RAPID RELEASE OF ANNEXIN A1 FOLLOWING HEAT-SHOCK RESPONSE IN LPS-STIMULATED MACROPHAGES

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Fever-induced heat shock response (HSR) is an evolutionarily conserved mechanism through which cells are able to tolerate environmental and inflammatory stressors. This protection is achieved through the synthesis and release of a family of proteins termed heat shock proteins (HSPs). As molecular chaperones, HSPs can bind to native intracellular proteins, protect them from denaturation, and facilitate their repair. Several studies have shown that prior induction of a heat shock response protected animals against the lethal effects of sepsis (1). Although not defining the mechanisms, these studies raise the intriguing possibility that the HSPs may deliver important endogenous mediators that protect the body against sepsis induced organ injury. Several studies have shown that endogenous Annexin A1 (ANXA1) mediates the antipyretic actions of dexamethasone (2); peripheral administration of ANXA1 inhibits fever and thermogenesis (3). We asked here whether ANXA1 might mediate the anti-inflammatory effects of the heat shock response in lipopolysaccharide (LPS) stimulated macrophages. RAW 264.7 macrophages were stimulated with LPS (1μg/ml) for different time (1-3 hours) at 37°C or 42°C to induce physiological thermal stress. Thereafter, both culture supernatants and cells were collected and the concentrations of ANXA1, HSP-70 and HSP-90 analysed by Western blotting. Stimulation of cells with LPS at 42°C induced decrease in intracellular ANXA1, HSP-70 and HSP-90 compared to cells incubated at 37°C. This was accompanied by an increased recovery of secreted ANXA1, HSP-70 and HSP-90 in the culture supernatant. Interestingly, immunoprecipitation of intracellular and extracellular ANXA1 followed by immunoblotting for either HP-70 or HSP-90 showed that these proteins might physically interact with each other. Similar results were obtained when we immunoprecipitated either HSP-70 or HSP-90 and immunoblot for ANXA1. These findings suggest that in RAW 264.7 macrophages ANXA1 exists as complex with both HSP-70 and HSP-90 and that these proteins might be responsible for its increased release upon thermal stress. Furthermore we propose that release of endogenous ANXA1 in fever-like conditions might contribute to the well-known anti-inflammatory effects of thermal stress on the immune response.

1. Wong H.R., (1998) New Horiz. 6: 194-200.
2. Strijbos *et al.*, (1992) Am. J. Physiol. 263: E632-E636.
3. Strijbos *et al.*, (1991) Br. J. Pharmacol. 102: 11P.