, p = 0.01
- 12.5 mio / ml: 123.7% ± 22.3, p = 0.05
- Dose-dependent effect: Tukey Post Hoc test: 25 mio/ml vs. 12.5 mio/ml: p = 0.02 (**Figure 6**)

→ Activated blood neutrophils degrade CEPs

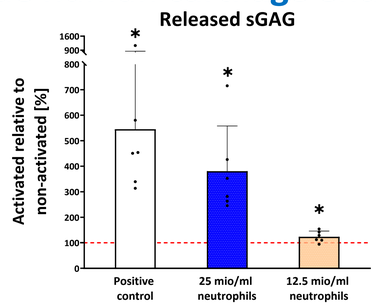

Figure 6: Relative sGAG released fro CEPs exposed to collagenase P (white bar) and activated neutrophil supernatants (blue: 25 mio/ml neutrophils; yellow: 12.5 mio/ml neutrophils).

MC1 bone marrow neutrophils have a pro-inflammatory transcriptome

MC1 bone marrow neutrophils:

- Enriched inflammatory / activation associated pathways associated (**Figure 3**)
- Upregulated calcium associated processes
  - Calcium ion transmembrane transport (FDR<0.001)
  - Positive regulation of cytosolic calcium ion concentration" (FDR<0.001)

→ suggesting increased neutrophil degranulation

→ a feature of activated neutrophils

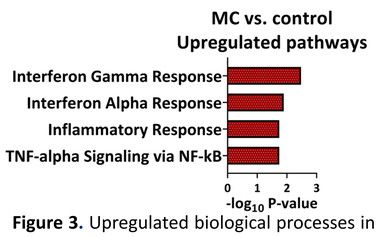

Figure 3. Upregulated biological processes in MC1 bone marrow neutrophils

Summary and discussion

- MC bone marrow neutrophils are activated and release more neutrophil elastase
- Activated blood neutrophils degrade CEPs
- plausible that inflammatory MC1 pathomechanisms include a vicious cycle of neutrophil activation and CEP damaging
- Whether MC1 neutrophil supernatant leads to increased sGAG release in vitro remains to be elucidated
- CEP damage promotes a pro-inflammatory disc/bone marrow crosstalk in MC1 and coincides with increased nerve fiber density.

Relevance

These findings could have implications for treatment strategies to mitigate inflammation and CEP damage in MC1

References: [1]Heggli I et al (2023). Annuals of the Rheumatic Diseases. 2 Dudli S et al. (2017). European Spine Journal.

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