Insight into the mechanism of mitochondrial DAMP release during sepsis

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Abstract

Recent studies have shown that mitochondria derived damage associated molecular pattern molecules (mtDAMP) are increased in the blood circulation of patients suffering from severe injuries and sepsis. DAMP's (including mitochondrial DNA and proteins) are considered to be pro-inflammatory and one of the important mediators of ongoing systemic inflammation during sepsis. The mechanism of mtDAMP release during sepsis is currently enigmatic. In this regards, the recent paper by Kana *et al.* [1] in *Autophagy* shows that upon lipopolysaccharide stimulation of primary hepatic cells, active extracellular release of mtDAMP occurs through the exocytosis of autolysosomes. Inhibition of the autophagy process attenuated the mtDAMP release from the cells. These data demonstrate the active role of autophagy in secretion of cellular proteins from the cells during inflammatory conditions like sepsis. This paper provides important insight into the mechanism of sepsis induced mtDAMP release and provides background for future investigations.

Keywords: Sepsis, mitochondria, DAMP, autophagy

The paper by Kana *et al.* demonstrates a mechanistic role for autophagy in the process of mtDAMP release from the cell [1]. The seminal work in the field by Hauser and colleagues demonstrated that severe trauma releases endogenous mitochondrial damage associated molecular pattern (mtDAMP) molecules (mitochondrial DNA, mtDNA) into the circulation, which can activate the innate immune cells and trigger a sepsis like inflammatory state [2]. Mitochondria are a source of several DAMP molecules, which act as endogenous danger signals, like mitochondrial DNA and proteins including N-formyl peptides cardiolipin, cytochrome c, and ATP to name a few[3]. Such mtDAMP's act similar to the bacterial antigens, leading to a state of systemic inflammatory response syndrome and ultimately multiple organ dysfunction and death in severe cases of trauma and sepsis [4, 5]. Similar to bacterial antigens, these mtDAMP molecules have been shown to activate the pattern recognition receptors on innate immune cells such as neutrophils and initiate inflammatory response and organ injury during trauma and sepsis [1-3, 6].

Sepsis is a state of severe systemic inflammatory response caused by microbial infection that can progress to multi-organ dysfunction. Studies show that the level of mtDAMP molecules is increased in the plasma of septic patients and correlate with the severity of sepsis [7, 8]. Similarly, animal studies also show that bacterial challenge increases the extracellular release of mtDAMP molecules such as mtDNA [4, 9]. It is conceivable that severe trauma can induce massive cell death and necrosis, which probably leads to a passive release of DAMP molecules into blood circulation [3]. Interestingly, organ histology has failed to demonstrate remarkable cellular damage (apoptotic or necrotic) in septic patients or animals that is consistent with simply passive release of DAMP molecules. Clearly, additional and more focused studies are needed to fully understand this process [10]. Therefore, the mechanism of sepsis induced mtDAMP release is poorly understood.

Autophagy-lysosomal pathway is an important cellular process which allows for recycling of essential constituents that are recovered during lysosomal degradation of cellular components

including mitochondria [11, 12]. Previous studies from the same laboratory have shown a significant induction of autophagy and extrusion of mitochondrial proteins in the plasma of LPS treated rats [13, 14]. LPS is a cell wall component of gram negative bacteria and is widely used in mechanistic research studies relating to sepsis. In an attempt to discover the mechanism of mtDAMP release during sepsis, Kana *et al*. now show that lipopolysaccharide (LPS) stimulation of primary hepatic cells as well as mouse embryonic fibroblasts leads to the mitochondrial degeneration, induction of autophagy, and extracellular release of not only mitochondrial DNA and proteins but also autolysosomal luminal proteins including LC3-II (microtubule – associated protein 1A/1B-light chain 3 - critical autophagy protein). PARK2 and PINK1 proteins, which are known to regulate mitochondrial autophagy (mitophagy), were also detected in the culture supernatants in LPS stimulated cells. Furthermore, pharmacological inhibition of autophagy and knockout of Atg5 (another essential protein for autophagy) attenuates the release of mtDAMP molecules and exposure of released mtDAMP to polymorphonuclear leucocytes activates them as evident by production of pro-inflammatory cytokines. Inhibition of autophagy did not cause cell toxicity. Moreover, use of lysosomal inhibitors also partially suppressed the release of LC3-II and mitochondrial proteins, implicating functionally active lysosomal involvement too in the secretion of mtDAMP molecules. Therefore, the major mechanistic conclusion drawn from their results is that mtDAMP molecules are released from the cells in an autophagy dependent manner. With respect to the above results, authors did not discuss the future therapeutic implications of these findings and if autophagy could be a therapeutic target during sepsis. But this study is definitely a good starting point for further exploration regarding pathogenic relationship between autophagy, mtDAMP and sepsis.

Although the findings present by Kana *et al* [1] provide a new insight into the mechanism of mtDAMP release during sepsis, still many questions remain unanswered and need further investigation. Though autophagy dependent release of mtDAMP was shown in both hepatic cells as well as mouse embryonic fibroblasts, it remain to be determined in future studies if this is universally true for all tissues. Sursal *et al.* showed that lethal sepsis in primates using anthrax bacillus caused sustained elevation of mtDNA with only a transient increase in bacterial DNA until death, suggesting continued tissue damage beyond bacterial clearance. Therefore, mtDAMP's are detrimental during sepsis. [4]. On the other hand, autophagy has also been shown to play an important role in pathogen elimination and host protection through a process called xenophagy (pathogen targeting to autophagosome) [15]. Mitochondrial dysfunction is known to contribute significantly to organ injury during sepsis [10, 16] and mitochondrial autophagy (mitophagy) aimed at clearing damaged mitochondria has been demonstrated to occur in the liver and lung during sepsis [17-19]. Autophagy thus seems to play a role both in mitochondrial quality control as well as mtDAMP release during sepsis. This raises important questions for future studies. Can autophagy be modulated in a way to attenuate mtDAMP release during sepsis without hindering the other processes such as mitophagy and xenophagy? Also, could the monitoring of autophagy proteins and mtDAMP's be used as prognostic biomarkers of sepsis to direct therapy? These are critical issues that require further experimentation.

Acknowledgements

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