The authors set out to test a hypothesis that HMGB1-complexes stimulate cytokine induction via dual receptor interaction. The authors postulated that HMGB1 signals via RAGE and the partner molecule via its reciprocal receptor and that the complexes bring these receptors in close proximity.

Methods Recombinant HMGB1 was complexed with low amounts of either the TLR4 ligand LPS or the TLR2 ligand Pam_3CSK_4. The complexes were used to stimulate intraperitoneal macrophages obtained either from mice deficient in RAGE, TLR2, TLR4, MyD88, TIRAP, TRIF or TRAM or from wild type mice on a C57BL/6 background. Supernatant IL-6 levels assessed by ELISA (R&D Systems) were used as the read-out method.

Results HMGB1-LPS and HMGB1-Pam_3CSK_4 complexes both stimulated IL-6 release in a synergistic mode compared to the individual components. Lack of TLR4 eliminated HMGB1-LPS complex-induced IL-6 production whereas absence of TLR2 eliminated that activated by HMGB1-Pam_3CSK_4 complexes. Deleting RAGE had however no effect on stimulation by either complex. To study whether the complex stimulation caused changes in the intracellular signaling the authors stimulated cells deficient in the adaptor molecules used by TLR2 (MyD88, TIRAP) or TLR4 (MyD88, TIRAP, TRIF, TRAM) with HMGB1-LPS and HMGB1-Pam_3CSK_4 complexes. Deletion of any of the adaptor molecules had similar effects on HMGB1 complex activation as compared with non-complexed LPS or Pam_3CSK_4.

Conclusions Cytokine production induced by LPS or Pam_3CSK_4 in complex with HMGB1 occurred via their reciprocal receptors. The authors could not demonstrate that HMGB1-RAGE interaction or changes in the signalling pathways was responsible for the synergistic cytokine release. Elucidating HMGB1 receptor usage in processes where HMGB1 acts alone or in complex with other molecules is essential for the understanding of basic HMGB1 biology and for designing HMGB1-targeted therapies.

19 HMGB1-PARTNER MOLECULE COMPLEXES ENHANCE CYTOKINE PRODUCTION BY SIGNALING THROUGH THE PARTNER MOLECULE RECEPTOR

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10.1136/annrheumdis-2011-201238.19

Background and objectives The pro-inflammatory protein HMGB1 is a pathogenic mediator in several inflammatory diseases as demonstrated by beneficial effects of HMGB1-blocking therapy in experimental models including arthritis. High levels of extracellular HMGB1 are found both in experimental models of arthritis and in synovial biopsies from RA patients. Furthermore, intraarticular injection of HMGB1 causes destructive synovitis in mice.

The authors and other groups have recently demonstrated that besides its endogenous pro-inflammatory properties, HMGB1 can induce cytokine release by complex formation with various proinflammatory molecules resulting in strong synergy. Direct HMGB1-mediated cytokine induction is mediated via TLR4. Receptor usage by HMGB1-complex formations is however an unresolved issue.