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SHORT COMMUNICATION

Nuclear histones: major virulence factors or just additional early sepsis markers? A comment

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Abstract In 2009, Xu et al. and Chaput et al. in Nature Medicine had argued that the main cause of death in sepsis is the release from neutrophil nets of nuclear histone, highly toxic to endothelial cells and that these polycations are major and unique virulence factors. Since 2009, numerous researchers have also suggested the involvement of histones in the pathophysiology of many clinical disorders. If histones are indeed major unique virulence toxic agents, then heparin, activated protein C and antibodies to histone should prove excellent antisepsis agents. However, this is provided that these agents are administered to patients early enough before the activation of the cytokine storms, immune responses and the coagulation cascades are irreversibly unleashed. This may not be practical, since a diagnosis of sepsis is usually made much later. Future identifications of novel early markers are therefore needed and a compilation of cocktails of antagonists may replace the faulty single antagonists tried for many years, but in vain, to prevent death in sepsis.

Keywords Nuclear histone · Sepsis · Septic shock · Post-infectious sequelae · Synergistic mechanisms

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Circulating histones in sepsis and in post-traumatic events

Two "breakthrough" articles in Nature Medicine from 2009 (Chaput and Zychlinsky 2009; Xu et al. 2009) had argued to be the first to suggest that the main cause of death in sepsis may be the release from neutrophil extracellular traps (NETosis) of highly cationic histones possessing high toxicity to endothelial cells. This commences immunological (cytokines storms) and coagulation cascades culminating in septic shock and death. In their study, Xu et al. showed that activated protein C (APC), a protease, cleaved histones and reduced lethality. However, blockade of APC activation exacerbated sub-lethal LPS challenge into lethality, which was reversed by antibody to histone. Chaput et al. assessed the protective effects of recombinant thrombomodulin (rTM), which was approved in Japan for the treatment of disseminated intravascular coagulation (DIC) and is currently undergoing a phase III clinical trial in the USA.

Both groups of investigators had concluded and advised that extracellular histones may be the potential molecular targets for therapeutics for sepsis and additional post-infectious inflammatory and traumatic manifestations. If substantiated, the possibility that histone neutralization by heparins, but especially by the newly reported non-anticoagulant heparin (Wildhagen et al. 2014), may be a blessed future hope for critical care patients combating post-infectious and inflammatory sequelae.

It may interest the readers that already during the years 1951–1965, Katchalski's group at the Weizmann Institute of Science in Rehovot, Israel, had described for the first time that the histone mimics poly-L-lysine and poly-L-arginine, injured blood vessels of rats, retarded blood coagulation and inhibited fibrinolysis (Biezunski et al.

1955; De Vries et al. 1953, 1956; Ginsburg et al. 1952). Also, during the years 1986–1996, investigators at the Department of Pathology, the University of Michigan, Ann Arbor, USA, at the Institute for Drug Research, School of Pharmacy and at the Institute for Dental Sciences at the Hebrew University of Jerusalem, Israel, had already described the killing of human umbilical cord endothelial cells by histone and by additional polycations, especially if combined synergistically with oxidants (Ginsburg et al. 1989; Ginsburg and Kohen 1995; Ginsburg et al. 1992, 1993). In those years, we never dreamed that many years later, we shall again address the histone issue thought central in the pathogenesis of so many clinical disorders.

It may be pictured that polymorphonuclear leukocytes (PMNs) recruited in sepsis, which are attracted to and adhere to endothelial cells, undergo activation and release not only highly cationic histones, LL-37 and elastase, but also a plethora of pro-inflammatory agonist such as oxidants, superoxide, nitric oxide, peroxynitrite, MPO-catalyzed hypochlorous acid, phospholipases A2 as well as numerous acid hydrolases, which may act synergistically to permeabilize and destroy endothelial cells.

Therefore, the concept that histones may exclusively act as a unique virulence factor should be doubted and reconsidered, since toxic histones probably never act on their own but always in synergy with many of the pro-inflammatory agonists released by activated neutrophils.

Since 2009, a large number of publications had also claimed a possible major involvement of nuclear histones, citrullinated histone and histone deacetylase in the pathogenesis of cardiovascular, hepatic, pulmonary and renal disorders as well as in post-traumatic episodes (Alhamdi and Toh 2016; Birmpas et al. 2015; Bosmann et al. 2013; Chen et al. 2014; Chong et al. 2012; Hirose et al. 2014; Huang et al. 2011; Kutcher et al. 2012; Saffarzadeh et al. 2012; Ward and Grailer 2014; Zhang et al. 2013, 2015; Zhao et al. 2014).

If toxic histone levels in plasma are significantly elevated in so many diverse clinical disorders, can this cationic peptide be considered: a novel "alarmin" virulence factor? Therefore, it may effectively be neutralized either by non-anticoagulant heparin (Wildhagen et al. 2014), activated protein C (a proteinase) or antibodies to histone (Xu et al. 2009).

However, since in clinical trials, activated protein C had failed to show effective protection against the aftermath of sepsis and was removed from use, maybe there are other reasons to explain their failure to affect the course of sepsis.

In reality, although non-anticoagulant heparins may prove promising "magic bullets", their efficacy may unfortunately be still quite limited since treatment of sepsis patients may start several hours–days after admission to the ICU and too late after the "horse had already left the stable". At later time points, heparins' action might be ineffective, since the deleterious effects of the immune cytokines storms and coagulation cascades may have already been unleashed. Therefore, a search for novel very early biomarkers might greatly improve early diagnosis and proper treatment may probably be by combinations between antibiotics and heparin (Reinhart et al. 2012; Shukla et al. 2014).

Since sepsis, septic shock, disseminated intravascular coagulopathy (DIC), adult respiratory distress syndrome ARDS and acute lung injury (ALI) as well as additional post-infectious and traumatic sequelae are all regarded as distinct multi-factorial episodes, it may clearly explain the futile attempts over so many years, to prevent mortality by the exclusive administration of only a single antagonist at a time. This definitely calls for the urgent development and testing of appropriate cocktails of inhibitors (Ginsburg 1999) to replace the ineffective single antagonists. After all, today the treatment of AIDS and tuberculosis is successfully controlled by cocktails of drugs.

Conclusions

If one concurs with the assumption that circulating histones from PMNs nets are indeed the major virulence factors in sepsis, then histone inhibitors such as activated protein C, heparin and anti-histone antibodies might prove as magic bullets. However, the main failure of these agent in practice is most probably the result of their late administration when all the cytokines and coagulation cascades had already been unleashed. Since sepsis septic shocks are distinct multi-factorial synergistic episodes, only appropriate early markers may allow early treatment by cocktails of antagonists yet to be established and to discontinue planning new trials to test again only single antagonists already proven to be totally ineffective.

It is surprising therefore that the recent article "Developing a new definition and assessing new clinical criteria for septic shock" (Shankar-Hari et al. 2016) did not include any comments about histone, heparins and their possible involvement in sepsis as published in scores of articles since 2009.

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