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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)

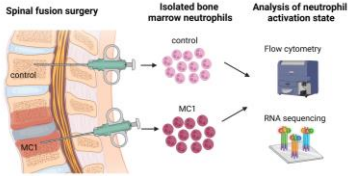

Figure 1. Bone marrow neutrophil collection and analysis.

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)

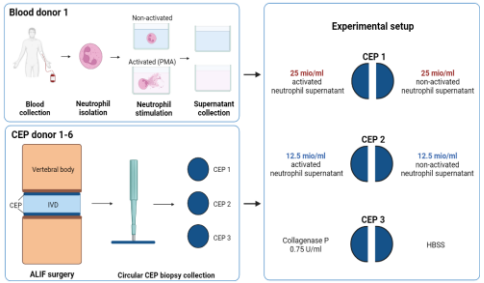

Figure 2. Neutrophil-mediated cartilage endplate (CEP) damage model.

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

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).

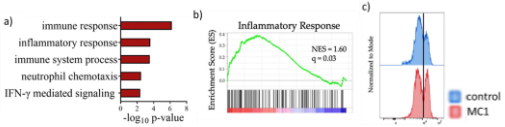

Figure 3. Transcriptomic analysis of MC1 and intra-patient control bone marrow neutrophils a) Gene ontology overrepresentation analysis: Top 5 upregulated biological processes in MC1 compared to intra-patient control bone marrow neutrophils. b) Gene set enrichment analysis: Enriched "inflammatory" gene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

Discussion

- **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

2. Activated blood neutrophils degrade human cartilage endplates (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

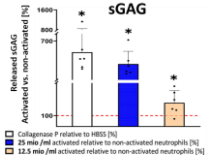

*Figure 4. Neutrophil-mediated CEP damage model. Relative sGAG released from CEP tissues exposed to positive control (white bars) or neutrophil supernatant from 25 mio/ml (blue) and 12.5 mio /ml blood neutrophils brown). sGAG release from half-biopsy specific control was set to 100 %. *P < 0.01.*

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**.

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