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Sepsis Pathogenicity and Histones: Are we "Re-discovering the Wheel"?

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Published Date: October 17, 2017

INTRODUCTION

It is alarming that today clinicians are still helpless trying to cope with life-threatening sequelae of severe microbial infections, which very often terminates in sepsis, septic shock and death. According to CDC (The Centers for Disease Control and Prevention) today the annual incidence of sepsis in the USA affects as many as 7,50,000 hospitalized patients and mortality rates are about 40% [1]. As of today, all the clinical trials of sepsis, which had tried the efficacy of only a single antagonist at a time, had failed to protect against septic shock, a disorder obviously caused by multi-factorial processes. Even the "hope of sepsis", activated protein C (**APC**), has recently been discontinued. Today, no effective treatment for sepsis is available and the morality rates are climbing steadily also because of the rapid acquisition of antibiotic resistance.

However, a possible "revolution" in our understanding of the pathophysiology of septic shock was offered in 2009 by two groups of investigators: Xu *et al* [2] and Chaput *et al* [3]. Both had argued that the main cause of death in sepsis might be related to the release from PMNs

neutrophils extra cellular traps (**NETs**) rich in highly cationic histones which injure endothelial cells **(ECs**), which commences cascades culminating in septic shock and death. In their study, Xu *et al* showed that APC "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* [3] assessed the protective effects of recombinant thrombomodulin (**rTM**). rTM was approved in Japan for the treatment of disseminated intravascular coagulation (**DIC**) and is currently undergoing a phase III clinical trial in the United States. Both groups of investigators concluded and advised that extracellular histones are probably the potential molecular targets for therapeutics for sepsis and other inflammatory diseases. Although the findings by Xu *et al* [2] and by Chaput *et al* [3] may be a blessed beginning of the establishment of a novel approach to combat mortality in sepsis and are therefore commended, some of their interpretations and originality of their findings about histone as toxic agents for ECs should be discussed and also reconsidered.

Analyzing the release from activated PMNs of toxic histones, it is reasonable to consider that concomitantly with the formation upon endothelial cells of neutron philextracellular traps (**NETs**) ("a lizard tongue effect?"), PMNs can also generate oxidants via NADPH oxidase and xanthine oxidase toxic hypochloric acid, myeloperoxidase, toxic cationic LL-37 as well as a plethora of lysosomal hydrolases, including the highly cationic elastase membrane-damaging phospholipase A2 and also trigger TH1cytokines production [4]. It is therefore reasonable to assume that several of these secreted agents may also act synergistically with the highly cationic histone to further intensify cell damage. It therefore stands to reason that the effect of APC as a possible anti-histone might be mitigated by a cocktail of inhibitors [5-7]. Highly anionic heparin is known to form stable complexes with cationic histone and as suggested by Xu *et al* [2] may be due to the ability of APC to abolish the synergy among histone and the additional pro-inflammatory agents released by activated neutrophils.

Already during the years 1986-1996, investigators from 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 shown the toxic effects of histone and additional polycations on HUVEC cells. Also, studies during the years 1951-1956 may perhaps call for the inclusion in any future therapy of sepsis, combinations among antioxidants, proteinase and phospholipase A2 inhibitors as well as of additional anti-inflammatory agents and also non anti-coagulant heparin [8,9]. Also, anti-bacteriolytic agents may reduce the toxic and phlogistic effects of microbial cell surface, membrane and cell-wall components, which can also be released following exposure to polycations and to certain antibiotics [10]. Since no such considerations had been proposed by neither groups of investigators [2,3]. The message they proposed was that histone is the sole "villain virulence factor" and therefore, there is no need to consider any additional neutrophil-derived pro-inflammatory agent as participants in cell damage as seen in sepsis.

Reading through the two articles in Nature Medicine, from 2009, It was surprising that none of a series of publications, which had already described the toxicity of histone to human primary umbilical cord endothelial cells (**HUVECs**) in culture, (see references below), had been cited In the articles by Xu *et al* and Chaput *et al* [2,3].

Many years ago, Katchalski's group from the Weizmann Institute of Science in Rehovot, Israel, described the toxic effects of the histone mimetics poly L-lysine and poly L-arginine on blood vessels of rats, human blood coagulation, platelets, fibrinolysis, phagocytosis, and bacteria [11-14]. It is obvious therefore, that the publications by Xu *et al* and Chaput *et al* [2,3], which claimed to be the first to describe the toxicity of histone to endothelial cells, may actually be an un-ethical "re-discovery of the wheel".

Paradoxically, the failure to cite already published information on histones toxicity to ECs also created a "Vicious Circle". This is because now, the authors of about 15 or more new publications since 2009 on the role played by histones in a variety of clinical disorders totally unrelated to sepsis were also unaware of any of the pioneering investigations on the subject published since 1952.

Taken together, if the highly cationic histone released from PMNs NETs is really the major virulence factor in the pathophysiology of septic shock then, the recent publication by Wildhagen *et al* [9] on the development of a non-anticoagulant heparin, which is still capable of neutralizing histones action, is a blessed new development in the struggle against the adverse effects of post-infectious sequelae. We have recently redefined sepsis as a multi factorial synergistic episode where no unique alarmin is generated, which if successfully inhibited might inhibit the deleterious biochemical and immunological cascades involved in tissue damage and patients' demise [15]. Being multi factorial it is thought that since all the clinical trials of sepsis which had administered only single antagonists at a time had failed [16], cocktails of antagonists should be tried as possible effective agents to cope with synergistic episodes [17]. Histones might not be considered as unique alarmins but just additional markers of cell damage [18].

OUR CAUTIONARY COMMENT

It is very obvious that today patients usually arriving at the intensive care unit (**ICU**) with a well-established sepsis when "all the horses have already left the stable", allowing immune system disruption and organ failure to take their toll. This might be further aggravated by the prolonged intravenous use of highly bacteriolytic antibiotics. Novel means of detecting early markers of sepsis should be available to every family physician to enable very early recognition and treatment of sepsis. Increased community awareness of sepsis will also aid early diagnosis and treatment. The inability to successfully control the deleterious aftermath of severe incurable microbial infections places sepsis and septic shock in a category of one of the least understood human disorders affecting a very large numbers of hospitalized patients.

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