Center of Experimental Rheumatology

Activated neutrophils degrade cartilage endplates

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Introduction

Modic changes (MC):

- Painful vertebral bone marrow lesions that occur around a degenerated intervertebral disc (IVD) and colocalize with endplate damage.
- **Signs for neutrophil contribution in MC[1,2]:** Granulation tissue at bone-disc junction and dysregulated neutrophil maturation in MC bone marrow

In rheumatoid arthritis: **Activated neutrophils** mediate articular **cartilage**[3]

Little **is known about the role of neutrophils in Modic changes and disc tissue damage**

Aims

1. **To show that MC1 bone marrow neutrophils are activated**
2. **To discover the effects of activated blood neutrophils on cartilage endplate (CEP) composition**

Methods

MC1 bone marrow derived neutrophil activation assessment

- Isolation of MC1 and intra-patient control bone marrow neutrophils from patients undergoing spinal fusion (n = 5+5)
- RNA isolation, sequencing, bioinformatic overrepresentation analysis (ORA) and gene set enrichment analysis (GSEA)
- Measurement of the neutrophil activation marker CD66b with flow cytometry (n =3+3) (Figure 1)

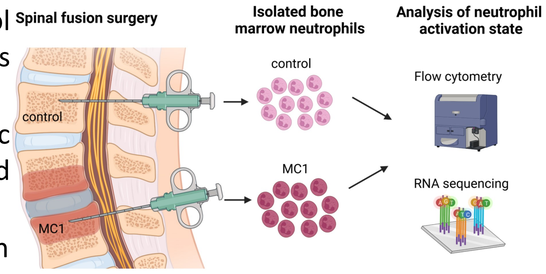

***Figure 1**. Bone marrow neutrophil collection and analysis.*

Neutrophil-mediated cartilage endplate (CEP) damage model

- Collection of 3 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 one donor
- CEP tissue released sulphated glycosaminoglycan (sGAG) and hydroxyproline (as collagen release measure) were assayed using dimethylmethylene blue and chloramine-T assay (release from half-biopsy specific control was set to 100 %) (Figure 2)

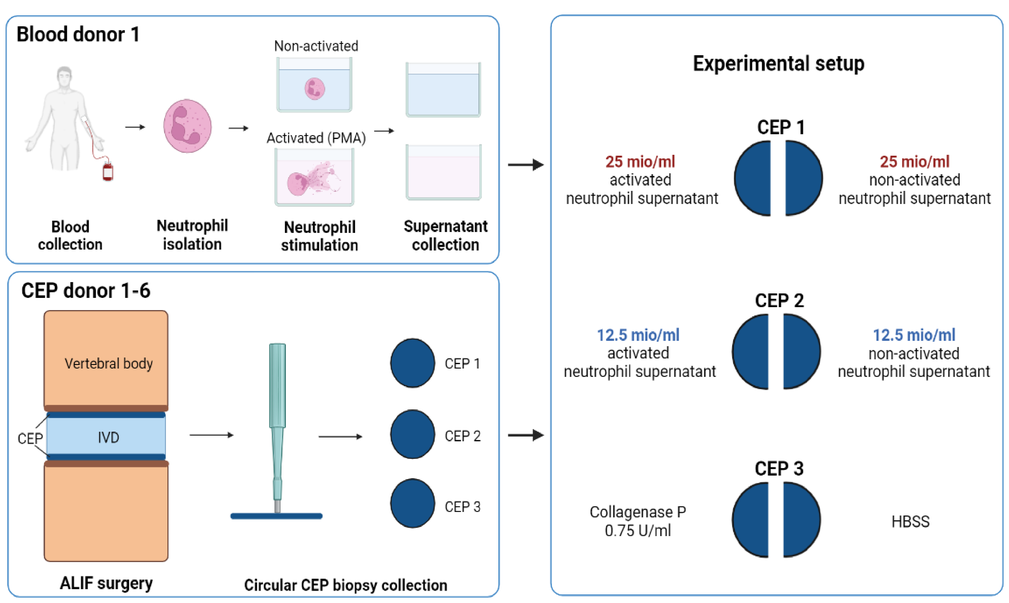

***Figure 2**. Neutrophil-mediated cartilage endplate (CEP) damage model.*

Results

1. MC1 bone marrow neutrophils have an activated pro-inflammatory phenotype

RNA sequencing:

- **185 differentially expressed genes** between MC1 and control bone marrow neutrophils
- **ORA: Top 5 upregulated gene ontology (GOs) in MC1:** All related to neutrophil activation (Figure 3a).
- **GSEA: Top enriched hallmark gene sets in MC1:** "inflammatory response" (p=0.001, normalized enrichment score (NES) =1.6) (Figure 3b), "IFN-α response (p=0.0, NES=2.2), and IFN-γ response (p=0.0, NES=1.9)

Flow cytometric analysis of CD66b expression:

- Significantly more activated neutrophils (measured as CD66^{high} expression) in MC1 (control: 43.1 % ± 15.7, MC1: 54.1 % ± 16.7, p=0.018) (Figure 3c).

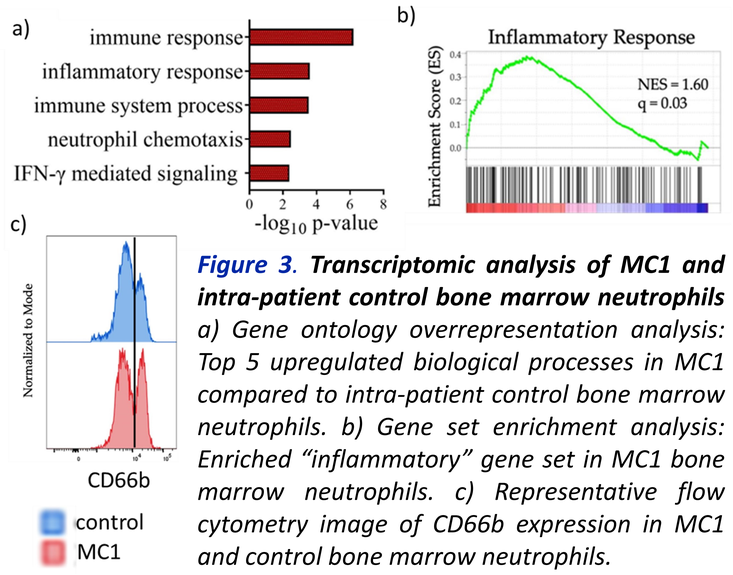

***Figure 3**. Transcriptomic analysis of MC1 and intra-patient control bone marrow neutrophils a) Gene ontology overrepresentation analysis: Top 5 upregulated biological processes in MC1 compared to intra-patient control bone marrow neutrophils. b) Gene set enrichment analysis: Enriched "inflammatory" gene set in MC1 bone marrow neutrophils. c) Representative flow cytometry image of CD66b expression in MC1 and control bone marrow neutrophils.*

2. Activated blood neutrophils degrade human CEPs

Exposure of CEP tissues to conditioned medium from activated neutrophils:

- **Significant increased sGAG release from CEP tissues in a dose-dependent manner** (Figure 4)
  - **25 mio/ml**: 380.1 % ± 177, p = 0.012;
  - **12.5 mio/ml**: 123.7 % ± 22.3, p = 0.048
  - Relative sGAG release: **3.1-fold higher** in the CEPs exposed to supernatant from 25 mio/ml neutrophils compared to 12.5 mio/ml neutrophils (25 vs. 12.5 mio/ml: p = 0.022)
- No significant effect of neutrophil supernatant on hydroxyproline release

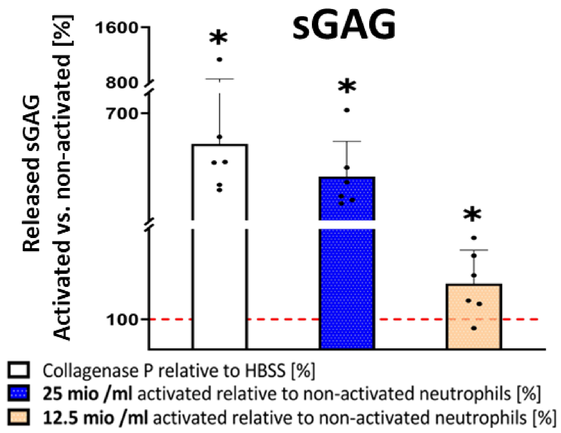

***Figure 4**. Neutrophil-mediated CEP damage model. Relative sGAG released from CEP tissues exposed to positive control (white bars) or neutrophil supernatant from 25 mio/ml (blue) and 12.5 mio /ml blood neutrophils brown). sGAG release from half-biopsy specific control was set to 100 %. *P < 0.01.*

Discussion & Conclusion

Implications

Activated MC1 bone marrow neutrophils may promote and exacerbate CEP damage, thereby **facilitating** enhanced **inflammatory disc/marrow crosstalk in MC1**.

CEP damage may also **increase nerve fibre density**.

These findings reveal a **potential novel MC1 pathomechanism** and could have implications for **treatment strategies** to **mitigate CEP damage in MC1**.

- **MC1 bone marrow neutrophils** have an **activated phenotype**
- Activated peripheral **blood neutrophils degrade proteoglycans** from CEPs
- **sGAG released** from **CEPs** exposed to **activated neutrophil** supernatant for only 18 hours is **similar to** the amount of **sGAG lost *in vivo* over 20 years** of natural ageing
- Whether activated MC1 bone marrow neutrophils can degrade CEP tissue remains to be elucidated

References: [1]*Modic et al., Radiology, 1988* [2]*Dudli et al., Eur Spine J, 2017* [3]*Carmona-Rivera et al., JCI Insight, 2020*

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