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MiniReview

Can we learn from the pathogenetic strategies of group A hemolytic streptococci how tissues are injured and organs fail in post-infectious and inflammatory sequelae?

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Abstract

The purpose of this review-hypothesis is to discuss the literature which had proposed the concept that the mechanisms by which infectious and inflammatory processes induce cell and tissue injury, in vivo, might paradoxically involve a deleterious synergistic 'cross-talk', among microbial- and host-derived pro-inflammatory agonists. This argument is based on studies of the mechanisms of tissue damage caused by catalase-negative group A hemolytic streptococci and also on a large body of evidence describing synergistic interactions among a multiplicity of agonists leading to cell and tissue damage in inflammatory and infectious processes. A very rapid cell damage (necrosis), accompanied by the release of large amounts of arachidonic acid and metabolites, could be induced when subtoxic amounts of oxidants (superoxide, oxidants generated by xanthine-xanthine oxidase, HOCl, NO), synergized with subtoxic amounts of a large series of membrane-perforating agents (streptococcal and other bacterial-derived hemolysins, phospholipases A_2 and C, lysophosphatides, cationic proteins, fatty acids, xenobiotics, the attack complex of complement and certain cytokines). Subtoxic amounts of proteinases (elastase, cathepsin G, plasmin, trypsin) very dramatically further enhanced cell damage induced by combinations between oxidants and the membrane perforators. Thus, irrespective of the source of agonists, whether derived from microorganisms or from the hosts, a triad comprised of an oxidant, a membrane perforator, and a proteinase constitutes a potent cytolytic cocktail the activity of which may be further enhanced by certain cytokines. The role played by non-biodegradable microbial cell wall components (lipopolysaccharide, lipoteichoic acid, peptidoglycan) released following polycation- and antibiotic-induced bacteriolysis in the activation of macrophages to release oxidants, cytolytic cytokines and NO is also discussed in relation to the pathophysiology of granulomatous inflammation and sepsis. The recent failures to prevent septic shock by the administration of only single antagonists is disconcerting. It suggests, however, that since tissue damage in post-infectious syndromes is caused by synergistic interactions among a multiplicity of agents, only cocktails of appropriate antagonists, if administered at the early phase of infection and to patients at high risk, might prevent the development of post-infectious syndromes.

Keywords: Streptococcus; Toxin; Lysosomal hydrolase; Synergism; Post-infectious sequela; Sepsis

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1. Prologue

Since the discovery of hemolytic streptococci by Billroth and Ehrelich in 1877 (see [1]) this elusive and enigmatic microorganism has occupied the attention of numerous investigators who attempted to unveil the mysteries behind its role in so many apparently diverse clinical manifestations. These span all the way from mild pharyngeal infections to severe debilitating and often fatal manifestations such as rheumatic fever, acute glomerulonephritis and culminating in myositis, fasciitis, better known today as the 'flesh-eating syndromes', septic shock and multiple organ failure [2–10]. Furthermore, the fact that group A hemolytic streptococcal cell wall and cytoplasmic structures also share common antigens with human tissues (molecular mimicry) might explain the pathogenesis of rheumatic fever and acute glomerulonephritis [7] both categorized as autoimmune and 'horror autotoxicus' phenomena.

The autoimmune nature of rheumatic fever and nephritis has led to attempts to develop an appropriate vaccine which, together with penicillin treatment, might prevent and also eradicate these post-infectious complications (discussed in [2,4,5,7]). On the other hand, no adequate therapies for the 'flesh-eating syndromes' have been developed. What makes streptococci so versatile is still enigmatic. However, the possibility that tissue injury following severe streptococcal infections might be induced not only as a result of synergism among their own metabolites, toxins and cell wall components, but mainly when these agents engage in a deleterious 'synergistic cross-talk' with the host's own defense systems, is paradoxical, but also highly realistic [2,11–14]. It is also important to note that an effective prevention of post-infectious and inflammatory sequelae, whether caused by Gram-positive or by Gram-negative bacteria, might depend on the development of adequate, quick, and inexpensive predictive markers, either in the outpatient clinic or at the bedside, which might herald their invasion of the bloodstream. Taken together, it is tempting to speculate that a deeper analysis of the pathophysiological events in streptococcal infections and their sequelae might also serve to evaluate the role played by synergism among a multiplicity of pro-inflammatory mediators in tissue damage caused by other types of microorganisms which may often result in sepsis, systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF) and the 'flesh-eating syndromes'.

1.1. Streptococcal hemolysins interact with oxidants and proteinases to kill targets

The notion that a 'cross-talk' among streptococcal toxins and proteinases and also among host-derived oxidants and several of the membrane-damaging agents and proteinases generated in infectious and

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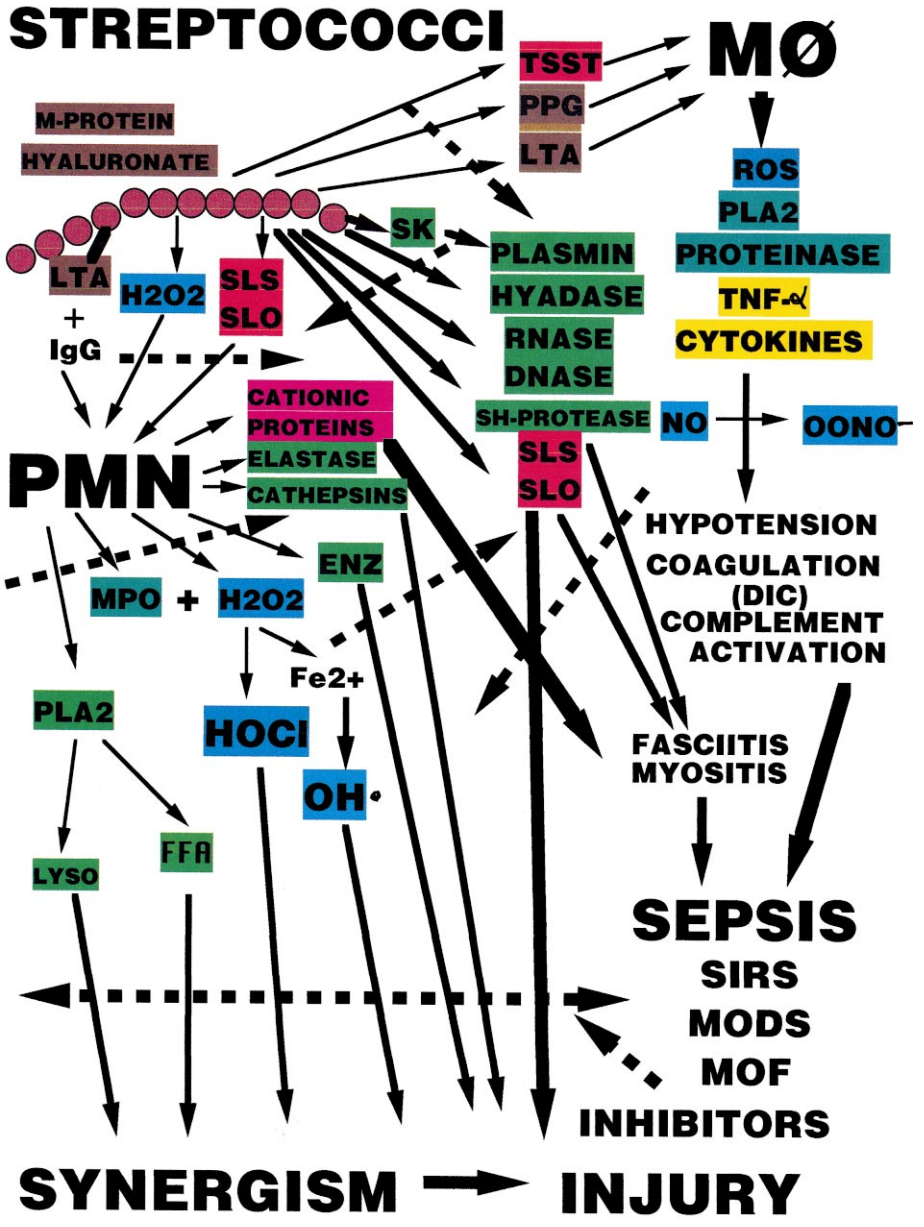
Fig. 1. Synergistic interactions in streptococcal sepsis. M-protein and -hyaluronate-positive group A hemolytic streptococci might adhere to target cells via LTA. Metabolizing catalase-negative streptococci might then release large amounts of H_2O_2, the highly cytolytic and cardiotoxic SLO, the non-immunogenic SLS, toxic shock syndrome toxin (TSST), streptokinase (SK) which can convert plasminogen to plasmin, SH-proteinase, the spreading enzymes hyaluronidase (HYADASE), ribonuclease (RNASE), deoxyribonuclease (DNASE) which might facilitate bacterial spreading causing cell injury, myositis, fasciitis and gangrene. Streptococcal SLO, SLS and binding of LTA-IgG complexes can degranulate PMNs to release lysosomal hydrolases (ENZ), activate NADPH-oxidase resulting in the accumulation of H_2O_2, which together with myeloperoxidase (MPO) catalyzes the formation of HOCl. H_2O_2 together with Fe^{2+} can generate the highly toxic OH radical. The PMNs also release the highly cationic and cytotoxic elastase and cathepsin G as well as bactericidal and cytotoxic cationic proteins. Secreted PLA_2 might release FFAs and highly cytolytic lyso-compounds (LYSO). All the extracellular agents might synergize among themselves to amplify cell damage. Chemotactic agents released at the site of streptococcal proliferation might recruit macrophages (MO), might be activated by streptococcal LTA, TSST and PPG to generate reactive oxygen species, PLA_2, proteinases, nitric oxide (NO) which might induce severe hypotension. NO might also interact with PMN-derived superoxide to form the highly cytotoxic, peroxynitrite ($OONO^-$). The activated cells might also generate large amounts of TNF-α and cytotoxic and chemotactic cytokines which trigger the activation coagulation (disseminated intravascular coagulopathy (DIC)) and the complement cascades. These multiple interactions might lead to septic shock, SIRS, MODS, and MOF. Since tissue damage is most probably caused by multiple synergistic interaction, and since clinical trials which had used single antagonists to prevent the onset of sepsis had invariably failed to prolong the lives of septic patients, only 'cocktails' of antagonists might prove beneficial to control sepsis and the flesh-eating syndromes. The dashed arrows indicate which pathways of the inflammatory processes might be inhibited by certified anti-inflammatory agents. Agents coloured: red, toxins; blue, oxidants; brown, streptococcal cell wall-associated agents; green, enzymes; yellow, cytokines; violet, cationic protein.



inflammatory foci originated in 1959 [15]. Catalase-negative group A hemolytic streptococci, which produced ample amounts of H_2O_2 and which also expressed on their surfaces the potent cell-bound membrane perforator streptolysin S (SLS), collaborated in a synergistic manner with a streptococcal serine proteinase to injure and disintegrate Ehrlich ascites tumor cells (a three-component synergistic system) (Fig. 1). These findings have recently been fully corroborated by showing synergy between streptolysin O (SLO) and a streptococcal proteinase in cell injury [16,17]. These observations established the basis for the "synergism concept of cellular injury in infectious and inflammatory processes" [13,14]. It hypothesizes that the catalase-negative hemolytic streptococcus, and the gangrene-inducing clostridial

species, seem to share with activated phagocytes strikingly similar pathogenetic properties. Both the bacteria and phagocytes possess adhesion molecules; they elaborate spreading enzymes (hyaluronidase, proteinases, nucleases) and secrete membrane-perforating toxins and enzymes. They both destroy extracellular matrix proteins and also spread to remote tissue sites. But above all, they inflict a deadly blow on cells by synergizing among themselves. Hemolytic streptococci also share common antigens with the human heart, kidney, connective tissue and brain (molecular mimicry) which might explain some of the autoimmune features of post-streptococcal sequelae. These observations led us to speculate that these microbial species and perhaps also certain of the Gram-negative toxigenic bacteria might perhaps be considered some kind of 'forefathers' of modern phagocytes (a converging evolutional phenomenon which does not necessarily involve the generation of identical gene products) [12].

These speculations led to a series of investigations which analyzed the role in cell damage of 'cocktails' composed of agents generated either by microorganisms or by the host's own defense systems. These included: streptococcal hemolysins and their cell wall components, reactive oxygen and nitrogen species, phospholipases, fatty acids, proteinases, cationic polyelectrolytes, cytokines and additional pro-inflammatory agents. All these agents might be simultaneously present in the milieu of infection and inflammation. It is believed that this approach might also shed new light on the complex events which lead to sepsis, septic shock, SIRS, MOF and adult respiratory distress syndrome (ARDS) (Fig. 1).

However, to protect against the synergistic effects of microbial and host-derived agonists there is urgency to develop novel, safe, and practical therapeutic approaches to deal with the deleterious effects caused by multifactorial systems, without at the same time compromising patients' lives. It is also important to stress that since inflammation is a double-edged sword [18,19], this issue constitutes a serious challenge to the future development of novel therapeutic measures to control severe infections and their sequelae.

The present review-hypothesis analyzes the publications which supported the supposition that tissue damage in infectious and inflammatory complications, whether caused by Gram-positive, Gram-negative [20] or other types of microbiota, involves a paradoxical synergistic deleterious 'cross-talk' among microbial and host-derived defense systems (Fig. 1).

2. Synergistic interactions among a multiplicity of pro-inflammatory agonists

2.1. The role of oxidants

Since the discovery of the respiratory burst in phagocytes [21–34], oxidants have been suspected and also claimed to be dominant pro-inflammatory agonists, and research efforts to unravel the mechanisms of cell damage in inflammation have focused mainly on the role of reactive oxygen species (ROS). This stemmed from the following observations. (a) In more than 100 human diseases, there was evidence for an enhanced generation of ROS either before or during the development of symptoms [25]. (b) Patients suffering from chronic granulomatous disease syndrome whose neutrophils (PMNs) and macrophages possess a defective oxidase [31], kill either catalase-positive bacteria or mammalian targets only with great difficulty. Nevertheless, catalase-negative bacteria (e.g. streptococci) are readily killed by CGD phagocytes presumably because the bacteria supply the missing H_2O_2 (a distinct collaboration). These observations, however, also suggested that some kind of beneficial/adverse cooperation between phagocytes and bacteria might take place (see below). (c) Supplementation of antioxidants markedly enhanced resistance to tissue damage caused by oxidative stress [28]. (d) Exposure of cells in culture to oxidants results in a steep decrease in ATP and antioxidant thiols which was accompanied by DNA damage characteristic of post-ionizing irradiation effects and by the inability to repair nuclear damage. These injuries could however be prevented by the supplementation of thiols and of additional antioxidants (reviewed in [33]).

It is also important to stress, however, that most of the reports indicated that killing targets in vitro, in media containing amino acids but devoid of serum proteins, necessitated the employment of millimolar (non-physiological) amounts either of H_2O_2 or of HOCl [34]. This was ascribed to the presence, in all

mammalian cells, of large amounts of a variety of membrane-associated antioxidants, mainly catalase. This was proven by the ability of the catalase inhibitors sodium azide and aminotriazole to destroy heme proteins and thus allow micromolar amounts of peroxide to injure the cells. A tight adhesion of activated phagocytes, via adhesion molecules upon their targets, was needed to deliver a 'kiss of death' via a synergism among their secreted agonists. This created a niche which eliminated inhibitory agents present in the medium, e.g. sulfur-containing amino acids, taurine, and proteinase inhibitors, which could inhibit both HOCl and elastase [34].

2.2. The role of combinations of oxidants, membrane perforators and proteinases

While large amounts of oxidants killed cells in a short period, many publications also stated that most mammalian cells, tested in vitro, were relatively resistant to high concentrations of a variety of proteinases (trypsin, elastase, cathepsin G, plasmin). These were capable of detaching cells from matrices by destroying extracellular matrices, but rarely killed them [13,14].

Perhaps, either for convenience or due to the wish to avoid studying complex systems, most of the published models on cell injury, today, usually tested the potential pathogenetic role played by single agonists, one at a time. However, several publications since 1988 which focused on synergistic mechanisms as a plausible explanation for cell damage in infections and inflammation will be reviewed and briefly discussed (Fig. 1).

Ehrlich ascites tumor cells injured by complement-dependent cytotoxic antibodies (also a porin-forming system) were rapidly disintegrated upon the addition of streptokinase-generated plasmin [35]. Tumor cells could also be killed more efficiently and in a synergistic manner by combinations between the attack complex of complement and hydrogen peroxide [36]. A variety of mammalian cells treated with subtoxic, physiological (micromolar) amounts of a variety of oxidants (peroxide, ROO, OH, HOCl, NO, oxidants generated by xanthine-xanthine oxidase, paraquat, menadione) were nevertheless rapidly killed (^{51}chromium and ^{3}H-arachidonate release assays), in a synergistic manner, by the presence of any of a large series of subtoxic amounts of membrane-damaging agents. These included: SLS [36–41] and SLO [37], phospholipases A_2 (PLA_2) and C, lysophosphatides, free fatty acids (reviewed in [13,14]), the cationic proteins, histone, defensin [37,41,42,44], and the attack complex of complement [32], taurocholic acid, the xenobiotics ethanol, methanol and lindane [42,43]. The latter also primed neutrophils to generate enhanced amounts of ROS (reviewed in [43]). Streptococcal hemolysins were shown to permeabilize neutrophil lysosomes resulting in the release of lysosomal enzymes and cationic proteins from which might injure adjacent host cells (discussed in [13,14]).

Cell damage induced by combinations of oxidants and membrane perforators was dramatically further enhanced, also in a synergistic manner, by the inclusion of a variety of subtoxic amounts of proteinases, e.g. plasmin, trypsin, elastase, carboxypeptidase [36,38–40]. Synergism among membrane perforators, oxidants and proteinases also resulted in the release of large amounts of arachidonic acid and metabolites However, it is of special note that maximal release of larger amounts of arachidonate always required the presence of a proteinase [38–40]. Therefore, it will not be surprising that other membrane-damaging agents, mainly of microbial origin, might also synergize with oxidants and proteinases to induce enhanced membrane injury [13,14]. Therefore it appears that the induction of even minor membrane damage was sufficient to overcome the potent antioxidant capacities of cells.

In a more recent study [45] diethyldithiocarbamate (DDC), a known anti-malarial agent, a SOD inhibitor and also a weak copper chelator, very significantly potentiated the killing of epithelial cells and the release of arachidonate by synergizing with a complex cocktail comprised of glucose oxidase (GO)-generated H_2O_2, peroxyl radical ($ROO^{\bullet}$), nitroprusside-generated NO, SLS, and histone. The role of a combination of exogenous PLA_2 and H_2O_2 to kill epithelial cells has been studied [46,47]. PLA_2, which was not cytolytic to epithelial cells, nevertheless became highly cytotoxic when combined with subtoxic amounts of peroxide. Peroxide treatment resulted in the removal from the cells of a large proportion of surface-associated glycosaminoglycans which exposed the 'naked' membrane to

an attack by non-cytotoxic amounts of PLA_2. It is also highly probable that arachidonate and additional fatty acids released by PLA_2 might also collaborate with peroxide to further enhance membrane damage as demonstrated with endothelial cells [36] (see below).

Cell damage induced by combinations of PLA_2 and peroxide was strongly inhibited by a novel non-penetrating PLA_2 inhibitor [47], a complex between carboxymethylcellulose and phosphatidylethanolamine (CME). CME also protected epithelial cells against killing by a highly cytotoxic cocktail comprised of SLS, SLO, peroxide, trypsin and DDC [48].

Neutrophil oxidants and elastase collaborated to induce cell injury [45–55] and to enhance the degradation of extracellular matrix proteolysis [56–58]. Neutrophil elastase and superoxide played an important role in leukotriene D4 (LTD4)-induced neutrophil-mediated increase in pulmonary microvascular permeability [59]. Cytolysis of target cells by PMA-activated neutrophils could result from the cooperative effects of oxygen radicals and a membrane-bound neutral serine proteinase [60]. Neutrophil-induced IgG binding due to the combined action of proteases and oxidants might explain the accelerated destruction of red blood cells in inflammatory diseases [61]. Proteinases derived from *Pseudomonas* strains extracted iron from transferrin which became available for the formation, by neutrophils, of the highly cytolytic hydroxyl radicals [62]. *Pseudomonas aeruginosa* proteinases collaborated with human neutrophil elastase to injure human respiratory epithelial cells [63]. Serine proteinases and defensins (neutrophil products) induced injury to lung epithelial cells and modulated interleukin-8 production by macrophages [64]. Neutrophil-derived oxidants and elastase were found to play a role in lipopolysaccharide (LPS)-mediated renal injury [65]. An enhancement of neutrophil-mediated injury to epithelial cells was induced by a leukotoxin derived from *Pasteurella haemolytica* [66].

Phospholipase C from *Clostridium perfringens* and *Bacillus cereus* elicited superoxide production by bovine PMNs which could injure adjacent targets [67] and PLC from *C. perfringens* also acted with oxidants to injure endothelial cells in culture [40].

Lysophosphatides 'primed ' human neutrophils for enhanced generation of superoxide [68] and, when combined with NO, enhanced cytolysis [69].

Cytosolic PLA_2 potentiated H_2O_2 cytotoxicity of kidney epithelial cells [70] and exogenous fatty acids modulated the function and cytotoxic responses to oxidant stress [71]. Arachidonic acid and additional free fatty acids synergized with peroxide, ROO and with trypsin to injure epithelial cells [36].

Tumor necrosis factor α (TNF-α) potentiated oxidant- and reperfusion-induced endothelial cell injury [72] and also altered the cytotoxic effects of peroxide on hepatocytes [73]. Neutrophil oxygen radicals also synergized with TNF and mono/polyunsaturated fatty acids [74]. The possibility that cytokines might also enhance the production and secretion of additional leukocyte-derived agonists (proteinases, cationic proteins, phospholipases, etc.) should be explored (Fig. 1). The mechanisms by which oxidants enhanced proteinase-mediated injury might be linked to the finding that oxidized proteins are more readily digested by proteinases [75,76]. Oxidants such as H_2O_2, which freely diffuse through the membrane, might also activate apoptosis, endogenous metalloproteinases [34] and also deplete anti-oxidant thiols to further amplify cell damage by apoptosis. However, a triad comprised of an oxidant, a membrane perforator and a proteinase seems to constitute a most powerful cell-damaging cocktail implicating its possible role in tissue destruction in infectious and inflammatory sites (see below). These findings also stress the paramount importance of 'anti-cocktail' therapies for post-infectious sequelae (see below and Fig. 1).

2.3. The role of microbial factors in apoptosis

Upon infection with pathogens, eukaryotic cells might undergo programmed cell death (apoptosis) as an ultimate response. Pathogens such as *Shigella flexneri* have the ability to induce apoptosis of macrophages which upon demise can elicit inflammation by releasing pro-inflammatory cytokines which might attract large numbers of neutrophils [77–79]. The molecular link between apoptosis and inflammation is the interleukin-1β-converting enzyme which is activated during macrophage apoptosis and binds to a secreted *Shigella* protein.

2.4. The role of microbial cell wall components and host-derived agonists

The main event which transpires following an invasion of the bloodstream whether by bacteria from the normal flora (opportunistic invasion) or by virulent microorganisms from exogenous sources is the encounter of their exotoxins (hemolysins, phospholipases) and of their cell wall components (LPS, lipoteichoic acid (LTA), peptidoglycan (PPG)) with humoral factor and particularly with phagocytic cells. These interactions are known to trigger the activation of deleterious cascades of reactive oxygen and nitrogen species, coagulation, cytolytic and chemotactic cytokines and the complement system. These multiple agents are thought to synergize among themselves to lead to severe sequelae such as sepsis, septic shock, SIRS, MODS and MOF. Several experimental models were devised to address these issues.

2.4.1. Experiments with LTA

This highly immunogenic but non-cytolytic agent can be released from Gram-positive bacteria either spontaneously or following treatment with neutrophil agents (see below). It binds to RBC membranes via its lipid moiety [7,8] and to endothelial cells via E-selectin. Human erythrocytes treated with proteinases (trypsin, papain), which presumably removed surface cell-associated glucose aminoglycans to expose membrane phospholipids [42], could bind excessive amounts of LTA [80]. Such cells were rapidly agglutinated and were also lysed by complement. Rabbits immunized with hemolytic streptococci, which developed high titers of anti-LTA antibodies, developed severe arthritis when LTA was injected into their knee joints [81]. LTA might then interact with antibodies to activate complement [82]. LTA-anti-LTA complexes caused massive degranulation of lysosomal enzymes from neutrophils and also induced the release of superoxide and peroxide generation [83]. However, a further enhancement of ROS generation also occurred if untreated PMNs were added suggesting that migrating neutrophils might amplify the respiratory burst by PMNs already coated with LTA. An amplification of superoxide generation by PMN-coated LTA was also observed when small amounts of nuclear histone were added together with anti-LTA IgG, suggesting that the cationic agent might have 'opsonized' the immunoglobulin and facilitated its interaction with the LTA-coated PMN surface. Enhanced amounts of superoxide and peroxide were also generated by PMNs which had been mixed either with LTA-coated fibroblasts or with LTA-coated epithelial cells suggesting that LTA could be presented to PMNs by non-neutrophilic cells provided that anti-LTA globulin was also present [83]. LTA-sensitized endothelial cells were killed in a synergistic manner by combinations of anti-LTA Ig and hydrogen peroxide.

LTA also stimulated the production of TNF-α and also caused shock [84], induced the production of TNF and NO [85,86] and also synergized with peptidoglycan to induce shock and MOF [86]. Finally, the presence of anti-LTA antibodies in sera of the population at large, and the fact that such antibodies cross-react with LTA derived from all Gram-positive bacteria, indicate that it might actively participate in tissue injury not only by 'sensitizing' cells to injury by antibodies and complement but also because of its capacity to stimulate macrophages to generate cytokines.

2.4.2. The role of bacteriolysis and cell wall PPG

An adverse collaboration among neutrophil and macrophage-derived agonists, microbial cell wall components, and certain antibiotics leading to enhanced tissue injury might occur in severe infectious conditions and in chronic granulomatous responses [13,14]. It is paradoxical, perhaps, that the neutrophil- and eosinophil-derived cationic proteins defensins, bacterial permeability increasing peptides [87], elastase, cathepsin G, and lysozyme (reviewed in [88–90]), which all function as distinct bactericidal agents, might also contribute to cell and tissue injury. Defensins acted in concert with peroxide to kill cells [44]. All these cationic agents and β-lactam antibiotics (reviewed in [91–93]) were capable of triggering the activation of autolytic wall enzymes (muramidases), resulting in bacteriolysis [88] and the massive release of LTA, LPS and PPG. Certain of the neutrophil-derived cationic agents might also mimic adhesion molecules capable of enhancing the binding of neutrophils to targets [94]. Histones and additional cationic polypeptides opsonized immune complexes to enhance tissue injury [95] and cationi-

zation of antigens might also increase their persistence in tissues causing chronic inflammation [89]. Polycations also acted as potent opsonins and as stimulators of the respiratory burst in human neutrophils [88,89], mimicking immunoglobulins [90]. Although enhancement of ROS generation by phagocytes might be beneficial for the host as it enhances the killing of virulent microorganisms, excessive amounts of ROS might also adversely injure host cells [13,14].

The role played by non-biodegradable microbial cell wall components (PPG-poly-PPG) as initiators of chronic inflammation and tissue destruction has been extensively reviewed [96–99]. Cell wall components of staphylococci, streptococci and mycobacteria are resistant to degradation by lysozyme and by additional lysosomal enzymes either because of the presence in the PPG of *O*-acetyl groups, (which deter lysozyme activity) or due to the presence in their cell walls of thick, impermeable layers of lipids and waxes (discussed in [98]). These properties contributed to the persistence, for long periods, of non-biodegradable wall components within macrophages which became 'chronically ' activated and caused low-turnover granulomas and to tissue destruction. Streptococcal cell wall components have also been shown to induce chronic arthritis in rats [100,101] which could be prevented by prior treatment with the muramidase mutanolysin, but not by lysozyme.

While staphylococci were readily autolysed, in vitro, by polycations (lysozyme, histones, elastase) presumably due to the activation of their autolytic wall enzymes (muramidases) (reviewed in [88]), no degradation of the cell wall occurred either within phagolysosomes of macrophages in culture [102] or in macrophages in granulomas [96–101]. This raised the suspicion that agents released into the phagolysosomes following phagocytosis might have interfered with the autolytic systems. Indeed staphylococci which had been treated in vitro either with H_2O_2 or with proteinases became highly refractory to autolysis by polycations [103]. Sulfated polyanions (heparin, dextran sulfate, suramine, Evans' blue, polyanethole sulfonate) very markedly inhibited autolysis of staphylococci induced either by cationic agents [104,105] or by β-lactam antibiotics [106–108]. This implies that in the milieu of inflammation the accumulation of sulfated polysaccharides due to the synergistic interaction between ROS and proteinases (see above) might retard microbial degradation. It is also of interest that sulfated polyanions inhibited a large variety of lysosomal hydrolases from neutrophils (reviewed and discussed in [98]). However, while on the one hand, the persistence of non-biodegradable cell wall components in tissues might initiate long-term destructive granulomas, on the other hand, inhibition of bacteriolysis, in vivo, might, paradoxically, also be beneficial as it might diminish the release of LTA, LPS and PPG from bacteria and thus might delay the onset of sepsis (see below). Non-biodegradable microbial cell wall components might also serve as immuno-adjuvants to enhance non-specific immunity against microbial infections and also against tumor cell proliferation [109,110]. One example is the capacity of a streptococcus-derived cell wall preparation, OK-432, to augment cytotoxicity to tumor cells by inducing the formation by blood monocytes of TNF-α, to induce synergism with rINF-α but at the same time to boost non-specific immunity to infections (discussed in [111]).

2.4.3. The role of LPS

Extensive studies on the chemical structure of LPS and its biological properties [112,113] led to an unprecedented explosion of basic science and clinical investigations to unravel its involvement in the initiation of sepsis and MOF. Numerous studies have also focused on the development of novel and sophisticated therapeutic strategies to cope with post-infectious sequelae (see below).

The mechanisms of LPS release from Gram-negative bacteria are still not fully known but bacteriolysis induced either by polycations [88] or by β-lactams [91–93] seem to be involved in this process during microbial invasion of the bloodstream. Undoubtedly, LPS is a scourge for clinicians because of its multiple and deleterious pathophysiological effects ranging all the way from neutrophil stimulation to release of excessive amounts of ROS [114], NO and cytotoxic cytokines which in turn activate macrophages to release enhanced amounts of ROS [115,116], to the activation of the cascades of coagulation and complement which might all act in synergy to induce sepsis, septic shock and eventually MOF (discussed and reviewed in detail in [116].

3. Why have effective therapies for post-infectious syndromes not been developed?

Paradoxically, perhaps, a coordinated deleterious 'cross-talk' among microbial exo- and endotoxins, bacterial cell wall components and host-derived agonists, might often occur in vivo. Therefore, the main dilemma which confronts clinicians today is the inability to effectively combat these complicated synergistic systems.

The reasons why treatment even with the most advanced and sophisticated anti-inflammatory strategies was not effective in sepsis, ARDS, ischemia-reperfusion, trauma, myositis, and fasciitis were recently extensively reviewed and discussed [116–131]. A recent review [132] also discussed the pros and cons of 33 different strategies which had been offered in the last few years to treat post-infectious complications. These therapeutic measures included: antibiotics, monoclonal and polyclonal antibodies against LPS, LPS binding proteins, polycations, interleukin-1 receptor antagonist, anticoagulants and inhibitors of complement, anti-adhesive molecules, inhibitors of reactive oxygen and nitrogen species, anti-proteinases, PLA_2 inhibitors, pentoxyfylline, tetracyclines, steroidal and non-steroidal anti-inflammatory agents, inhibitors of bacterial translocation from gut, hemofiltration techniques, and these in addition to conservative supportive measures.

Several explanations might be offered as to why therapeutic measures reported in numerous clinical trials of sepsis invariably failed to show any significant effects to control post-infectious sequelae caused especially by Gram-negatives. (1) Immune strategies might inhibit both deleterious (damaging host tissues) and beneficial (augmenting host defense) effects of inflammatory mediators. No easy solutions to cope with this dilemma have been found [116,117, 128,130,131]. (2) The lack of reliable, quick, and inexpensive early predictive markers to herald the onset of sepsis delays effective treatment to a point of no return [131]. (3) Most of the large-scale clinical trials in sepsis had attempted therapies by administering only one antagonist at at time [128,130–133]. Disappointingly, however, multidrug therapies achieved only a limited success [128]. Combination therapies might worsen the patient's condition [134]; therefore, the slogan "you are damned if you do, but you are also damned if you don't" might be very appropriate to express the frustrations and the helplessness of clinicians who witness their patients going into irreversible shock. It is of interest, however, that γ-globulin therapy has shown promise in the treatment of patients suffering from neonatal sepsis and toxic shock syndrome caused by Gram-positive toxigenic bacteria [135–140]. The relative success in therapy might presumably be ascribed to the fact that antibodies present in γ-globulin preparations could neutralize the main highly immunogenic, extracellular membrane-active factors (hemolysins, proteinases and spreading enzymes LTA+antibodies+complement and the toxic shock syndrome toxin which might act synergistically to injure issues [2,8,13, 14,48]. It might, however, also be speculated that based on the 'synergism concept of cellular injury', a successful therapy of the serious 'flesh-eating bacterial syndromes', and perhaps also of septic shock, might benefit from the use of antibiotics combined with 'cocktails' comprised of γ-globulin, anti-proteinases, anti-oxidants and anti-cytokines, i.e. a multidrug strategy [4,8,128,130,132].

To illustrate the complex interactions which might occur following the invasion of the bloodstream and also the soft tissues by virulent group A hemolytic streptococci Fig. 1 depicts which synergistic pathways of cellular injury might occur and which stages might perhaps be inhibited by combinations of appropriate anti-inflammatory agents. These might comprise γ-globulin as a potential source of antibodies to most if not to all of the extracellular products of hemolytic streptococci, including LTA and PPG, anti-oxidants, proteinase inhibitors, PLA_2 inhibitors, monoclonal and polyclonal antibodies against pro-inflammatory cytokines, inhibitors of LPS, anti-coagulants, inhibitors of NO synthase, polyanions to bind and neutralize the highly cytolytic cationic proteins, etc. Novel drugs which might be effective to attenuate bacteriolysis and the massive release of microbial cell wall components capable of activating macrophages to release cytokines and NO might help clinicians to control the deleterious consequences of post-infectious sequelae (Ginsburg, submitted).

4. Epilogue

Undoubtedly, the search for effective and safe 'magic bullets' to control post-infectious complications is, today, one of the most urgent and burning tasks of clinical medicine especially in the ailing antibiotic era. The hemolytic streptococcus might serve as a useful model to evaluate the role played by multiple synergistic interactions among pro-inflammatory agonists generated by the bacteria and by the host, as main causes of the deleterious sequelae seen after so many kinds of microbial infection. Therefore, there is an urgency to develop drugs and combinations of drugs which might be able to: diminish the translocation of bacteria from the gut into the bloodstream, attenuate and also control bacteriolysis induced either by cationic agents from phagocytes or by certain antibiotics to neutralize microbial hemolysins, toxins and endotoxins, oxidants, proteinases, phospholipases and the consequences of coagulation and complement activation. The recent reports that both the tyrosine kinase inhibitors tyrphostins [141,142] and dexabinol (HU-211), an anadamite derivative [143], were protective even if administered to animals several hours after delivering a lethal dose of LPS, are encouraging. Finally it is hoped that the establishment of a brains trust and 'cross-talk' among researchers in the basic and clinical sciences might provide the chance to devise safe and efficient therapies for a large group of post-infectious and often lethal manifestations against which we still stand helpless.

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