A94 INTERLEUKIN 1β AND TLR LIGANDS GIVE ENHANCED CYTOKINE PRODUCTION BY THEIR INTERACTION WITH HMGB1

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Background The proinflammatory protein HMGB1 was recently identified as a mediator of rheumatoid arthritis (RA) and of other inflammatory diseases. This is demonstrated by extracellular HMGB1 expression both in experimental models of arthritis and in synovial biopsies from patients with RA. Furthermore, intra-articular injection of HMGB1 causes destructive synovitis in mice and HMGB1-targeted treatment ameliorates experimental arthritis. Recent data have indicated that HMGB1 alone does not have proinflammatory properties while complexes of HMGB1 and the TLR ligands CpG DNA and lipopolysaccharide (LPS), as well as interleukin 1 (IL1) and nucleosomes, have enhanced cytokine-inducing abilities compared with the ligands alone.

Aim To study the mechanisms of HMGB1 complexes and their effect on the proinflammatory cytokines found in RA. Furthermore, to answer the question whenther the proposed receptors for HMGB1, RAGE, TLR2 and TLR4 mediate the complex signalling.

Methods HMGB1 and low doses of TLR4 ligand (LPS), TLR9 ligand (CpG DNA), TLR2 ligand (Pam3CSK4) or IL1β were incubated together at 4°C overnight. Human PBMCs, synovial fibroblasts from patients with RA (RASF) and osteoarthritis (OASF) or peritoneal mouse macrophages were stimulated with complexes overnight and cytokine levels measured in the supernatants by ELISA or CBA. Inhibitors were added 1–2<h before stimulation.

Results HMGB1-LPS complexes had synergistic and enhancing effects on the production of IL6, tumour necrosis factor (TNF), IL10 and IL1β from PBMCs compared with HMGB1 or LPS alone. The authors also show that HMGB1 can directly associate to LPS and that the stimulatory effect can be eliminated by denaturing the complexes before stimulation. HMGB1 in complex formation with either CpG DNA or Pam3 also induced IL6 production in a synergistic way, although not as strongly as HMGB1-LPS complexes. On the other hand, stimulation of IL1 receptor (IL1R) expressing RASF and OASF with HMGB1-IL1β complexes gave 10–100-fold enhancement in IL6 and IL8 secretion. Blocking studies using IL1R antagonist (anakinra) showed that HMGB1-IL1β but not HMGB1-LPS complexes mediated their effect through the IL1R. Furthermore, detoxified LPS, a TLR4 antagonist, significantly inhibited the effect of HMGB1-LPS complexes. Receptor usage was also studied in mice deficient for RAGE, TLR2 or TLR4 receptor. Preliminary data demonstrate that TLR4 deficiency inhibits HMGB1-LPS-induced IL6 production while RAGE or TLR2 deficiency has no effect.

Conclusion Our results show that HMGB1 interacts with different immunostimulatory molecules to enhance their activity, using different receptors depending on the ligand. Thus, the complexes enhance production of the proinflammatory cytokines that have previously been associated with arthritis disease activity.

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