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Isaac Ginsburg & Erez Koren

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REVIEW ARTICLE

Bacteriolysis – a mere laboratory curiosity?

Isaac Ginsburg[a] and Erez Koren[b]

[a]Institute for Dental sciences, The Hebrew University Hadassah Faculty of Dental Medicine, Ein Kerem Campus, Jerusalem, Israel;
[b]Teva Pharmaceutical Industries Ltd., Kfar Saba, Israel

ABSTRACT

The role of bacteriolysis in the pathophysiology of microbial infections dates back to 1893 when Buchner and Pfeiffer reported for the first time the lysis of bacteria by immune serum and related this phenomenon to the immune response. Later on, basic anti-microbial peptides and certain beta-lactam antibiotics have been shown not only to kill microorganisms but also to induce bacteriolysis and the release of cell-wall components.

In 2009, a novel paradigm was offered suggesting that the main cause of death in sepsis is due to the exclusive release from activated human phagocytic neutrophils (PMNs) traps adhering upon endothelial cells of highly toxic nuclear histone. Since activated PMNs also release a plethora of pro-inflammatory agonists, it stands to reason that these may act in synergy with histone to damage cells. Since certain beta lactam antibiotics may induce bacteriolysis, it is questioned whether these may aggravate sepsis patient's condition. Enigmatically, since the term bacteriolysis and its possible involvement in sepsis is hardly ever mentioned in the extensive clinical articles and reviews dealing with critical care, we hereby aim to refresh the concept of bacteriolysis and its possible role in the pathogenesis of post infectious sequelae.

1. Definition of bacteriolysis

Bacteriolysis can be defined as an event that may occur when normal microbial multiplication is altered due to an uncontrolled activation of a series of autolytic cell-wall breaking enzymes (muramidases), which control cell multiplication.

Morphologically, two main patterns of bacterial cell degradation under various physiological and pathological conditions have been defined:

a. The term plasmolysis was proposed when a significant degradation of cytoplasmic constituents occurred, leaving apparently intact cell walls.
b. The term bacteriolysis was proposed when a significant breakdown and degradation of the rigid cell walls occur, presumably due to the un-controlled activation of autolytic wall enzymes (muramidases). Peptidoglycan hydrolysis can lead to the rupture of the murein sacculus due to its high osmotic pressure leading to the release of cytoplasmic constituents and cell-wall fragments (Ginsburg and Lahav 1983; Ginsburg 2002a) (Figures 1 and 2).

2. Early history of bacteriolysis

The phenomenon of bacteriolysis was first reported in 1893 by Buchner and Pfeiffer [reviewed in 1] who showed that fresh and immune sera could kill and also lyse certain bacterial species which was correlated with protection against infection. In 1922, Fleming described the role of the bacteriolytic enzyme, lysozyme in microbial lysis (Fleming 1922) and in 1969 Glynn studied the complement lysozyme sequence in immune bacteriolysis in the Gram negative *Escherichia coli* (Glynn 1969). He suggested that human complement acted on the outer lipoprotein–lipopolysaccharide layers of the bacterial cell-wall and so allows lysozyme access to the deeper mucopeptide (Salton 1958; Glynn 1969). However, a deeper insight into the nature and mechanisms of bacteriolysis and its possible role in inflammation, infection, in post infectious manifestations and also in tissue trauma, had emerged mainly from numerous pioneering publications studying the basic structures and functions of microbial cell constituents (Ginsburg 1987, 2002a), from studies on the mechanisms of action by beta lactam antibiotics (Salton 1958; Tomasz 1979; Wecke et al. 1982; Bera et al. 2005) and
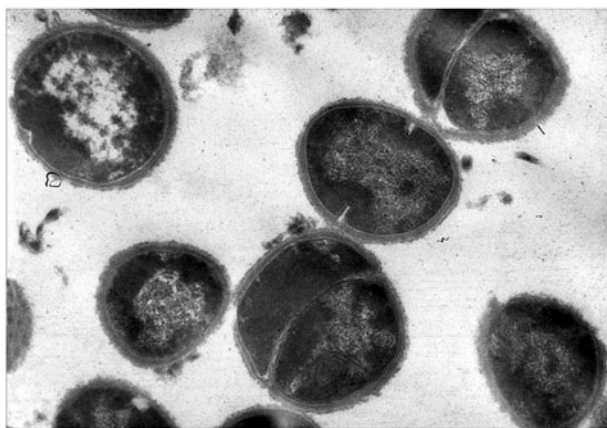
Figure 1. *Staphylococcus aureus* (logarithmic) incubated for 14 h in acetate buffer (pH 5.0) (×24,000) (Ginsburg and Lahav 1983).
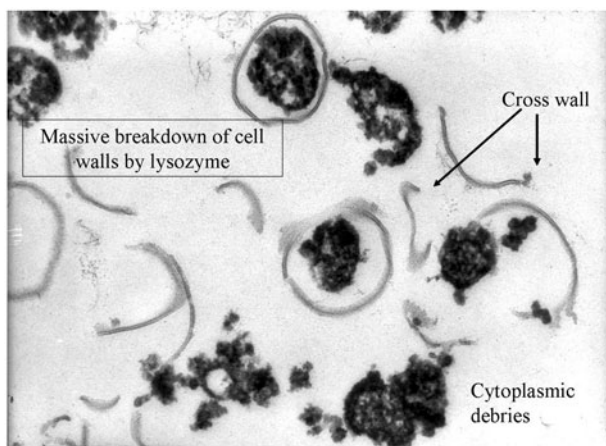

Figure 2. *Staphylococcus aureus* (logarithmic) incubated for 14 h in acetate buffer (pH 5.0) in presence of lysozyme. Note: breakdown of cell walls and cytoplasmic constituents by bacteriolysis (×24,000) (Ginsburg and Lahav 1983).

also of anti-microbial peptides (Katchalski et al. 1952; Ginsburg 1987; Ginsburg 2002a, 2004; Ginsburg and Koren 2008).

3. Role of cationic peptides in the induction of bacteriolysis

The production of new antibodies against environmental microbiota necessitates the release by bacteriolysis of antigenic microbial entities. These include capsular polysaccharides, endotoxin (LPS) from gGram negatives (Glynn 1969), Lipoteichoic acids (LTAs) from gram positives (Ginsburg 1987, 2002a) and from all other microbial species than Gram positive and gram negative of the rigid cell-wall structure peptidoglycan (PPG) [*N*-acetylglucosamine and *N*-acetylmuramic acid] (Ginsburg 1987, 2002a). PPG can theoretically be further

hydrolyzed by the cationic enzyme lysozyme but as will be discussed later on, only for certain non-pathogenic microorganisms (Wecke et al. 1982; Bera et al. 2005). Following the access of microorganisms to the blood stream, there is a massive recruitment of phagocytic neutrophils (PMNs), possessing a huge arsenal of oxidants polycations and hydrolases (Hampton et al. 1998; Kolaczkowska and Kubes 2013). PMNs, which phagocytosed bacteria, can also kill them intracellularly by oxidants and by cationic peptides and release into the surrounding media the major microbial structures LPS, LTA and PPG possessing pro-inflammatory properties (Katchalski et al. 1952; Ginsburg 1987, 2002a, 2002b, 2004; Ginsburg and Koren 2008).

Plasma and mammalian cells express over 100 cationic peptides found in skin, eyes, ears, mouths, gut, immune, nervous and urinary systems. These peptides vary from 10 to 150 amino acids with a net charge between −3 and +20 and a hydrophobic content below 60%. These agents include defensins, lysozyme, Cathelicidin, LL-37, histatin and nuclear histone. A detailed account on anti-microbial peptides and their possible future use to replace antibiotics can be found in recent reports (Guaní-Guerra et al. 2010; Phoenix et al. 2012; Seo et al. 2012).

4. Bactericidal versus bacteriolyic activities

One should differentiate between a bactericidal and a bacteriolytic event. While when suspended in physiological media, killing of bacteria by various cationic agents may take several minutes, bacteriolysis and release of the major cell-wall components may take several hours and depends on the pH of medium (Wecke et al. 1982; Ginsburg 1987, 2002a). Therefore if not waited long enough, one may thus miss the bacteriolysis end point (Figure 1).

In vitro, microbial viability can be established either by counting the number of viable colonies surviving after growth on agar plates using serial dilutions or also by using fluorescent markers to differentiate between viable and non-viable bacteria (Haugland 2005). Bacteriolysis *in vitro* can be measured by growing bacteria in a medium containing 3H *N*-acetyl glucosamine, a part of the backbone of the peptidoglycan (PPG). The amounts of soluble radioactivity released following treatment can indicate the degree of cell-wall lysis. In tissue sections, bacteriolysis can be visualized by transmission electron microscopy (Katchalski et al. 1952; Wecke et al. 1982; Ginsburg 1987, 2002a, 2004; Ginsburg and Koren 2008).

5. Inhibition of bacteriolysis by oxidants and by proteinases

Since not all microbial cells may be totally degraded by leukocytes factors, their intact cell-walls might persist for long periods intracellularly and to induce chronic inflammation. One explanation given for the survival of microbial un-degraded pro-inflammatory wall components within phagocytes may be the inactivation of the microbial autolytic enzymes (muramidases) by oxidants and by proteinases (Ginsburg 1989). Oxidants are generated via NADPH oxidase (Hampton et al. 1998). It was also shown that neutrophils-mediated myeloperoxidase (MPO) –H_2O_2/–HOCl production inactivated in *E. coli*, *Staphylococcus aureus*, and in *Pseudomonas aeruginosa*, of a class of cytoplasmic membrane enzymes (penicillin-binding proteins, PBPs) that covalently bind beta-lactam antibiotics to their active sites with loss of enzymatic activity (Rakita and Rosen 1991). Antibiotics also inhibited the respiratory burst in neutrophils (Hand et al. 1990). This may also contribute to the survival of microbial constituents causing protracted inflammation.

6. Effect of various agents on bacteriolysis

Several poly-anionic agents may slow down bacteriolysis induced by antibiotics. It was shown that the degradation of *S. aureus in vitro* by beta lactams was markedly inhibited by the poly-anions suramine and Evans blue (Wecke et al. 1990) and also by polyanethole sulfonate (a heparin-like potent sulphated anti-coagulant) (Wecke et al. 1986) suggesting that the accumulation of sulphated polysaccharides in inflammatory sites, might also interfere with bacteriolysis. Clindamycin- (Wecke et al. 1990) and chloramphenicol- (Reinicke et al. 1983) treated *S. aureus* caused a remarkable thickening of their cell-walls due to increased numbers of 0-acetyl groups in the murein (PPG). This made the bacterial wall much more resistant to lytic enzymes [see (Wecke et al. 1982; Bera et al. 2005)]. This was also shown in bone marrow-derived macrophages and was clearly revealed by electron microscopy and by radio-labeling experiments. Such reduced wall degradation may increase the survival of highly phlogistic cell-walls in inflammatory sites (see below).

7. The lysozyme riddle: is this enzyme a genuine and an effective bacteriolyic agent?

In 1922, Alexander Fleming discovered the enzyme lysozyme (*N*-acetylmuramide glycanhydrolase) (Fleming 1922). Lysozyme is a 139 amino acids cationic protein found in neutrophils, macrophages, saliva, mucous, egg white, in milk and in additional body fluids. A diagnosis of myeloid leukaemia was helped by measuring lysozyme in urine using a suspension of *Micrococcus lysodeikticus* as a highly sensitive substrate (Ginsburg un-published data). It was anticipated that lysozyme might kill, lyse and biodegrade all pathogenic microorganisms. Surprisingly, however, while lysozyme can very rapidly (within 1–2 min) lyse certain non-pathogenic gram-positive cocci (e.g. *M. lysodeikticus*) and also spore-bearing aerobic bacilli) possessing a "simple" peptidoglycan – PPG, lysis of *S. aureus*, which possesses a more complex peptidoglycan, may take up to 6–12 h and is effective mainly in acidic buffers (Figures 1 and 2). This allows protonation of lysozyme making it more cationic. It is also of clinical significance that lysozyme rarely lyses haemolytic streptococci, *Streptococcus viridans*, and Listeriae species, but does it very slowly with enteric bacteria. However, while *S. aureus* was freely lysed by lysozyme (Wecke et al. 1982), group A streptococci could be partially lysed by a synergism among lysozyme, lysolecithin (LL) and phospholipase C (Lahav et al. 1979). Also, haemolytic streptococci cultivated in presence of sub-inhibitory concentrations of penicillin-G lost their membrane-associated phospholipids to a large extent following treatment with small concentrations of LL and lysozyme (Efrati et al. 1976). Neutrophil extracts, rich in many lysosomal hydrolases and lysozyme, released LTA from group A streptococci and *Streptococcus mutans* (Sela et al. 1977). Human neutrophils, which had been incubated with LTA from haemolytic streptococci, generated large amounts of superoxide and H_2O_2 when challenged with anti-LTA antibodies. This was further potentiated by proteinases (Ginsburg et al. 1988). LTA released from group A streptococci may also further interact with bystander non-sensitized PMNs resulting in enhanced oxidants generation. Damage can also be inflicted to fibroblasts and to epithelial cells treated by anti-LTA and by proteases. PMNs incubated with LTA also released lysosomal enzymes following treatment with anti-LTA antibodies (Ginsburg et al. 1988). In general, while lysozyme alone failed to induce bacteriolysis, it can nevertheless do it synergistically if combined with oxidants and with other hydrolases, released from activated PMNs. The main reason for the high resistance to lysozyme of *S. aureus* and perhaps also of many other pathogenic microorganisms, may be ascribed to the presence in their peptidoglycans of *O*-acetyl groups, which hinder the interaction of lysozyme with the *N*-acetylglucosamine–*N*-acetyl muramic acid (NAG–NAM) linkages in the PPG (Wecke et al. 1982). However, *in vitro*, deacetylation by mild alkaline solutions rendered such cell-walls highly digestible by egg-white lysozyme

(Wellman-Labadie et al. 2008). Nevertheless, if neither neutrophils nor macrophages engulfing pathogenic bacteria can shake off the *O*-acetyl groups from the bacterial cell-walls (Wecke et al. 1982), it may explain why their un-degraded rigid walls persist for long periods within phagolysosomes to contribute to chronic inflammation. Taken together, it may be concluded that the activity of lysozyme may not be associated with its enzymatic activity but rather due to its cationic properties. Like other highly basic anti-microbial peptides, lysozyme is capable of activating autolytic wall enzymes in bacteria (Ginsburg 1987; Cottagnoud and Tomasz 1993; Ginsburg 2002a) but being highly cationic, these agents may also function as double-edge swords also to injure mammalian cells (Ginsburg and Koren 2008) (see below Section 11 dealing with histones and endothelial cells in sepsis).

8. Pathological changes induced by microbial cell-wall components

A large series of important pioneering investigations by Schwab et al. at the University of North Carolina had studied in great detail the interaction of peptidoglycan polysaccharide polymers (PG-APS) of group A streptococci in arthritis models (Schwab et al. 1967; Ginsburg and Trost 1971; Cromartie et al. 1977; Clark et al. 1979; Dalldorf et al. 1980; Fox et al. 1982; Janusz et al. 1984; Leong et al. 1984; Esser et al. 1986; Stimpson et al. 1986; Schwab 1993). Only a few articles will be described in some detail. Arthritis was induced in rats by a single intraperitoneal injection of an aqueous suspension of PG-APS fragments derived by sonication. This was followed by a chronic, remittent, erosive arthritis lasting several months (Schwab 1993). The intraperitoneal injection of peptidoglycan–carbohydrate fragments from group A streptococci produces a chronic, poly-articular, erosive synovitis in rats (Stimpson et al. 1986). The cell-wall material accumulates rapidly in the liver, spleen, and lymph nodes, where it caused little injury. At the same time, selective localization and persistence of the material in the synovial and periarticular tissues occurs. Its presence in the joint was associated with acute and recurrent inflammation with focal synovitis, pannus formation, joint destruction, and ankylosis. Cell-wall fragments become localized in the synovial and periarticular tissues at a time when there were leukocytes in the blood stream, which appear to contain the material (Janusz et al. 1984). Another paper focusing on infection theory was published in 1971 (Ginsburg and Trost 1971) stressing the ability of non-biodegradable wall components to persist and induce chronic relapsing joint lesions. Intact and un-degraded bacterial walls can be translocated to inflammatory sites and to be involved also in arthritis. Rabbits were first injected intra-articularly by the streptococcal toxin streptolysin S to induce acute damage. Then, streptococci labelled with FITC were injected into their tonsils. One day later, streptococci were found to localize in the injured joints (Rickles et al. 1969). We also found persistence of labelled streptococci in the heart and muscle of mice (Ginsburg et al. 1969; Ginsburg and Trost 1971). In these experiment rabbits were injected intravenously with extracellular products ("toxins") of group A streptococci to induced myocardial, muscular, and hepatic lesions. When such animals were then challenged with FITC-labelled group A streptococci or with titanium oxide particles, the labelled bacteria and the particles localized within phagocytic cells in the tissue lesions induced by the toxins. It is therefore proposed that a combined mechanism of injury and localization of bacteria in damaged tissues may be responsible for post streptococcal sequelae and other chronic inflammatory diseases. This led in 1977 to a publication of a review article under the title: "Can chronic and self-perpetuating arthritis in the human be caused by arthrotropic un-degraded microbial cell wall constituents? A working hypothesis" (Ginsburg 1977).

9. Role of bacteriolysis in inflammation, infection and in sepsis

Clinically, observations on the bacteriolysis phenomenon date back to 1895 when the Jarisch–Herxheimer phenomenon (Belum et al. 2013) was described. It showed that patients suffering of syphilis who were treated with mercury and also later on with other drugs including certain antibiotics caused the release from dying spirochaets of a "Toxin". This cascade caused a clinical syndrome composed of some of the following features: Abrupt onset of fever, chills, myalgias, tachycardia, vasodilatation with flushing, exacerbated skin rash, or hypotension. Amazingly, these symptoms are nearly identical with those seen today in gram negative and gram positive septic patients who were administered intravenously with certain bacteriolytic beta lactams, where release of LPS from gram negatives occurred (Shenep et al. 1988; Hurley 1992, 1995; Prins et al. 1994). While endotoxin release secondary to antibiotic therapy for gram-negative bacteria likely occurs, its significance has been difficult to detect, when specifically looked for. In one study clear differences in endotoxin levels and pro-inflammatory cytokine levels were detectable between highly bacteriolytic, ceftazidime treatment vs. low level bacteriolytic imipenem treatment, but its clinical relevance was not different in

terms of mortality or other clinical outcomes (Simpson et al. 2000). This tended to aggravate patients conditions and raises the important question whether these antibiotics should be avoided?

10. Role of microbial cell-wall components in sepsis

The rapid development of resistance to antibiotics lead to an increase in the annual incidence of sepsis and septic shock diagnosed in the USA in more than 750,000 hospitalized patients with a mortality of about 35%. Worldwide, sepsis is one of the common deadliest and the least understood disorder and globally, 5–10 million patients are estimated to be afflicted every year (Opal and Cohen 1999; Angus and Van der Poll 2013; Liesenfeld et al. 2014; Opal 2014). Septic shock can be defined as a multi-factorial synergistic event involving the invasion of the blood stream by various microbial species, which upon bacteriolysis, release cell-wall components. These can activate neutrophils (PMNs) and coagulation cascades, especially when the exaggerated immune responses also go into overdrive, as they try to fight an infection (Opal and Cohen 1999; Angus and Van der Poll 2013; Liesenfeld et al. 2014; Opal 2014; Koren and Ginsburg 2015). In sepsis, no single major alarmin-virulence factor has been identified in pathogenicity which, if specifically inhibited, might prevent patients' demise (Koren and Ginsburg 2015).

Screening the voluminous literature on sepsis treatment showed that over the years all repeated efforts to save patients' lives by administering antibiotics combined only with singly selected antagonists, had failed and even the most promising activated protein C, the "miracle drug", had been removed from use. Today, there is no specific effective treatment for sepsis and septic shock (see below). Despite improved critical care, the incidence and mortality from severe sepsis remains unacceptably high. However, a proposal was made in 1999 to try to replace single antagonists by cocktails of anti-inflammatory agents, remained un-noticed (Ginsburg 1999).

In 2000, an important symposium was held at the Rockefeller Institute (Horn et al. 2000) in which the discussants were requested to evaluate the role played by the major microbial cell-wall components, LPS, LTA, PPG and combinations among them, which had been released by bacteriolysis as major agents implicated in the pathogenesis of sepsis. The participants were requested to comment and discuss on the question "is sepsis a 1-hit, a 2-hit or a multiple hit (synergistic) episode among the microbial components as major events

in the pathogenesis of sepsis?". Surprisingly, none of the publications arguing the role of synergism in post-infectious sequelae had been mentioned by the participants (Ginsburg and Kohen 1995a). Also discussed whether antibiotics can induce the release of pro-inflammatory constituents from both Gram negative and Gram positive microbes to offer a potential opportunity to limit the extent of inflammation by selection of antibiotics. This is based on how microbes are killed or inhibited from growth, as well as the extent to which the host mounts an inflammatory response to the antibiotic–microbe interaction. Experimental evidences support the concept that different antibiotics that manifest similar efficacy in their ability to kill gram-negative microbes may show significant differences in their capacity to cause the release of soluble endotoxin. Multiple experimental *in vitro* and *ex vivo* studies have documented the major differences among antibiotics with respect to endotoxin release, production of endotoxin-induced pro-inflammatory cytokines, induction of cytokines, and differential levels of survival in experimental animal models of gram-negative sepsis in response to antibiotic chemotherapy (Horn et al. 1996). Moreover, additional studies suggested that experimental approaches used to assess antibiotic therapeutic efficacy within the framework of microbe-induced inflammatory mediators may also extend to gram-positive microorganisms (Horn et al. 2000). In these respects, the Jarisch–Herxheimer phenomenon (Belum et al. 2013) raises a very important dilemma whether highly bacteriolytic antibiotics could seriously aggravate sepsis patients' conditions and therefore, should be avoided. However, there are conflicting ideas on this subject as clinicians at emergency facilities and in ICUs argued that the mortality rates among septic patients administered bacteriolytic agents were not significantly greater than those receiving non-bacteriolytic antibiotics. However, since surviving patients who are exposed to constant and prolonged infusions of antibiotics, which also can lyse microorganisms, may also develop unanticipated metabolic changes and also injury to internal organs. Indeed, one common complication in gram negative sepsis is tubular necrosis (Richman et al. 1981; Cunningham et al. 2002). This is when circulating microbial cell-wall agents, mainly PPG released by antibiotics, may generate toxic cytokines such as TNF alfa and additional toxic agents generated by PMNs. Surprisingly, after publishing the minutes of the Rockefeller Symposium from 2000, it was anticipated that new articles supporting or refuting the synergism concept of sepsis pathogenicity be published but as of today, no related publication has appeared in the clinical literature.

11. Histones as possible major participant in the pathophysiology of septic shock

A possible turning point in the understanding of the pathophysiology of septic shock was offered in 2009 by two teams of investigators, who published their papers in Nature Medicine (Chaput and Zychlinsky 2009; Xu et al. 2009). These authors suggested that microorganisms invading the blood steam either via catheters or open wounds interact with recruited neutrophils (PMNs) adhering to endothelial cells. As a result, PMNs shed out oxidant-depended nets (Branzk and Papayannopoulos 2013) rich in highly bactericidal nuclear histones, which injures and causes dysregulation of the endothelial cells. This was claimed to be the main cause of death in hospitalized sepsis patients. However, these events could be inhibited to some extent either by antibodies to histone, activated protein C or by heparin (Chaput and Zychlinsky 2009; Xu et al. 2009). Heparin is however problematic because of its bleeding effects. Unfortunately, the promising activated protein C was finally removed from use because it failed to significantly affect patents demise (Opal 2014). However, in the future it may be possible that the use of a non-anti-coagulant heparin might prove safer than regular heparin and more efficient since it can still retain its ability to neutralize histones toxic effect (Wildhagen et al. 2014). At this point, it may interest the readers that toxicity of histone and of additional polycations to human umbilical cord endothelial and to additional cell types in culture was found to be markedly further enhanced, especially if combined synergistically with oxidants and proteinases. These studies had been published years ago (Varani et al. 1989; Ginsburg et al. 1992; Varani et al. 1992; Ginsburg et al. 1993; Ginsburg and Kohen 1995a, 1995b) but rarely cited in sepsis literature.

Taken together, it is unreasonable to accept that the "exclusive" release of a unique toxic histone – alarmin from activated PMNs nets adhering to endothelial cells can be the main cause of death in sepsis as reported (Chaput and Zychlinsky 2009; Xu et al. 2009). Is it not more sensible to assume that highly toxic histone and additional toxic polycations generated by activated PMNs such as LL-37, elastase and cathepsins may actually act in synergy with the plethora of pro-inflammatory agents also discharged from activated PMNs? Thus, very possibly, heparin and especially non anti-coagulant heparin (Wildhagen et al. 2014) may inhibit the synergy among histone and the additional agents generated by activated PMNs. However, histones toxicity may also be further amplified by combination with microbial cell-wall products released. Therefore, cocktails of anti-inflammatory agents (Ginsburg 1999) may be more effective if also combined with antibodies to microbial cell-wall components. However, the possible unique role of histone claimed in 2009 to be the main agent causing death in sepsis (Chaput and Zychlinsky 2009; Xu et al. 2009) is also seriously questioned. This is because since 2009, many clinical publications unrelated to sepsis had also reported the presence of high-levels of circulating histones in plasma in many clinical disorders (Kutcher et al. 2012; Zhang et al. 2013; Hirose et al. 2014; Ward and Grailer 2014; Zhang et al. 2015; Alhamdi and Toh 2016). These findings raise a crucial question whether circulating highly toxic cationic histones are real unique alarmins or just additional markers of tissue damage (Ginsburg et al. 2016). Surprisingly, we could not find the terms histone or bacteriolysis in any of the numerous articles on sepsis published since 2009 in Critical Care Journals. Also, we could not find either the term histone or bacteriolysis in the consensus report on sepsis, published in JAMA in 2016 (Singer et al. 2016). Is it possible that the histone story may still be considered too "novel and pre-mature" to be appreciated and considered in therapy of sepsis?

12. Epilogue

Today, after the demise of activated protein C (Opal 2014), numerous strategies focusing on the pathophysiology of severe sepsis are being pursued (Pugin 2008; Lehár et al. 2009; Martinez de Tejada et al. 2012; Vincent and Beumier 2013; Ekaney et al. 2014; Marini et al. 2015; Schork 2015; Douglas and Roussel 2016; Flores and Guillen-Guio 2016; Maslove and Marshall 2016; Russell 2016). Bacteria and bacterial products such as LPS, LTA and PPG released following bacteriolysis (Horn et al. 2000; Ginsburg 2002a; Ginsburg et al. 2015) are appreciated as central in the pathophysiology of sepsis. During gram-positive and negative infections, PPG and LPS which reach the circulation, possibly by bacterial breakdown, induce nearly all the classical features of infectious illness and may cause systemic inflammation with organ failure in animal models. PPG and LTA interact with the innate immune system through receptors mainly expressed on monocytes/macrophages but may also induce inflammatory changes in other cell types as well (Kengatharan et al. 1998; Myhre et al. 2006). Therefore, PPG and LTA may perhaps be targeted via antibodies. LTA may also bind to neutrophils which in presence of anti LTA antibodies induces the release of superoxide and H_2O_2 (Ginsburg et al. 1988).

Taken together, it stands to reason that bacteriolysis is not a mere laboratory curiosity but an active seminal

phenomenon in sepsis and septic shock. Nevertheless, suggesting that the main cause of death in sepsis may be the unique release from PMNs nets of highly toxic histone is un-reasonable. This is because concomitantly with the release of histone, numerous additional pro-inflammatory agents are also discharged from activated PMNs adhering to endothelial cells, which can synergize with histone to injure the endothelium (Varani et al. 1989; Ginsburg et al. 1992; Varani et al. 1992; Ginsburg et al. 1993; Ginsburg and Kohen 1995a, 1995b). Furthermore, substantial amounts of the microbial cell-wall components LPS, LTA and PPG released by beta lactams may also be found in the circulation of sepsis patients, which can also synergize with histone and with the numerous leukocyte pro-inflammatory agents released by activated PMNs. Such combinations may perhaps be targeted by non-anti-coagulant heparin (Wildhagen et al. 2014), with cocktails of antagonists and with immunoglobulins rich in antibodies to TH1 cytokines and microbial cell-wall components (Ginsburg and Kohen 1995a; Ginsburg 1999; Koren and Ginsburg 2015).

The main pro-inflammatory cytokines produced during an infection are tumour necrosis factor-α (TNF-α), interleukin-1α (IL-1α), IL-1β, IL-12 and IL-18. They transmit danger signals, which alert the various components of the host defence. As proposed by Netea et al. (2003) "innate deficiency in cytokine release during acute severe infections leads to a rapid multiplication of the invading microorganism, and to a secondary reaction of the host consisting of systemic inflammatory and anti-inflammatory reactions [systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS)], which could ultimately lead to shock and death".

We argue that septic shock is a distinct multifactorial synergistic episode (Ginsburg and Trost 1971; Richman et al. 1981; Ginsburg and Kohen 1995a; Ginsburg 1999; Horn et al. 2000; Cunningham et al. 2002; Chaput and Zychlinsky 2009; Xu et al. 2009; Koren and Ginsburg 2015), in which no unique alarmin is generated, and which if successfully inhibited might stop the toxic cascades. Currently, clinical efforts are made by testing various signalling antagonists. Excessive inflammation is being addressed with the use of specific blocking antibodies for injurious cytokines as well as devices and molecules that address the pro-inflammatory cytokine pathways. Replacement strategies for protective molecules exhausted during the sepsis episode are being examined and strategies to reverse the immunosuppression of sepsis are being revived (Pugin 2008; Lehár et al. 2009; Martinez de Tejada et al. 2012; Vincent and Beumier 2013; Ekaney et al. 2014; Marini et al. 2015;

Schork 2015; Douglas and Roussel 2016; Flores and Guillen-Guio 2016; Maslove and Marshall 2016; Russell 2016). Finally, as a cautionary note, the main stumbling block for an effective treatment of sepsis is the relatively late arrival of patients to ICUs when effective early treatments can be instituted. Unfortunately, at that time "many of the main pro-inflammatory horses have already left the stables". Prompt diagnosis, intervention and risk assessment are critical in caring for septic patient but remain difficult with currently available methods. Biomarkers may become useful adjuncts to clinicians and ultimately serve as targets for future therapeutic trials in sepsis. The most relevant markers were previously reviewed by Vincent and Beumier (2013) and include interleukin-6, C-reactive protein, pro-calcitonin, triggering receptor expressed on myeloid cells-1, and biomarker panels (Xing et al. 2008). Also, many biomarkers have been proposed and assessed clinically, but none alone is specific enough to definitively determine diagnosis. The future direction of research is most likely a greater focus on the use of panels or combinations of markers with clinical signs (Lehár et al. 2009; Vincent and Beumier 2013).

We may hope that in the future, a series of new biomarker tests should be available not only for use in ICUs or at the office of the family physician but even in every first-aid household kit. One such an example may be a simple diagnostic devise to measure early markers similar to that used at home to evaluate blood glucose levels in diabetic patients. These will definitely shorten the time to allow better diagnosis and an earlier effective treatment in ICUs.

Finally, the bacteriolysis phenomenon, which can occur either following treatment by certain antibiotics, by cationic peptides released from activated neutrophils or from injured tissues, is not a mere laboratory curiosity. Therefore, it should be considered in future clinical treatments of post infectious sequelae (Ginsburg and Lahav 1983; Ginsburg 2002a).

Disclosure statement

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