

Rapid release of high mobility group box protein-1 (HMGB-1) in transient arthritis

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Recently, extracellularly released high mobility group box protein (HMGB)-1 was shown to play an important regulatory and pro-inflammatory role in inflamed joint tissues (1, 2). Once released from articular cells (1, 3), extracellular HMGB-1 may act as a master cytokine, causing the release of TNF- α , IL-1 β and IL-6 from macrophages and synovial lining cells (4-6), while vice versa, TNF- α and IL-1 β may themselves induce HMGB-1 release (7, 8). Little remains known about the relative timing of local HMGB-1 release at the onset of acute joint inflammation, and we sought to investigate these response kinetics.

For this purpose, samples were obtained from a previous study assessing the effects of inflammation on synovial fluid (SF) biomarkers (9). In short, after approval by the Utrecht University Ethics Committee for Animal Experimentation, sterile transient joint inflammation was induced in the intercarpal joint of six healthy horses by injection of 0.5 ng lipopolysaccharide (LPS). Synovial fluid and plasma samples were obtained just prior to LPS injection at post-injection hour (PIH) 0, and at PIH 8, 24 and 168.

Aliquots (10 μ l) of SF and plasma were analysed using a commercially available HMGB-1 ELISA (HMGB-1 ELISA kit II, Shino-test corporation, Kanagawa, Japan) that cross-reacts with equine HMGB-1 (T. Heinola, unpublished data). TNF- α was determined by means of a sandwich ELISA (Equine TNF- α screening set, Endogen corporation, Rockford, IL, USA). Synovial fluid was digested with hyaluronidase (0.05 mg/ml, 15 mins at 37°C) and diluted 4x but still exhibited matrix interference (non-parallelism); hence standard curves were constructed in pooled normal SF. One-way repeated measures ANOVA was used for all sample comparisons (with $p < 0.05$).

As previously reported, intra-articular LPS injection caused transient severe arthritis (SF WBC: $215.4 \pm 15.9 \times 10^9$ at PIH 8 and $64.6 \pm 6.7 \times 10^9$ at PIH 24, mean \pm SEM), with all clinical and SF parameters returning to normal baseline values at PIH 168 (9). Synovial fluid HMGB-1 was significantly elevated at PIH 8 and 24 ($p < 0.0001$) compared to baseline and PIH 168 (Fig. 1A), while plasma levels remained below the detection limit (< 2.5 ng/ml) throughout (Fig. 1B). Only 2 horses had detectable SF TNF- α concentration at PIH 8, and a single horse accounted for 3 out of 4 positive SF samples (Fig. 1C). This horse was also an outlier with respect to plasma TNF- α concentration (Fig. 1D).

These data reveal a surprisingly rapid release of HMGB-1 into synovial fluid at the onset of acute joint inflammation, with peak

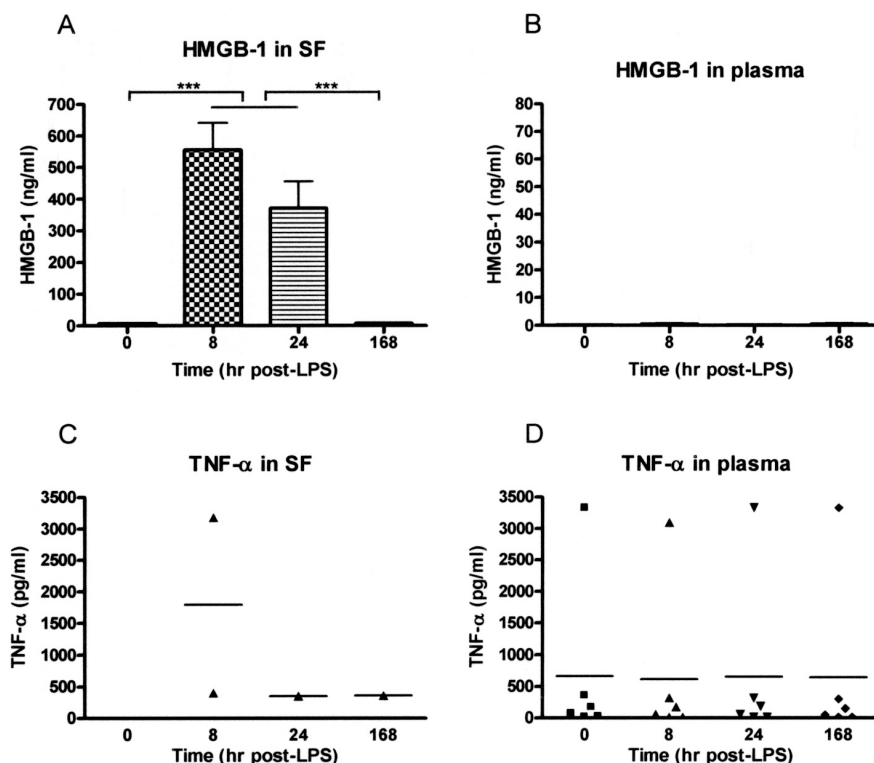


Fig. 1. Mean (\pm SEM) concentrations of high mobility group box protein (HMGB)-1 in synovial fluid (A) and plasma (B), and TNF- α concentrations (mean and scatter) in synovial fluid (C) and plasma (D). Transient arthritis was induced in the intercarpal joint of horses ($n=6$) at $t=0$ hr by IA injection of 0.5 ng *E.Coli* LPS. *** $p < 0.0001$ compared to baseline ($t=0$) and $t=168$.

concentrations already measured at 8 hours post-LPS injection. At this time, SF TNF- α concentration was low in all but 1 horse, and any surge is likely to have preceded this time point, peak SF TNF- α levels having been reported at PIH 2 in this model (10) followed by IL-1 β peak at PIH 6. Previous reports on HMGB-1 release from cultured macrophages in response to endotoxin, TNF- α or other pro-inflammatory cytokines suggested HMGB-1 to be a late responder, with release shown at 18-24 hrs post-stimulation (4, 7, 8), as opposed to IL-1 β and TNF- α , which are produced within the first few hours after exposure to endotoxin (3). The timing of local HMGB-1 release in the current work is however compatible with preformed HMGB-1 being available within the joint tissues for rapid mobilisation following stimulation (7), either directly by LPS or indirectly by TNF- α . Our findings thus suggest that in intact joints *in vivo*, timing of extracellular HMGB-1 release may be less temporally distinct from that of the rapidly mobilised cytokines like IL-1 β and TNF- α . Future studies, including even earlier sampling time points, will have to resolve the exact order of appearance of this triad of cytokines in arthritis.

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