22 THE PRO-INFLAMMATORY EFFECT OF HMGB1, A MEDIATOR OF INFLAMMATION IN ARTHRITIS, IS DEPENDENT ON THE REDOX STATUS OF THE PROTEIN

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Background and objectives High mobility group box protein 1 (HMGB1) is a nuclear protein involved in chromatin architecture and is present in all cells. HMGB1 also functions as a prototype alarmin and is released passively during necrotic cell death, as well as actively secreted from certain immunocompetent cells. Levels of HMGB1 are highly elevated in synovial tissue of rheumatoid arthritis patients, and the protein is an important mediator of different inflammatory processes. Blocking the inflammatory effect of HMGB1 with antibodies or specific inhibitors ameliorates disease in different experimental arthritis models.

In this study, the authors wanted to investigate if the redox status of HMGB1 is important for the pro-inflammatory effect of the protein. We also investigated if the presence of the redox sensitive cysteine is necessary for binding of HMGB1 to its receptor TLR4/MD2.

Methods To determine the redox status of HMGB1, meaning the three cysteines present in the molecule, the authors used tryptic digestion followed by liquid chromatography electrospray tandem mass spectrometry. To investigate the pro-inflammatory effect of HMGB1 and HMGB1-mutants, primary human and murine macrophages and macrophage-like RAW 264.7-cells were stimulated with HMGB1 and cytokine production was measured by ELISA. Binding studies of HMGB1 to its receptor TLR4/MD2, and investigation of the importance of redox status, was performed by Surface-Plasmon Resonance analysis.

Results The authors could determine that, in order for HMGB1 to have a pro-inflammatory effect, the redox sensitive cysteine at position 106 (C106) needs to be in a reduced thiol state and the cysteines at position 23 and 45 have to form a disulfide bridge. Oxidation of C106 or lack of a disulfide bridge between C23 and C45 then causes HMGB1 to lose its pro-inflammatory

effect. In Surface-Plasmon Resonance experiments the authors could demonstrate that a substitution of C106 for an alanine or a serine inhibited the binding of HMGB1 to its receptor TLR4/MD2.

Conclusion The pro-inflammatory effect of HMGB1 is dependent on the redox status of all three present cysteines. The reduced C106 is necessary for the binding of HMGB1 to its receptor TLR4/MD2 explaining the lack of cytokine inducing effect of HMGB1 where C106 has been substituted for another amino acid or if the cysteine has been oxidised. The knowledge of the importance of the redox status of HMGB1 can promote the development of therapies targeted at only blocking HMGB1 when it is in its pro-inflammatory form.