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Synergistic Aspects to Explain the Pathophysiology of Sepsis and Septic Shock-An Opinion

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Introduction

It is disconcerting and also alarming that today clinicians are still bewildered and helpless when trying to cope with life-threatening sequelae of severe microbial infections, which very often culminate in sepsis, septic shock and death. According to CDC (the Centers for Disease Control and Prevention), the annual incidence of sepsis in the USA affects as many as 750,000 hospitalized patients and mortality rate is about 40% [1,2]. It was found in 2 complementary inpatient cohorts that up to 50% of hospital deaths were linked to sepsis [3]. Worldwide, sepsis is one of the common deadly diseases. It is one of the few conditions to strike with equal ferocity in resource-poor areas and in the developed world. Globally, 20 to 30 million patients are estimated to be afflicted every year. Every hour, about 1,000 people and each day around 24,000 people die from sepsis worldwide and sepsis is one of the least well known diseases. In the developing world, sepsis accounts for 60-80% of lost lives in childhood, with more than 6 million neonates and children affected by sepsis annually. Sepsis is responsible for >100,000 cases of maternal sepsis each year and in some countries is now a greater threat in pregnancy than bleeding or thromboembolism [4,5]. Screening the voluminous literature on sepsis treatment revealed unsuccessful efforts to save patients' lives by administering antibiotics but only a signally-chosen antagonist at a time. The numbers of anti-inflammatory agents tested ineffectively over the years is phenomenal (see below) and today even the most promising activated protein C, the "miracle drug" was recently discontinued [6-9]. The initial reactions to infection are generalized pro-and anti-inflammatory responses. These usually starts by activation by microorganisms and some of their products of neutrophils, macrophages and monocytes, which are followed by toxic effects on vascular endothelial cells via pathogen recognition receptors, leading to endothelial disruption. Why have all the therapeutic strategies tested invariably failed to cope with the sequelae of severe microbial infections and what future approaches might break the stalemate leading to a better understanding of the pathophysiology of the "horror autotoxicus" phenomena of sepsis? [10].

Reviewing the "glorious history" of medical microbiology revealed that immunoglobulins rich in anti-toxins activities proved very effective to cope with those maladies where a single virulence agent, such as the toxin of diphtheria, tetanus and botulism, are the main pathogenetic virulence agents. Also, anti-viral vaccines are the hallmark of the prevention of many childes viral diseases and of viral hepatitis. On the other hand, no single major virulence factor is identified in the majority of Gram positives Gram negatives, fungal and Mycobacterial pathogens. Therefore, it stands to reason that cell and tissue damage inflicted by these microorganisms may be a result of a coordinated "cross-talk" (synergism) among host factors and a multiplicity of pro-inflammatory agents generated during the proliferation of bacteria, mainly in the blood stream. These may include: extracellular pore-forming and membrane-permeabilizing hemolysins, capsular polysaccharides, LPS (endotoxin), the membrane-associated lipoteichoic acid (LTA), the rigid cell-wall peptidoglycan (PPG), leukocyte-derived oxygen and nitrogen species, anti-microbial cationic peptides, phospholipases, cationic proteinases, growth factors, cytokines and chemokines and many others. All these agents might be generated in various stages of inflammation and infection by microbes and by the host response. Furthermore, certain life-saving antibiotics might also act as "double-edged swords" by enhancing the release of microbial products (LPS, LTA, PPG, capsular polysaccharides, intra cellular toxins), resulting from to the activation of nascent autolytic wall enzymes released leading to bacteriolysis [11,12].

How Sepsis Progresses to Septic Shock?

Sepsis may commence when microorganism gain access to the blood circulation. Neutrophils recruited bind to endothelial cells to form NETs (neutrophil extracellular traps) [13,14] and release DNA/Histone complexes highly toxic to bacteria and also to endothelial cells. Activated phagocytes can then release reactive oxygen and nitrogen species, lysosomal acid hydrolases, cationic peptides (e.g. LL-37) and permeability increasing factor, cationic peptides, which may not only kill bacteria but may also activate their nascent autolytic wall enzymes to induce bacteriolysis and the release of microbial pro-inflammatory components [15,16].

The hard-to-degrade microbial cell-wall peptidoglycan (PPG), LPS and LTA from Gram positives were shown to trigger some of the symptoms and pathologies associated with experimental sepsis in animal models. Also, plasma complement might act on circulating Gram negatives to release LPS, a process which may lead to disseminated intravascular coagulopathy (DIC) [17,18]. Furthermore, certain beta-lactam antibiotics are highly bacteriolytic and capable of disintegrating cell-walls of Gram positive and negative bacteria to release pro-inflammatory agents [19,20]. Meningococci reaching the end of the logarithmic phase of growth tend to undergo spontaneous autolysis releasing massive amounts of LPS and PPG causing severe meningeal damage [21]. Therefore, the choice of antibiotics selected for treatment of suspected sepsis should be selected with care.

It is enigmatic why none of the extensive reviews on the etiology of sepsis and the clinical aspects of septic shock published in the last 10 years hardly ever quoted any publications on bacteriolysis and its possible obvious role as major actors in the pathogenesis of post infectious sequelae? [12].

In numerous clinical trials of sepsis conducted, a plethora of agents have been tested as possible antidotes (see references [22] and [23] for a detailed list of publications covering this area).

Looking like supermarket shelves packed with groceries, the following agents were tested in well controlled clinical trials of sepsis. These included: gamma globulins, antibiotics, LPS-binding proteins, monoclonal and polyclonal antibodies against LPS, TNF-α and cytokines, receptor antagonists, microbial permeability enhancing cationic peptides (BPI), polymyxin B, lysozyme, proteinases inhibitors, azo dyes, lipids and phospholipids, prostacyclines, sulphated anti-coagulants, anti-thrombin III, plasminogen activator inhibitor (PAl-l), scavengers of reactive oxygen and of nitrogen species, inhibitors of NO synthase, tyrosine kinase inhibitors, anandamites, pentoxyphilline, PAF antagonists, inhibitors of adhesion molecules, steroids and amino steroids, NSAIDs, inhibitors of the nuclear factor NFkB, PLA2 inhibitors, angiotensin converting enzymes (ACE), high volume hemofiltration techniques, lactulose, glucans, bradykinin and histamine antagonists, lactoferrin feeding and colony stimulating factors (GCSF, GMCSF), tetracyclines, heparin, IL-10 and additional anti-cytokine antibodies, and finally activated protein C, other anticoagulants and additional agents.

Several of these agents had previously been proven effective to prevent shock and organ failure in small laboratory animals, mainly in mice, provided that these agents had been administered before the injection either of LPS or after the performance of caecal-ligation and puncture, a common method to induce septic shock and organ failure. This clearly indicates that once the deleterious biochemical, pharmacological and immunological cascades generated by microbial agents were activated, no singly-administered antagonist was effective to prevent the aftermath of the invasion of microbial cells into the blood stream.

As a result of the failure to come up with a "miracle" drug, scores of "desperate letters to the editors and viewpoints on the subject attempted to explain these failures. It was finally suggested that clinicians and basic scientists should get together, go back to the drawing board, to propose novel approaches of therapies of septic shock [2,24]. It has also been questioned whether the continuation of clinical trials with only a marginal benefit, is ethical [1]. These pessimistic stands might stem from the realization that no single identified omnipotent pro-inflammatory agonist exists, which if effectively administered in the early phases of sepsis, might perhaps stop the deleterious cascade of events often leading to patients demise. Therefore, will cocktails of antidotes be more effective life savers? [22].

The Synergism Concept of Cellular Injury

The concept that tissue damage initiated following microbial invasion of the blood stream might be caused by interactions among a multiplicity of pro-inflammatory agonists had emerged form observations on the pathophysiology induced by group A hemolytic streptococci. This microorganism generates membrane-damaging toxins such as streptolysins O and S, intracellular hemolysin, proteinase and spreading enzymes such hyaluronidase, 4 RNAses, DNAse, super antigens, anti-phagocytic M-protein, cross reactive antigens and highly phlogistic peptidoglycan. It may therefore be argued, that synergism among a multiplicity of similar agents might also be the main cause of damage inflicted also in septic shock [25,26]. Several publications, which may have direct relevance to septic shock, were published along the years [27-40]. Many of the studies employed human umbilical cord endothelial cells labeled by arachidonic acid,

which had been treated by combinations among oxidants, the highly cationic histone, proteinases, phospholipases and additional agents. It is enigmatic why none of these publications are ever cited in the Critical Care literature. In other studies, it was shown that lipoteichoic acid (LTA), a regulator of autolytic wall enzymes in Gram positive bacteria, induced neutrophil activations and the release of superoxide and H_2O_2 following treatment by anti-streptococcal antibodies [16].

Taken together, it is highly plausible that, *in vivo*, synergies among microbial-derived agents and agents generated by the immune responses of the host, but not only a single agonist, might explain how cells and tissues are destroyed in post-infectious sequelae [22, 27-40]. It is again enigmatic that the synergism concept of cell damage in post-inflammatory sequelae is constantly disregarded.

Are there Novel Recent Breakthroughs in the Understanding of the Pathophysiology of Sepsis? A Man or a Mouse Model?

Today, the availability of sophisticated genetic tools to analyze agents (genes) involved in infection and inflammation has recently raised a controversial issue. Are mouse models of sepsis valid in sepsis research in humans and if not, what might be an alternative? In 2013, Seok et al. [41] published a paper where they stated that "A cornerstone of modern biomedical research is the use of mouse models to explore basic pathophysiological mechanisms, evaluate new therapeutic approaches, and make go or no-go decisions to carry new drug candidates forward into clinical trials. Systematic studies evaluating how well murine models mimic human inflammatory diseases are nonexistent". The authors showed that although acute inflammatory stresses from different etiologies result in highly similar genomic responses in humans, the responses in corresponding mouse models correlate poorly with the human conditions and also, one another. Among genes changed significantly in humans, the murine orthologs were found to be close to random in matching their human counterparts (e.g. R^2 between 0.0 and 0.1). The authors also suggested for translational medical research to focus on the more complex human conditions rather than relying on mouse models to study human inflammatory diseases. However, before the ink on this article pages dried up, a challenging paper strongly supported the assumption that the mouse model answers all the demands and explained the pathogenesis for septic shock in humans [42]. In this study, Takao and Miyakawa re-evaluated the same gene expression datasets analyzed by Seok et al. using more conventional statistical methods and found a few critical differences in the analysis methods used between the previous study and theirs. They focused on genes whose expression levels were significantly changed in both humans and mice. The study by Seok et al. analyzed sets of genes that were significantly changed in the human conditions regardless of the significance of the changes in mouse models for comparison, which is not a conventional method of comparing two gene expression datasets. Assuming that mouse models would mimic only partial aspects of human disorders, inclusion of genes that showed no significant response would generally decrease the sensitivity to detect the responses shared by the disorders and their models. For this reason, the researchers excluded such genes from their analysis. Takao and Miyakawa concluded that based on their analysis, genomic responses in mouse models greatly mimic human inflammatory diseases. Thus, this strong debate over the exclusive use of mice as the animal of choice in sepsis is going on and is awaiting a further clarification.

"Miracle Histones": Is it a Breakthrough in Sepsis Research?

An outstanding new approach to explain the pathogenesis of septic shock in humans has recently emerged since neutrophil traps had been described [43,44] and histone released from activated neutrophils NETs was incriminated as the major cause of death in sepsis [45,46] presumably due to its high toxicity to endothelial cells. However, the possibility that concomitantly with the breakdown of the nuclei and the release of histone from neutrophils NETs, the cell also release into the surrounding media oxidants, scores of acid lysosomal hydrolases and cationic peptides is not considered. Furthermore, being a highly "sticky" molecule, histone and possibly other cationic antimicrobial peptides eg. LL-37, can perhaps also bind to and activate the respiratory burst in marginating PMNs via NADPH oxidase to induce the generation of toxic oxidants [47,48]. Also, there is an obvious possibility that the activation of the endothelial xanthine-xanthine oxidase system, which generates superoxide and H_2O_2, can be turned on upon the linings of the blood vessels [49,50]. These oxidants may also act synergistically with scores of lysosomal hydrolases accumulated upon endothelial cells to induce cell damage. However, again, no such a considerations were suggested in the two "breakthrough" papers [45,46], and therefore will histone remain the "exclusive virulence factor" causing death in sepsis?

These "breakthrough" papers on histone and sepsis were soon followed by a "burst" of articles describing the possible involvement of cationic histones also in a series of additional human disorders (see below). We are now also waiting for the development of effective anti-histone strategies to verify that this polycation is indeed the major "virulence factor" but maybe that histone does not function on its own, but in synergy with additional agonists. The following recent publications on histone and sepsis are worth reading [51-61].

Are the Publications Suggesting the Key-role of Histone in Septic Shock New Concepts, or a "Re-discovery of the Wheel"?

The exciting story about histone and sepsis brings us back to 1951 when Katchalski and colleagues at the Weizmann Institute in Israel synthesized for the first time linear polymers of amino acids. This is when the highly cationic poly L-Lysine and poly L-arginine (histone mimics?) came into life. These investigators contributed pioneering studies showing the role of cationic poly electrolytes in microbiology, membranology, infection, blood coagulation and fibrinolysis, all relevant to the understanding how the cationic protein histone can induce a catastrophic disease [62-74].

Additional properties of histone relevant to sepsis were also described. Since the early nineteen eighties, it was shown that histone acted in synergy with pro-inflammatory agents to injure endothelial cells in culture (see [27-40]). Arginine- and lysine-rich polymers tend to form stable complexes with negatively-charged membranes of blood cells to induce cell agglutination and lysis, to kill endothelial cells particularly when combined with oxidants and also to form stable complexes with polyanions. These agents include heparin and polyanethole sulfonate, which both neutralize these polymers' strong cationic properties, their toxicity to cells and also their ability to activate the complement cascade. However, heparin might also function mainly by abolishing the synergy between histone and additional pro-inflammatory agonists [32,75,76]. A critical issue still to be clarified is how early after sepsis is suspected and diagnosed, should heparin be administered? This is important since any delay in its

administration might allow more and more histone to be released from disintegrating PMNs which will continue to increase endothelial damage. In this respect, calf thymus histone was also shown to act as a potent opsonin [77], an activator of the respiratory burst in PMNs releasing superoxide and peroxide [78] and histone bound to streptococci, also activated T-cells to generated TH1 cytokines [79]. Finally, engulfment (endocytosis) of histone-coated *Candida albicans* by fibro-sarcoma cells markedly facilitated their metastasis to the lungs of mice, presumably by recruiting PMNs [80]. Cationic poly L-histidine was shown to form stable insoluble complexes with catalase, SOD and with glucose oxidases [81] and poly L-arginine also acted in synergy with a variety of agents to induce intense luminescence (generation of reactive oxygen species) in human blood leukocytes [82].

Epilogue: Where do we go from here?

In view of the failure to prevent the aftermath of severe microbial infections by using only single antagonists, we should go back to the drawing board and think "outside the box". How wrong can one be by assuming that the pathological processes seen in sepsis are caused by a synergism among a multiplicity of pro-inflammatory agonist, which may perhaps be dealt more efficiently by cocktails of adequate antagonists yet to be devised? Furthermore, if histone of neutrophil origin is considered the main "virulence factor" then a possible effective treatment could be the administration of polyanions (e.g. heparin, polyanethole sulfonate) combined with cocktails of antagonists (e.g. gamma globulin, antioxidants, anti-proteases and additional agents), which should all be administered not later than 1-6 hours after sepsis diagnosis. Such combinations might hopefully prevent the ensuing deleterious cascades leading of septic shock and death. It will also be of interest to find out how soon after the histone story may sinks in, new instructions and recommendations will be proposed by the Critical Care Council to alter procedures of sepsis treatment, and this, based on the alleged role of the exclusive role histone argued as a main "virulent factor"?

Regarding the "re-discovery of the wheel phenomenon", it might be very beneficial and also ethical if every editorial board of a scientific Journal consults "old" referees who still recall and respect pioneering publications, which today are often abused since they employed old fashion technologies and are therefore obsolete. One advise to "younger" investigators regarding "re-discovery of the wheel syndrome" and the constant avoidance to cite already relevant published information. It is highly recommended that they read about the "Disregard Syndrome" and how it might be a menace to honest science in order to avoid the "Diseased science" phenomenon [83,84].

Conclusion

It is tragic that today millions of human beings may still succumb to the sequelae of severe microbial infections. Although histone was previously suggested to be the major cause of death due to sepsis [45,46], we still argue that no single virulence factor has been clearly identified to be responsible for death in sepsis. Therefore, it is highly plausible that only multidrug strategies might be helpful and this is due to the multifactorial nature of sepsis and septic shock [22]. Furthermore, the dangers of sepsis and septic shock is also due to the rapid development of antibiotic resistance, housing and hospital crowding and in many countries to wars, poverty, ignorance and lack of adequate health care.

We wonder: is the World Health Organization (WHO) doing its utmost to combat human misery? How many resources are allocated to deal with the "epidemic of sepsis and septic shock" which kills millions?

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