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HMGB1 as a prognostic biomarker of SJS/TEN and its putative role in the pathogenesis due to keratinocyte cell death.

G. B. Nwikue, D. F. Carr, J. Lea, N. Harper, D. J. Antoine, M. Pirmohamed. Clinical and Molecular Pharmacology, University of Liverpool, Liverpool, UNITED KINGDOM.

Introduction: Stevens Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but serious life threatening severe immune-mediated cutaneous reactions with a manifestation of extensive epidermal detachment predominantly due to keratinocyte death with mortality ranging from 10-30% [1]. To date, no generic mechanism-based biomarker for SJS/TEN has been identified or validated. This study investigated whether high mobility group box-1 (HMGB1), a well validated cell death marker[2] represents a valid, utilisable diagnostic and prognostic biomarker for drug-induced SJS/TEN.

Method: Sera from nevirapine-treated Malawian HIV patients presenting with maculopapular exanthema (MPE, n = 27), drug rash with eosinophilia and systemic symptoms (DRESS, n = 12), SJS/TEN, n = 12 and matched tolerant controls, n = 114 were analysed for total HMGB1 by ELISA. Additionally, an *in vitro* keratinocyte cell-line (HaCaT) model was used to investigate mechanisms of keratinocyte cell death occurring in SJS/TEN and the subsequent effect on HMGB1 dynamics. To model necroptosis, HaCaT was stably transfected with RIPK3 cloned into a pCW vector. The aim was to selectively activate TNF-α dependent RIPK3 regulatory switch that would favour necroptosis.

Results: Mean total serum HMGB1 was significantly elevated in MPE (6.0ng/ml), DRESS (6.3ng/ml) and SJS/TEN (15.9ng/ml) acute sera versus tolerant controls (1.3ng/ml) ($P<0.001$). An *in vitro* model of Fas-mediated keratinocyte apoptosis (using NOC-18) resulted in a dose-dependent elevation of both HMGB1 secretion at 10mM versus vehicle exposure ($P<0.05$), and nuclear to cytosolic translocation by dying keratinocytes. Similarly, flow cytometry (annexin V and propidium iodide) demonstrated that TNF-α, thought to have a role in SJS/TEN pathogenesis, induced apoptosis but not necroptosis, as well as HMGB1 secretion in cIAPs-inhibited HaCaTs. However, there was a significant induction of necroptosis in HaCaTs transfected with RIPK3, a key modulator of necroptosis compared to unstimulated control ($P<0.05$).

Conclusion: Injury to keratinocytes following both apoptosis and necroptosis in SJS/TEN mediates the secretion and release of HMGB1, a potent mediator of the innate immune response. Further work is underway to evaluate HMGB1 isoforms and further elucidate the source of HMGB1 and its isoforms and their potential role in the pathogenesis of SJS/TEN.

References:

1. Roujeau JC. (2005). *Toxicology* **209**: 123-129.

2. Antoine D.J., *et al.* (2002). *J Hepatol* **56**: 1070-9.