Center of Experimental Rheumatology

Activated neutrophils degrade cartilage endplates

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INTRODUCTION

Modic changes (MC) are painful vertebral endplate bone marrow (BM) lesions that occur around a degenerated intervertebral disc and colocalize with endplate damage. Neutrophils are activated in MC BM. Activated neutrophils mediate articular cartilage damage in rheumatoid arthritis. However, little is known about the role of neutrophils in disc tissue damage.

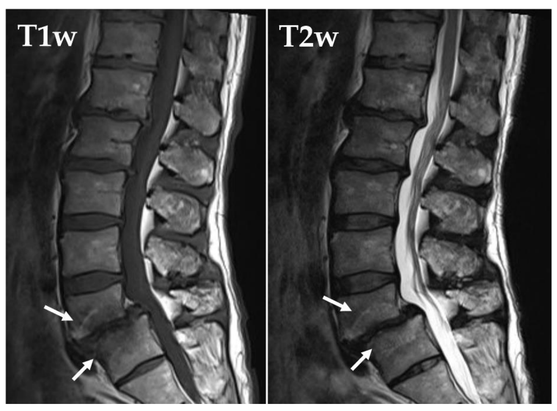


***Figure 1**. Modic changes on T1-weighted and T2-weighted magnet resonance images (MRI). White arrow indicate Modic lesion.*

AIM

The aim of this study was to discover the effects of activated neutrophils on cartilage endplate (CEP) composition.

METHODS

Circular 4 mm CEP biopsies from L4/5 and L5/S1 (n=6) were collected from patients undergoing anterior lumbar interbody fusion surgery at the University of California San Francisco (Figure 2).

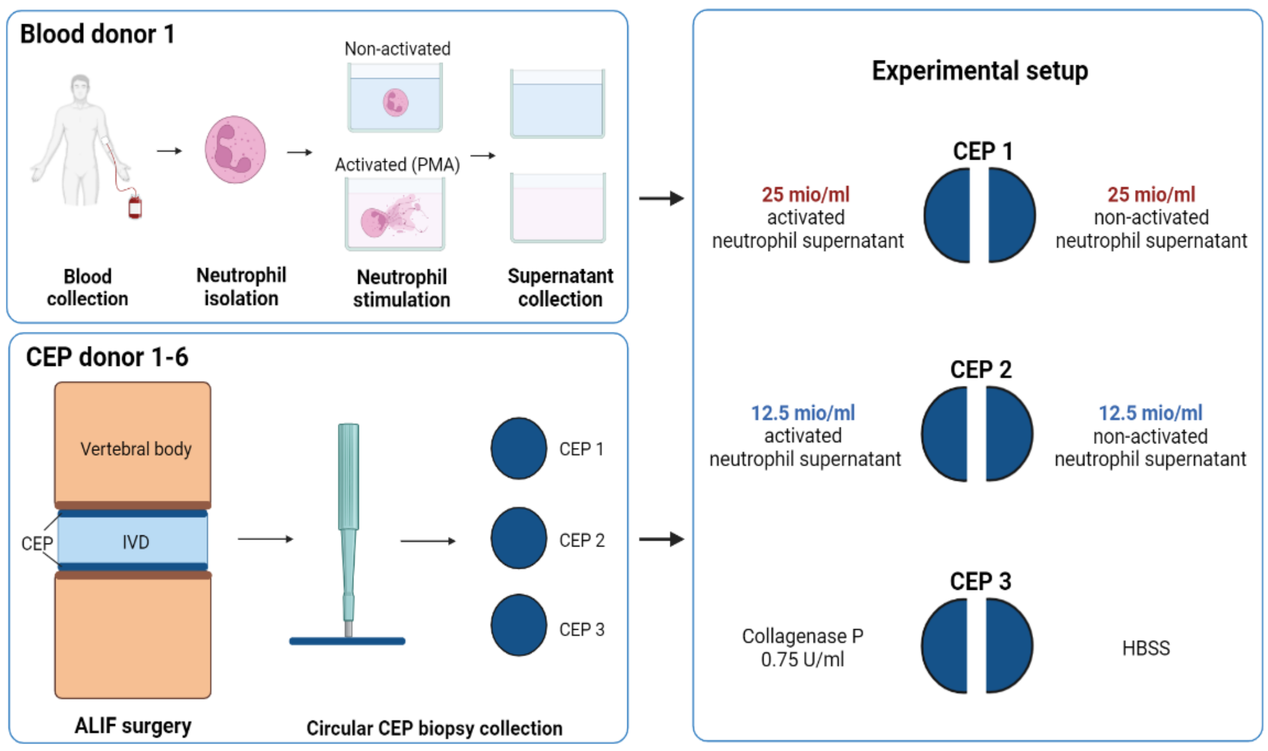


***Figure 2**. Sample collection and experimental design.*

From each donor, 3 CEP biopsies were halved. One half-biopsy from each pair was exposed to either: 1) 0.75 U/ml collagenase P (positive control); 2) 25 mio/ml activated (100nM PMA, 3h, 37°C) neutrophil supernatant; or 3) 12.5 mio/ml activated neutrophil supernatant. The other half-biopsy from each pair was used as control and exposed to: 1) HBSS buffer; 2) 25 mio/ml non-activated neutrophil supernatant; or 3) 12.5 mio/ml non-activated neutrophil supernatant. Exposure supernatant and CEP tissues were collected after 18h and assayed for sulphated glycosaminoglycans (sGAG) and hydroxyproline using a dimethylmethylene blue and chloramine-T assay. Relative sGAG / hydroxy-proline release from the biopsies were determined by normalizing sGAG / hydroxyproline release from the half-biopsy exposed to activated neutrophil supernatants to that released from the half-biopsy exposed to the biopsy-specific control (100%). Relative release was tested against null hypothesis (μ0=100%) using a one sample t-test. P-values<0.05 were considered statistically significant.

RESULTS

Exposure of CEP tissues to neutrophil supernatants caused significant release of sGAG from the CEP tissues in a dose-dependent manner (25 mio/ml: 380.1% ± 177, p=0.012; 12.5 mio/ml: 123.7 % ± 22.3, p=0.048, positive control: 545.0 % ± 302.8, p=0.016) (Figure 3).

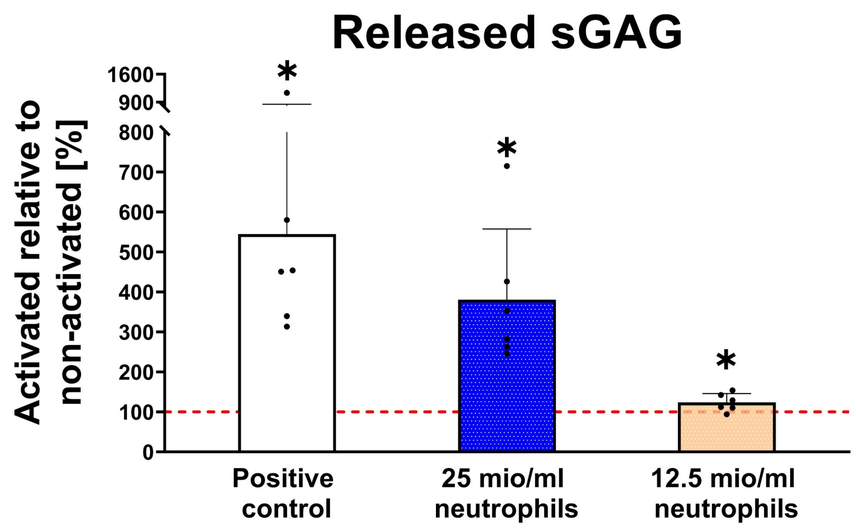
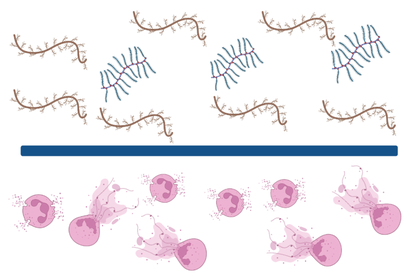


***Figure 3**. Relative sGAG released from CEP tissues exposed to collagenase P (white bar) or neutrophil supernatants (blue/yellow bar). Relative sGAG release was compared with one sample t-test. *P<0.05, CEP donors: n=6. Relative sGAG release was 3.1-fold higher in CEPs exposed to supernatant from 25mio/ml neutrophils compared to 12.5mio/ml (P=0.022).*

In contrast, there was no significant effect of neutrophil supernatant on hydroxyproline release (25 mio/ml: 162 % ± 90.74, p=0.155; 12.5 mio/ml: 110.1 % ± 117.9, p=0.842; positive control: 2536 % ± 1321, p=0.006) (not shown).

DISCUSSION&CONCLUSION

- Establishment of a **neutrophil-mediated CEP damage model**
- CEP exposure to activated neutrophil supernatant leads to a **significant loss of proteoglycans**.



- **Relative sGAG release** following exposure to **activated neutrophil** supernatant for only 18 hours is similar to relative **sGAG lost *in-vivo* over 20 years of natural ageing**.

→ **Activated neutrophils** might exacerbate **CEP damage** present in **Modic changes**

IMPLICATIONS

CEP damage

- **is** a facilitator of **enhanced inflammatory disc/marrow crosstalk** and
- coincides with **increased nerve fibre density**.

→ These findings could have implications for **treatment strategies** to **mitigate CEP damage in MC.**

Targeted treatment for Modic changes

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