

## The Pathogenesis of Sepsis: “If We Cannot beat them Alone Join Them?”

Korem Maya<sup>1</sup>, Koren Erez<sup>2</sup> and Ginsburg Isaac<sup>2</sup>

<sup>1</sup>Department of Clinical Microbiology and Infectious Diseases, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

<sup>2</sup>Institute for Dental Research, The Hebrew University – Hadassah Faculty of Dental Medicine, Jerusalem, Israel.

### \*Correspondence:

Isaac Ginsburg, Institute for Dental Sciences, Hadassah Faculty of Dental Medicine, Hebrew University-Hadassah Medical Center, 91120, Jerusalem, Israel, E-mail: ginsburg@mail.huji.ac.il.

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### ABSTRACT

*Sepsis and septic shock are probably the least understood human disorders which worldwide take the lives of millions of patients. Sepsis may be defined as a multifactorial synergistic phenomenon where no unique damage-associated molecular patterns –alarms are identified which if successfully neutralized, might mitigate and protect against death in sepsis.*

*Microorganisms which invade the blood stream may activate neutrophils to adhere to endothelial cells and to form oxidant – dependent nets rich in highly toxic nuclear histones claimed to be the main cause of death in sepsis due to the dysregulation of endothelial functions. However, the histone saga was recently critically debated since high levels circulating histones are also found in many clinical disorders unrelated to sepsis, therefore, histones may not be considered as a unique damage-associated molecular patterns- alarms but as additional markers of severe cell damage.*

*We hereby argue that the main cause of tissue damage in sepsis may be an end result of a synergism between the numerous neutrophils pro inflammatory agents and the multiplicity of similar pro inflammatory agents generated by hemolytic streptococci and by additional pathogenic microorganism which recruit large numbers PMNs to the inflammatory sites. It is recommended that in sepsis caused by hemolytic streptococci and by additional toxigenic bacteria, a use of cocktails of antagonists might be more beneficial therapeutic strategies and this in view of the total failure to treat sepsis only by administrations of single antagonists. Also, targeting PMNs by immunological strategies should be sought for, to mitigate synergies between leukocytes and microbial cells.*

### Keywords

Sepsis, Shock, Inflammation, Pathogenesis.

### Introduction

Screening the vast literature attempting to explain the pathogenesis of post inflammatory and infectious sequelae such as septic shock, reveals a gloomy situation. As of today, no effective therapies are available to prevent the deleterious aftermath of severe microbial infections [1-4]. Septic shock is most probably the least understood human disorder, which is getting even worse due to the development of high resistance to antibiotics. Being a typical multifactorial episode, it is not surprising that as of today, practically all the clinical trial of sepsis conducted which have administered to patients only single antagonists, have been ineffective. In 2014 Opal et al. have questioned: What is the next

after the demise of recombinant human activated protein C ? [4]. Worldwide, more than one million sepsis cases are reported annually with a mortality rate of 10-40% when shock is present [5].

### Re definition of sepsis

Recently, we have re defined sepsis and septic shock as synergistic multi factorial episodes where no single damage-associated molecular patterns are generated which if successfully inhibited, could abolish the devastating consequences when microorganisms invade the blood stream [6]. Therefore, it stands to reason that being multifactorial episode, the clinical use in sepsis therapies should include cocktails of adequate antagonists [7]. After all, cocktails of drugs are currently employed very effectively against Human Immunodeficiency Virus (HIV), tuberculosis and many malignancies.

An invasion of the blood stream by microorganisms may lead to a massive neutrophil accumulation [8]. Polymorphonuclears (PMNs) adhering upon endothelial cells, may generate an oxidant – dependent nets rich in the highly toxic cationic nuclear histone [9-11]. Injury by the highly toxic histone may dysregulate endothelial cells function, eventually leading to severe immunological and metabolic changes culminating in death [1-3]. These events can be further amplified by the release of the microbial cell wall components endotoxin (LPS), lipoteichoic acid (LTA) and peptidoglycan (PPG) following bacteriolysis induced by PMNs cationic peptides and also by certain antibiotics [12-14]. All these events are most probably synergistic in nature [6].

The support for the synergism concept of tissue damage in post infectious and inflammatory conditions such as septic shock was actually derived from studies of the pathophysiology of group A hemolytic streptococcal infections, classical multifactorial pathogens [15,16]. Clinically, group A Streptococci can induce acute tonsillitis, pharyngitis, acute necrotizing fasciitis, rheumatic fever, arthritis, chorea and sepsis [15-21].

Group A hemolytic streptococci (GAS) are notorious for their ability to elaborate a huge arsenal of highly toxic pro inflammatory agents [15-19]. These include: membrane – perforating streptolysins S and O, the cell bound hemolysin which induces a "kiss of death", proteinase, DNase, RNase, hyaluronidase, streptokinase generated plasmin [15,16,19]. Therefore, the pathological lesions induced by streptococci are most probably initiated mainly by a synergy among their secreted agonists. However, these agents may also collaborate in a synergistic manner with the plethora of pro inflammatory agents released from PMNs recruited in huge numbers to the infected sites. This probably occurs when the phagocytes succumb and autolyze due to the effect of GAS hemolysins. The toxic agents elaborated by activated PMNs include: superoxide,  $H_2O_2$ , hydroxyl radical, peroxynitrite, HOCl, the cationic peptides histone, LL37, elastase, cationic proteinase, cathepsins and proinflammatory TH1 cytokines [8,22]. It is highly likely, therefore, that these agents may injure tissues not by acting alone but mainly in a synergistic manner with the streptococcal agonists to injure cells and tissues [6].

### **The synergism paradigm and sepsis**

Historically, the synergistic phenomenon and mechanisms of cell damage induced in sepsis and most probably also in additional microbial infectious sequelae, have also been examined on tumor cells. In 1958 [23] and in 1960 [24] it was demonstrated that if the cell membranes of Ehrlich ascites tumor cells used as targets were punctured by membrane-damaging agents such as cytotoxic antibodies and complement or by streptolysin S, the cells were disintegrated by the addition of proteinases. Since then, many studies had also shown the combined/ synergistic cytotoxic effects induced by oxidants,  $PLA_2$ , cationic peptides, such as histone, LL37, and extracts from PMNs on human umbilical cord endothelial cells, epithelial cells and also in animal models [25-33]. Following bacteriolysis, streptococcal membrane - associated lipoteichoic acid (LTA) is released and can combine with anti - LTA

antibodies to produce superoxide and  $H_2O_2$  due to the activation of PMNs [34,35]. Complexes of bacterial polysaccharides and peptidoglycan induced severe chronic arthritis [36]. These toxic agents may hopefully be contained by cocktails of antagonists yet to be introduced clinically for human use [7].

### **The possible role of cationic histone in sepsis pathogenicity**

In 2009 two novel studies in Nature Medicine offered by Xu et al. [10] and Chapout et al. [11] introduced a novel approach to explain the pathogenicity of sepsis. These authors proposed that the main cause of death in sepsis may be the release from PMNs nets (Netosis) adhering upon endothelial cells (EC) [9] of highly toxic oxidant-dependent nuclear histone which dysregulated the cells and led to patients demise. However they have also shown that treatment either by activated protein C (which cleaved histones), by highly anionic heparin or also by antibodies to histone, protected cells as well as animal models against death in sepsis. These exciting but also provocative results seemed to be “too good to be totally reasonable”. Since 2009, scores of publications have also reported the presence of high levels of circulating histones in many human disorders totally unrelated to sepsis. The results by Xu et al. and by Chapout et al. [10,11] have also been recently challenged by several publications which had doubted whether histone is the unique “evil culprit” or just an additional marker of cell damage [37,38]. We have proposed that since activated PMNs adhering to EC may also simultaneously release into the surrounding media a plethora of pro inflammatory agonists including oxidants, histone, LL37, highly cationic elastase, cathepsins and proteinase, the treatment by the highly anionic heparin, may have actually neutralized not only cationic histone activity but mainly the synergies among the various agents. A paper recently published described a non-anticoagulant heparin which may have a promising effect in sepsis treatment since it lacks a pro coagulant activity but can still neutralize cationic histone [39].

### **Identification of Sepsis in hospitals**

Warning early signs of sepsis are usually first identified either by the family physician, or later on in the emergency room or in the intensive care unit (ICU). There, measurements of the levels of main key markers are made. These include: C- reactive protein, RBC, WBC, platelet count, hemoglobin levels, plasma pH, bicarbonate,  $Pco_2$ ,  $Pao_2$ , arterial blood gases, lactate, liver function tests and blood cultures. However, and most importantly, we may one day come up with even simpler tests for sepsis using strips similar to pH paper and urine test strips. These may rapidly identify 3-5 of the major warning markers of sepsis and may be identified even by a non-professional household member before the patient is rushed to the emergency room or to ICU. In the emergency room or ICU, the patient may be further treated by proper non- bacteriolytic antibiotics and especially by the non-anti-coagulant heparin [39]. The latter may rapidly neutralize circulating histone and additional toxic polycations suggested as major toxic agents involved in the pathogenesis of sepsis [7,10,11].

However, if we accept the synergism concept of sepsis pathogenicity we may also include as antidotes: anti-oxidants

such as ascorbate, N- acetyl cysteine, glutathione, the proteinase inhibitors aprotinin, antibodies against TH1 cytokines [40,41] and also a pool of IgG [7]. Several investigators have also suggested the use of corticosteroids.

Care should be taken to refrain from using highly bacteriolytic antibiotics [13,14] which may induce the Jarisch Hexheimerl- like phenomenon [39]. The endotoxin released into the circulation can cause erythema migrans, fever, malaise, fatigue, headache, myalgias and arthralgias, as well as neurologic, musculoskeletal, or cardiovascular symptoms. Severe sepsis induced by Gram negatives may also cause severe tubular necrosis.

Excessive neutrophil migration during the early stages of sepsis may lead to an exaggerated inflammatory response with associated tissue damage and subsequent organ dysfunction [1-3]. On the other hand, dysregulation of migration and insufficient migratory response that occurs during the latter stages of severe sepsis contributes to neutrophils' inability to contain and control infection and impaired wound healing.

Despite the intensive efforts to develop therapies for sepsis we are still hovering in the dark especially when a rapid development of antibiotic resistance occurs [1-3]. The synergism phenomenon of cell damage described may most probably not be mainly restricted to hemolytic Streptococci and other toxigenic bacteria but might also occur with many additional microbial species mainly characterized by their ability to recruit large numbers of PMNs. These include infections by Staphylococci, *E.coli*, *Pneumococci*, *Meningococci*, *Shigella*, *Pseudomonas* and in the inflammatory bowel diseases such as ulcerative colitis and Crohn disease. In all these cases, cocktails of antagonists might be more effective especially if combined with PMNs targeting [42].

Since Neutrophils play a major role in controlling infection targeting the cells [42] may be a reasonable approach to mitigate the adverse effects of PMNs agents . Since the systemic activation of Toll-like receptors and high levels of TNF- $\alpha$  and nitric oxide are involved in the reduction of neutrophil recruitment may down-regulate CXCR2 in neutrophils [42].

### Neutropenia and hematological malignancies in sepsis

Not in all clinical disorders, there is a normal chemotaxis and accumulation of PMNs. Intensive chemotherapy for hematological malignancies can induce the breakdown of mucosal barriers leading to high-risk neutropenic fever. It is estimated that 13-37% of neutropenic patients develop bacteremia [43]; The depth and duration of neutropenia is directly related to incidence of serious bacterial infections that can progress rapidly, leading to hypotension and life threatening complications with a mortality rate of 50% [44]. Because neutropenic patients are unable to mount robust inflammatory responses, serious infection can occur with minimal symptoms and signs. In such patients, fever is often the only sign of infection because the endogenous pyrogen (IL-1) is produced by mononuclear cells, not by granulocytes, and these mononuclear cells include also fixed – tissue macrophages that persist after

chemotherapy [45]. The pathophysiology of sepsis in neutropenic patients cannot be attributed solely to an initial bacterial stimulus provoking an early systemic inflammatory response syndrome (SIRS) or a "cytokine storm" characterized by a systemic release of inflammatory cytokines as IL-1, IL-6, TNF- $\alpha$ , and IFN- $\gamma$  [46]. During immune suppression, the systemic release of inflammatory cytokines is either markedly lowered [47] or shows the absence of selective cytokines [48]. Sepsis in neutropenic patients therefore, is probably the consequence of an imbalance between pro and anti-inflammatory cytokines as suggested by the multimodal hypothesis of sepsis[49] and the direct effect of invading microorganisms and their toxic products [49]. For example, endotoxins from gram negative bacteria cell wall are detectable in blood of septic patients and reproduces many of the features of sepsis when it is infused into humans, including activation of the complement, coagulation and fibrinolytic systems. This effects may lead to microvascular thrombosis and the production of vasoactive products such as bradykinin [50].

For these reasons, it is critical to recognize neutropenic fever early and to initiate empiric systemic antibacterial therapy promptly in order to avoid progression to a sepsis syndrome and possibly death [47].

### Summary

The incidence of sepsis ranges from 149-240/100,000 to 13-300/100 000. Case-fatality rate depends on the setting and severity of disease. It can reach up to 30% for sepsis, 50% for severe sepsis and 80% for septic shock. These numbers may be further increased by the emergence of antibiotic resistance, the excessive prescriptions of unnecessary antibiotics, crowded hospitals and emergency rooms, lack of sufficient isolation areas, shortage of trained nurses and medical staff, the lack of disposable coats and finally the unskilled use of sterile infusion needles and catheter. However one of the main mishaps in sepsis treatments is the very late arrival of patients at the emergency rooms when: “all the horses have already left the stable”. Can we find suitable very early markers to diagnose sepsis? Fortunately, developed countries may have much lower incidences of cross infections and their sequelae. Unfortunately the rest of the world will have to sill cope with the disastrous consequences of severe microbial infections.

Taken together, the resemblance of activated PMNs to group A hemolytic streptococci, staphylococci and to other toxigenic and invasive bacteria led to hypothesize that we may perhaps learn a lot from the pathophysiology of these toxigenic bacteria how cells and tissues are destroyed in infectious and in inflammatory sites [16,51]. Therefore, can highly toxigenic and invasive bacteria be perhaps also considered as some kind of “fore fathers of modern PMNs”. Finally, why not also take a lesson from the ants "you sluggard, consider their ways and become wise “??? (The Bible book of Proverbs 6:6).

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