REVIEW ARTICLE

New paradigms in sepsis: from prevention to protection of failing microcirculation

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Summary. Sepsis, also known as septicemia, is one of the 10 leading causes of death worldwide. The rising tide of sepsis due to bacterial, fungal and viral infections cannot be stemmed by current antimicrobial therapies and supportive measures. New paradigms for the mechanism and resolution of sepsis and consequences for sepsis survivors are emerging. Consistent with Benjamin Franklin's dictum 'an ounce of prevention is worth a pound of cure', sepsis can be prevented by vaccinations against pneumococci and meningococci. Recently, the NIH NHLBI Panel redefined sepsis as 'severe endothelial dysfunction syndrome in response to intravascular and extravascular infections causing reversible or irreversible injury to the microcirculation responsible for multiple organ failure'. Microvascular endothelial injury underlies sepsis-associated hypotension, edema, disseminated intravascular coagulation, acute respiratory distress syndrome and acute kidney injury. Microbial genome products trigger 'genome wars' in sepsis that reprogram the human genome and culminate in a 'genomic storm' in blood and vascular cells. Sepsis can be averted experimentally by endothelial cytoprotection through targeting nuclear signaling that mediates inflammation and deranged metabolism. Endothelial 'rheostats' (e.g. inhibitors of NF-κB, A20 protein, CRADD/RAIDD protein and microRNAs) regulate endothelial signaling. Physiologic 'extinguishers' (e.g. suppressor of cytokine signaling 3) can be replenished through intracellular protein therapy. Lipid mediators (e.g. resolvin D1) hasten sepsis resolution. As sepsis cases rose from 387 330 in 1996 to 1.1 million in

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2011, and are estimated to reach 2 million by 2020 in the US, mortality due to sepsis approaches that of heart attacks and exceeds deaths from stroke. More preventive vaccines and therapeutic measures are urgently needed.

'The main factor in all inflammatory states consists in a lesion of the vessels which are attacked by the irritating cause.'(Elias Metchnikoff, 1845–1916) [1]

Current and emerging definitions of sepsis

Consistent with Metchnikoff's prescience, the key event in inflammation as a mechanism of disease is damage to blood vessels attacked by an 'irritating cause'. Runaway infections damage microcirculation, in which endothelium represents the main interface for blood-tissue exchange. Microcirculation comprises the smallest blood vessels, the pre-capillary arterioles, capillaries and post-capillary venules, embedded in the body's organs, thereby regulating blood flow, tissue perfusion and oxygenation, blood pressure and tissue temperature. Microvascular endothelium constitutes the largest surface area of human circulation [2] (Fig. 1). Hence, infections that cause a lesion in microcirculation can potentially compromise the function of multiple organs, including the lungs, heart, liver, gut, kidneys and brain, leading to hypotension and myocardial dysfunction, microvascular leak, thrombocytopenia without or with disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS), acute kidney injury (AKI) and acute brain injury. The common denominator for multi-organ dysfunction in sepsis is endothelial microvascular injury [3–6].

Therefore, the Working Group on Blood Systems Response to Sepsis convened by the NIH NHLBI Division of Blood Diseases and Resources in 2010 redefined sepsis
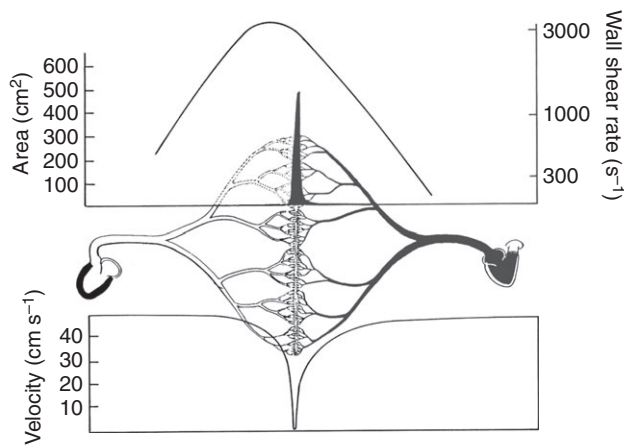
Fig. 1. A global view of circulation. Arterial circulation is on the left and venous on the right. Microcirculation comprises the greatest endothelial surface area (left axis of the upper panel), as represented by the central solid peak. The upper panel right axis represents wall shear rate (s^{-1}), with the curve reaching its highest values on the arterial side of microcirculation (pre-capillary arterioles). The lower panel comprises the velocity of blood flow, and indicates that the slowest values correlate with post-capillary venules, the terrain of white blood cell emigration (adapted from [2]).

as a severe endothelial dysfunction syndrome in response to intravascular and extravascular infections leading to reversible or irreversible injury to microcirculation responsible for multiple organ failure [7,8]. This definition emphasizes the central role of microvascular endothelial injury in systemic (intravascular) or localized (extravascular, e.g. urosepsis) infections by bacterial, fungal or viral agents that damage the integrity of microcirculation in multiple organs. It was developed amid growing dissatisfaction with the existing definition, proposed in 1992, which was 'a systemic inflammatory response to infection' (SIRS), popularly described as a 'cytokine storm' [9]. The 1992 definition has been repeatedly criticized as non-specific [10,11], while a decade later an attempted change was not introduced [12]. 'Systemic inflammatory response' is the mechanism of many acute and chronic diseases caused by non-microbial 'irritating causes', such as autoimmune, metabolic or physical insults. Yet, these patients usually do not exhibit the hypotension characteristic of septic shock. Hence, SIRS does not adequately explain the fundamental nature of sepsis, which is caused by insufficiently controlled bacterial, fungal and viral infections manifested by impairment or collapse of microcirculation. The collapse, known as septic shock, underlies multiple organ failure, culminating in hypotension that is refractory to resuscitation measures recommended by the Surviving Sepsis Campaign [13].

How microbial agents damage microcirculation and cause sepsis – from Ebola to MRSA

Microbial injury to microcirculation can commence through direct invasion of endothelial cells by microbial agents or indirectly through attack by their products ('virulence factors'). For example, the Ebola virus employs a virion glycoprotein that preferentially binds to endothelial cells, causing their death within 12–16 h [14]. To add insult to injury, the virus deploys VP24 protein to disarm the host's innate immune response and sabotages antiviral antibody production by expressing a defensive decoy made of truncated virion glycoprotein [15,16]. Clearly, the Ebola virus, in this confrontation with the human host, outsmarts its genome. The Ebola virus [17], along with its 'relative', the Marburg virus, and rickettsiae, which cause epidemic typhus and Rocky Mountain spotted fever, demonstrates astonishingly effective virulence directed against microvascular endothelium to rapidly produce signs of septic shock.

Other microbes cause indirect damage to microvascular endothelium. In the absence of adequate antibiotics, bacteria rapidly proliferate to reach a quorum that bursts with virulence factors. These factors, such as anthrax toxins and cytolytic toxins from methicillin-resistant *Staphylococcus aureus* (MRSA), penetrate the cell membrane of endothelial (and epithelial) cells and kill them. Other staphylococcal and streptococcal virulence factors (e.g. protein A, protein G, clumping factor and streptokinase) interact with plasma proteins (immunoglobulin G Fc fragment, fibrinogen, plasminogen and von Willebrand factor), disarming phagocytosis, counteracting antibody responses and causing tissue necrosis [18–20]. Lipopolysaccharide (LPS, 'endotoxin') is a very potent proinflammatory virulence factor of Gram-negative bacteria [21], the cause of sepsis in two-thirds of patients either alone or in combination with other microbes [22]. Fungi and viruses have also been found to play an increasingly important role in sepsis etiology [22]. These microbial agents are sensed by toll-like receptors (TLRs), which are the mainstay of innate immunity and inflammation [23] (see below).

The damage to microvascular endothelium is aggravated by host-produced inflammatory mediators: complement, cytokines, chemokines, adhesion molecules, inducible cyclooxygenase 2 (COX2) and nitric oxide (NO) synthase metabolites. In addition, host endogenous products (HEPs) released from human blood and vascular cells (e.g. cell-free hemoglobin [24], high mobility group box 1 protein [25], histones [26] and neutrophil extracellular traps [27]) are detrimental. Cumulatively, they can inflict a deadly blow to microvascular endothelial cells through 'anoikis', apoptosis of endothelial cells detached from extracellular matrix. These 'homeless' cells can be detected and counted in the circulation [28], being a potential source of genomic information.

Persistent hypotension (vasoplegia) and microvascular leak in sepsis

A drop in blood pressure associated with incipient or ongoing infection, manifested by fever, chills and prostra-

tion, represents a hallmark of sepsis, prompting vigorous resuscitation measures and rapid administration of empiric antimicrobial therapy until the infectious agent is identified [29, 30]. Hypotension in the setting of sepsis reflects endothelial instability manifested by hyporesponsiveness to catecholamines [31]. Production of NO by endothelial NO synthase (eNOS), and especially inducible NO synthase (iNOS), and generation of prostacyclin by cyclooxygenase (COX) 2 are proposed as the main triggers of hypotension in sepsis. However, NOS inhibitors were not uniformly effective in improving sepsis-induced hypotension [32].

The blood tissue barrier comprises microvascular endothelial cells and extracellular matrix. Tight junctions and adherence junctions keep endothelial cells together [33]. Microvascular leak results from the direct action of microbial virulence factors (e.g. LPS [4], staphylococcal alpha toxin [34] or Ebola virus glycoprotein [14]), which produce gaps in these junctions. Hence, blood plasma escapes and causes edema, one of the five cardinal signs of inflammation [33]. In addition, overproduced pleiotropic cytokine interleukin (IL)-6, chemokine monocyte chemotactic protein 1 (MCP-1) [35] and vascular endothelial growth factor (VEGF), known as 'vascular permeability factor', open up endothelial adherence junctions by uncoupling the VE-cadherin-p120 catenin complex. The blood protein Slit, recognized by endothelial Robo receptor 4, stabilizes endothelial junctions [5]. Recombinant Slit protein increased survival in polymicrobial sepsis whereas cytokines remained elevated. Similarly, selective targeting of transcription factor nuclear factor kappa B (NF-κB) in endothelial cells prevented microvascular leak in polymicrobial sepsis and endotoxemia, thereby underscoring the central role of microvascular endothelial cytoprotection in experimental sepsis attenuation [4,5].

The human genome's 'weak spots' for sepsis

The outcome of sepsis caused by the Sudan species of the Ebola virus has been linked to the human leukocyte antigen-B locus [36]. Alleles B*67 and B*15 were associated with fatal outcomes whereas B*07 and B*14 predicted survival, indicating that the polymorphic genome region, which encodes HLA proteins, may determine the outcome of sepsis. Mutations in human genes encoding IRAK-4 (49 patients), MyD88 (22 patients), NF-κB essential modulator (NEMO, 100 patients) or IkBα (five patients) are linked to microbial infections, some of them lethal [37]. They encompassed a wide spectrum of bacteria, environmental mycobacteria, fungi (Candida), pneumocystis and viruses. The new paradigm of inborn errors of immunity formulated by J. L. Casanova and his collaborators was further corroborated by discovery of at least 34 new gene defects responsible for immunodeficiency [38]. They teach us that not only defects in adaptive ('specific') immunity but also defects in innate ('non-specific') immunity predispose to sepsis. Thus, genomic diagnosis, which is increasingly affordable, can be broadly applied to prevention and precise treatment of sepsis [39].

Lifting the curse of sepsis: vaccinate!

It is clear that the best hope against the potentially deadly sepsis caused by the Ebola virus is vaccination. Such vaccines are imminent [40]. Vaccines against other causes of sepsis are more difficult to come by. However, *Streptococcus pneumoniae*, which is responsible for pneumococcal sepsis, which complicates pneumonia, asplenia and sickle cell anemia, is being contained by a pneumococcal vaccine primarily administered to children [41]. A striking reduction in hospitalization for pneumonia and its invasive complication, sepsis, was also observed among non-vaccinated older adults, including the 65–74 and 75–84-year-old age groups, a compelling example of 'herd immunity'! Similarly, patients with IRAK-4 and MyD88 deficiencies should be immunized with *S. pneumoniae* conjugated and non-conjugated vaccines, *Haemophilus influenzae* conjugated vaccine, and *Neisseria meningitides* conjugated and non-conjugated vaccines [37]. A vaccine against group B *Neisseria meningitides* reduced the incidence of meningococcal sepsis, manifested by *Purpura fulminans*, one of the most challenging forms of sepsis to treat. Despite antibiotic therapy, it often causes hearing loss, neurologic damage and loss of limbs due to amputation in young survivors [42].

An anti-staphylococcal combination vaccine promises a new measure of protection against staphylococcal sepsis in immune-compromised patients [34]. Staphylococcal protein A subverts the ability of anti-staphylococcal vaccines to mount an effective antibody response, again giving microbes the upper hand in this battle of the genome wars [18].

Genome wars in sepsis

Increasingly belligerent microbial agents unleash their genomic prowess to challenge potentially weak spots in human genome immune programming. The concept of genome wars in sepsis includes confrontation between the microbial genome that encodes virulence factors and the human genome that responds by immune reprogramming, and their stochastic interactions (Fig. 2) [8]. These interactions encompass: (i) variable expression of host proteins and microbial virulence factors that subvert the host's innate immunity, and (ii) variable time elapsing from incipient infection to effective clearance of causative microbial agents. This clearance is executed by TLR-mediated and antibody/complement-mediated phagocytosis, supported by very early antimicrobial therapy [29,30].

The host genome's immune reprograming in response to infections is orchestrated by signaling to the nucleus. The first checkpoint in this signaling comprises TLRs
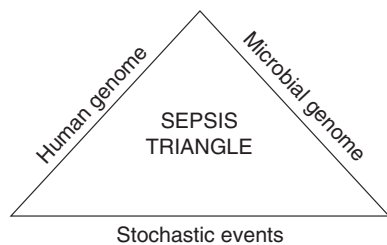
Fig. 2. A conceptual depiction of the 'sepsis triangle'. The outcome of the confrontation between the human genome and the microbial genome depends on stochastic events that represent 'a black box' in the sepsis conundrum. These events are influenced by variable expression of genes in both human and microbial genomes, as well as by variations in the time course of these genomic responses (adapted from [8]).

responsible for activation of phagocytes, non-phagocytic innate lymphoid cells and endothelial cells by LPS and other ligands (IL-1β, IL-18, bacteria, fungi and viral nucleic acids) (Fig. 3). The 'family of five' adaptors led by the myeloid differentiation primary response 88 (MyD88) 'staffs' this checkpoint [23,43]. Signals generated by different TLRs are integrated into the second checkpoint, downstream of the MyD88 family. It is a nuclear transport 'relay station' comprised of nuclear transport adaptors, termed importins/karyopherins alpha and beta (importins α and β). They shuttle NF-κB, activator protein-1 (AP-1) and other stress-responsive transcription factors (SRTFs) to the nucleus. Once inside the nucleus, SRTFs are freed to bind their cognate promoters and initiate gene transcription, leading to genome reprogramming from a resting to an activated state. SRTFs, either alone or in various combinations, regulate the genomic response to microbial agents, as well as to signaling pathways emanating from cytokine/chemokine receptors [44]. In addition, the nuclear transport checkpoint integrates metabolic signals conveyed by sterol regulatory element-binding proteins (SREBPs) shuttled solely by importin β1 [45].
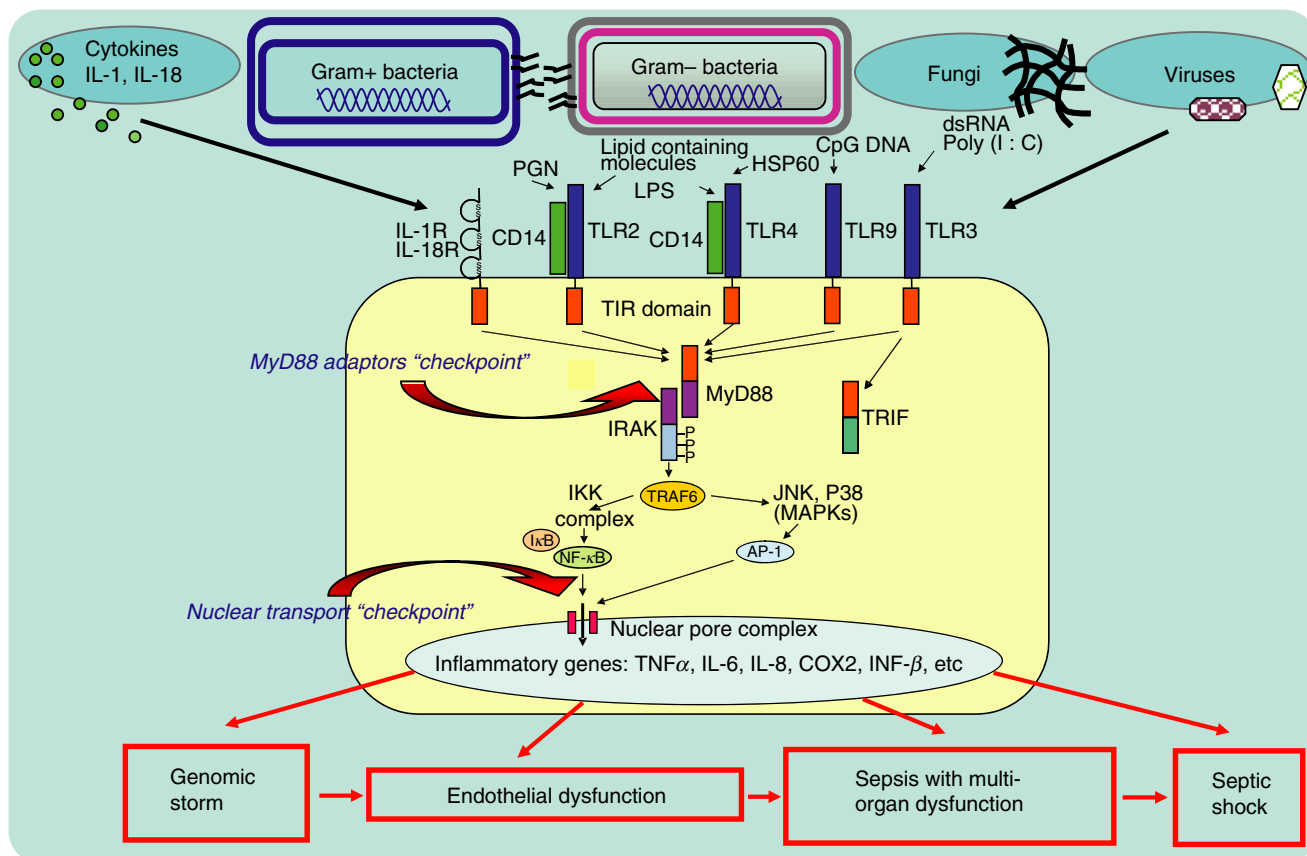

Fig. 3. Sepsis-causing microbes are sensed by toll-like receptors (TLRs), the mainstays of innate immunity. These receptors are expressed on immune and non-immune cells (endothelial and epithelial cells). Signals generated upon encounter with invading microbes are conveyed by innate immunity adaptors that belong to the MyD88 family of five (the first signaling checkpoint). Subsequent activation of proinflammatory stress responsive transcription factors (SRTFs), including nuclear factor kappa B (NF-κB), activator protein-1 (AP-1, comprised of cFOS and cJUN) and others, allows them to be ferried to the nucleus by nuclear transport shuttles (a second signaling checkpoint). These specialized proteins, termed importins/karyopherins alpha and beta, loaded with their SRTF cargos, are poised to cross nuclear pores. In the nucleus, SRTFs find their cognate sites in the promoter regions of multiple genes that encode mediators of inflammation and apoptosis. Massive activation of these genes, termed a 'genomic storm', follows. The wave of mediators of endothelial damage hits the microcirculation, causing endothelial dysfunction, injury and apoptosis. Sepsis with multi-organ dysfunction ensues, culminating, in some cases, in septic shock.

The 'genomic storm' and its consequences

In response to virulence factors encoded by microbial genomes, the human genome expresses or represses a plethora of genes. Up-regulated genes encode inflammatory cytokines and chemokines, signal transducers (COX2 and NOS, both mediators of vascular hyporeactivity) and cell adhesion molecules. The concept of a 'genomic storm', originally based on the response of white blood cells to trauma and burns in critically injured patients was extended to encompass human volunteers responding to the extremely low doses of endotoxin (2–4 ng kg^{-1}). Therefore, it represents a fundamental human response to severe inflammatory stress [46].

A tidal wave of up-regulated gene products raises their levels in blood and vascular cells. They include cytokines and chemokines and other mediators of microvascular injury. Subsequently, IL-6, IL-1β and TNFα induce an 'acute phase' protein response characterized by a burst of protein synthesis in the liver involving C-reactive protein (CRP), serum amyloid proteins, coagulation proteins, fibrinogen and factor VIII, and complement proteins. CRP is a widely used biomarker of systemic and localized inflammation.

Chemokines induce increased trafficking of neutrophils, monocytes and macrophages to infected tissue. Activated neutrophils and monocytes penetrate the blood tissue barrier and produce reactive oxygen intermediates ('free radicals') and isoprostanes [47]. They potentiate damage to endothelium, which then loses its barrier function and anticoagulant mechanism. The latter is mediated by the thrombomodulin–protein C axis [48]. Thus, a 'genomic storm' disturbs the microvascular homeostasis maintained in endothelial cells by physiologic suppressors of inflammation and coagulation. Multiple organ dysfunction ensues, culminating in persistent hypotension despite adequate fluid resuscitation along with vasopressors (septic shock). Perfusion abnormalities include lactic acidosis, oliguria and an acute alteration in mental status [49]. Some of these changes are linked to mitochondrial dysfunction [50].

Intracellular signaling pathways in endothelial cells, as well as immune cells involved in the sepsis mechanism, are also controlled by short, non-coding RNAs, known as microRNAs (miRNAs). They regulate gene expression by repressing translation or degrading mRNA. The extracellular miR-223 transported by high-density lipoproteins is delivered to endothelial cells to reduce expression of intercellular adhesion molecule 1 and granulocyte-macrophage colony stimulating factor 2 [51]. Other miRNAs, such as miR-181b, have been linked to regulation of expression of nuclear transport shuttles (e.g. importin α3) in sepsis [52].

Calming the 'genomic storm'

Rapid initiation of antimicrobial therapy in patients with hypotension, fever and other signs of infection is crucial in limiting its runaway course [29,30]. This is accompanied by fluid replacement and vasopressors [13]. Novel methods of microbial detection based on fluorescent *in situ* hybridization, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry and real-time polymerase chain reaction allowed a turnaround time of 30–150 min for staphylococci and their antibacterial resistance elements [53]. Thus, antimicrobial treatment in the first 'golden hour', including pre-hospital settings, is the goal to avert widespread microvascular changes in patients suspected of harboring sepsis-causing infections. Insidiously, virulence factors (e.g. LPS, staphylococcal and streptococcal toxins, anthrax toxins) can remain in microcirculation following initiation of antimicrobial therapy and continue to target blood cells and microvascular endothelial cells [54,55]. Disarming the microbial genome with small molecule compounds to suppress expression of its virulence factors, such as inhibiting streptokinase expression in group A streptococci, is a new paradigm in antimicrobial genomic therapy [56].

A parallel concept is to calm the host's 'genomic storm' by targeting the nuclear transport checkpoint (Fig. 3), [21,45]. This approach would counteract uncontrolled production of potentially harmful inflammatory mediators (TNFα, interferon gamma, IL-6, IL-8 and MCP-1) that appear in plasma within 90–120 min after LPS administration in human volunteers [57]. As shown in Fig. 3, signaling pathways emanating from LPS-stimulated TLR4, other TLRs, and from subsequently stimulated cognate receptors for cytokines and chemokines converge at the nuclear transport checkpoint [44]. Importins α and β shuttle SRTFs and SREBPs through nuclear pores. In the nucleus, SRTFs bind and activate the regulatory elements in at least 46 human genes encoding mediators of inflammation [21]. SREBPs, master regulators of lipid homeostasis, activate >30 genes encoding proteins responsible for synthesis and uptake of cholesterol, fatty acids and triglycerides [45]. The concept of targeting this key checkpoint for SRTFs and SREBPs has been tested by using nuclear transport modifiers (NTMs) such as cSN50.1, a highly soluble 28 amino acid cell-penetrating peptide [45] with dual specificity toward importin α5 and importin β1 [58]. NTMs sufficiently reduce nuclear translocation of transcription factors, thereby attenuating the hyperinflammatory and hypermetabolic responses underlying microvascular injury [21,45,59,60]. NTM is able to extinguish production of multiple inflammatory mediators at once. In contrast, monoclonal antibodies can neutralize only a single target (e.g. TNFα), which is not enough to attenuate human sepsis though effective in some animal models [32]. Significantly, NTM attenuated plasma levels of 23 out of 26 LPS-induced proinflammatory cytokines, chemokines and growth factors, and dramatically increased survival in a murine model of LPS-induced systemic microbial inflammation (lethal endotoxic shock) (Fig. 4). Some of these suppressed mediators (e.g. GM-CSF) are produced
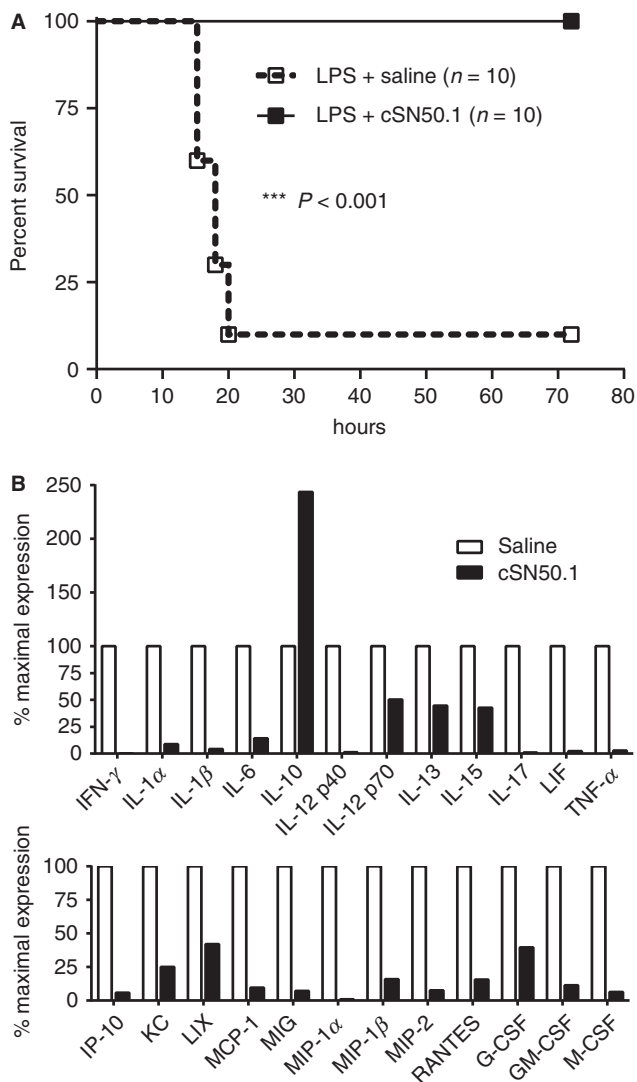
Fig. 4. Calming the 'genomic storm' induced by lipopolysaccharide (LPS). Nuclear transport modifier (NTM) was administered intraperitoneally to mice before and after a high dose of LPS. (A) Modulation of nuclear transport of SRTFs by NTM enhances survival from lethal endotoxic shock. (B) Twenty-three cytokines, chemokines and hemopoietic growth factors in plasma are reduced by NTM. Notably, expression of the anti-inflammatory cytokine IL-10 was enhanced by NTM (adapted from [21]).

by innate response activator B cells that either protect from or, paradoxically, contribute via IL-3 to experimental and clinical sepsis [61,62]. Moreover, NTM reduced neutrophil trafficking to lungs and suppressed production of chemokines, cytokines and VEGF in LPS-challenged lungs [21]. In other studies, NTM (cSN50) reduced intravascular platelet thrombi, improved thrombocytopenia and normalized fibrin-related markers and plasminogen activator inhibitor-1 in LPS-induced hemorrhagic necrosis and apoptosis of the liver [60], and reduced microvascular leak caused by staphylococcal superantigen SEB [59]. The concept of targeting nuclear transport of SRTFs was extended to the control of experimental sepsis associated with airway infection by anthrax spores [54].

The metabolic profile of sepsis non-survivors is marked by metabolites linked to fatty acid transport and β-oxidation, and gluconeogenesis [63]. These pathways depend on transcriptional regulation, not only by peroxisome proliferator-activated receptors α, β and γ, but also by SREBPs, which are reduced in the nucleus by NTM [45]. Thus, attenuating genomic derangements of inflammatory, coagulant and metabolic pathways with NTMs offers a new strategy for combating sepsis (Fig. 5).

Thrombocytopenia in sepsis

A drop in blood platelets to less than $150\,000\ \mathrm{uL}^{-1}$ has long been recognized as a hallmark of Gram-negative bacteremia and ensuing sepsis [55]. Thrombocytopenia in sepsis may be centered in bone marrow suppression of platelet-producing megakaryocytes with a decreased immature platelet fraction, or peripherally be due to trapping of circulating platelets in zones of endothelial injury [64]. Platelets are also susceptible to cytolytic microbial toxins or the membrane attack complex of complement activated by microbial agents [65]. Heparin may induce antibodies against platelet factor 4, a hallmark of heparin-induced thrombocytopenia, and antimicrobial therapy may lead to drug-induced thrombocytopenia [66]. In a retrospective study of 304 patients (mean age 68.8 ± 15.8 years) with severe sepsis or septic shock, 47.6% developed thrombocytopenia, which was drug induced in 17.9% of patients [66]. Significantly, thrombocytopenic patients suffered more episodes of bleeding and were more prone to AKI and ARDS. Moreover, they had elevated serum lactic acid and prolonged requirement for vasopressors, suggesting more severe microvascular endothelial dysfunction.

DIC in sepsis

Rapid and widespread microbial injury to microvascular endothelium in sepsis sets the stage for DIC, reported in 37% of 145 patients with thrombocytopenia as a complication of severe sepsis and septic shock, diagnosed in 304 patients [66]. In another study using the International Society on Thrombosis and Haemostasis DIC score to evaluate 40 patients with severe sepsis or septic shock, 95% had fibrin-related markers (fibrin monomer and D-dimer) [67]. Therefore, the importance of prolonged prothrombin time and thrombocytopenia in predicting disease severity and survival was highlighted.

While the mechanism of DIC remains enigmatic, recent advances indicate that injury to endothelium directly activates the coagulation cascade through assembly of a prothrombinase complex linked to phosphatidyl serine exposed on injured endothelial cell membrane [68]. This mechanism of thrombin formation in microcirculation seems to obviate
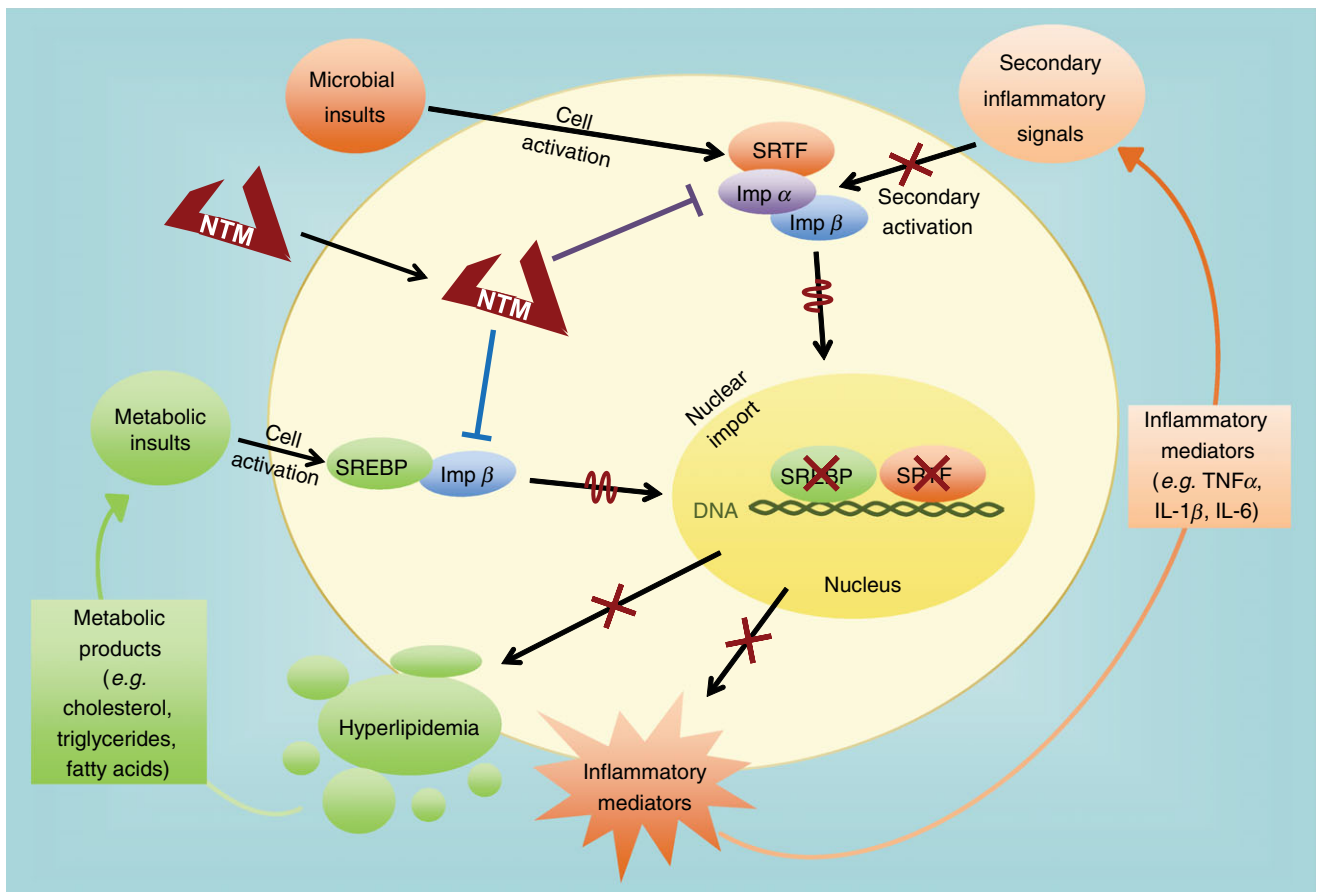
Fig. 5. Nuclear transport of stress-responsive transcription factors (SRTFs) and sterol regulatory element-binding proteins (SREBPs) is modulated by a dual specificity NTM, cSN50.1 peptide, which binds nuclear import proteins (importin α5 and importin β1, respectively). Attenuation of nuclear transport of SRTFs (as shown by wiggly line) reduces production of inflammatory mediators (cytokines, chemokines as shown by X). Expression of their target genes, induced by a feed-forward activation loop, is likewise suppressed (as shown by X). Attenuation of nuclear transport of SREBPs (as shown by wiggly line) reduces expression of their target genes (as shown by X) that encode proteins involved in synthesis of cholesterol, triglycerides and fatty acids. Both groups of transcription factors, SRTFs and SREBPs, contribute to the 'genomic storm' induced by endotoxin and other microbial virulence factors involved in sepsis (for details see [21] and [45]).

the need for tissue factor pathway that was unsuccessfully targeted in clinical sepsis trials [32]. LPS-induced thrombocytopenia and DIC with microvascular fibrin deposition were independent of thrombin-induced platelet stimulation and the associated procoagulant activity, as was observed in mice deficient in protease activated receptor (PAR)-1, -2 and -4 and PAR-2/PAR-4 and PAR-1/PAR-2 [69]. Thrombin generated on the surface of injured endothelium activates an physiologic anticoagulant mechanism that is vested in the thrombomodulin-protein C axis [48]. Surprisingly, in humans, a supra-normal dose of protein C zymogen concentrate had no significant effect on LPS-induced biomarkers of coagulation, fibrinolysis and inflammation, whereas significant protein C activation and production of tumor necrosis factor alpha (TNFα) were detected [70]. Likewise, heparin administration remains controversial, awaiting a well-designed prospective study. A retrospective propensity-matched cohort study indicated that early intravenous administration of unfractionated heparin was associated

with a 12% relative risk reduction in mortality [71]. As only a fraction of patients with severe sepsis and septic shock displays signs of DIC, this level of effectiveness is not surprising. Similarly, clinical trials of antithrombin and thrombomodulin were ineffective [32].

Thrombin activates microvascular endothelial cells through PAR1, leading to a loss of barrier function. This signaling pathway culminates in nuclear transport of NF-κB and AP-1 [72]. They mediate production of barrier-disrupting cytokine IL-6 and MCP-1 [21,35]. PAR1-derived cell-penetrating, membrane-tethered peptide ('pepducin') reduced lung vascular leakage and increased survival in a murine polymicrobial sepsis model [73]. However, mice deficient in PAR-1, PAR-2, PAR-4, PAR-2/PAR-4 and PAR-1/PAR-2 succumbed to LPS-induced lethal shock [69]. Imatinib, an inhibitor of the tyrosine kinase Abl-related gene, attenuated thrombin-induced endothelial barrier dysfunction by stabilizing cell matrix interactions and reduced organ edema in a

murine polymicrobial sepsis model [74], while exacerbating ventilator-induced lung injury in a mouse model [75].

Why physiologic anti-inflammatory regulators fail in sepsis

Proinflammatory signaling in response to microbial virulence factors activates feedback systems designed to 'put on the brakes', encompassing extracellular and intracellular regulators. Extracellular anti-inflammatory cytokines IL-4, IL-10, IL-11, IL-13 and IL-1 receptor antagonist counteract the deleterious action of proinflammatory cytokines and chemokines. Their production represents a compensatory anti-inflammatory response syndrome (CARS), which is linked to the 'immunoparalysis' seen in the later stage of sepsis [76]. Transition to an immunosuppressive phenotype is mediated in part by transcription factor hypoxia-inducible factor 1α in human monocytes derived from patients with sepsis [77].

Intracellular inhibitory proteins, such as IL-1 receptor-associated kinase (IRAK)-M, inhibitors of NF-κB (IκB), suppressors of cytokine signaling (SOCS) and A20 protein, which regulates the NF-κB pathway, have evolved to limit the duration and strength of proinflammatory signaling pathways emanating from TLRs and cytokine receptors in immune and non-immune cells (e.g. endothelial cells). Their role in the host response to endogenous or exogenous microbial insults in experimental models is emerging. Selective expression of degradation-resistant transgenic IκBα in endothelial cells was remarkably protective in a polymicrobial sepsis model [4].

A20 protein protects endothelial and epithelial cells from microbial inflammation in multiple organs. A20 protein deficiency causes dissemination of commensal intestinal microbes (gut microbiome) through the intestinal barrier due to uncontrolled proinflammatory signaling from TLRs and the MyD88 adaptor axis [78]. This signaling culminates in nuclear transport of NF-κB and other SRTFs that are responsible for a 'genomic storm' [21].

Another newer endothelial 'rheostat', caspase and receptor interacting protein adaptor with death domain (CRADD/RAIDD), controls signaling from TLRs and G protein-coupled receptors activated by LPS and thrombin, respectively. This signaling is dependent on cytoplasmic B-cell lymphoma/leukemia 10 (BCL10) in immune and non-immune cells [35].

SOCS 1 and SOCS 3 target cytoplasmic segments of cytokine receptors and/or Janus kinases for ubiquitin-mediated proteosomal degradation and are consumed in the process by the same mechanism [79]. Intracellular protein therapy with bioengineered cell-penetrating (CP)-SOCS3 was developed to replenish intracellular stores of endogenous SOCS3. Microvascular injury (apoptosis and hemorrhagic necrosis) caused by LPS or superantigenic staphylococcal enterotoxin B in murine liver was sup-pressed, proving the concept of relative depletion of physiologic anti-inflammatory regulators [80]. CP-SOCS3, with an extended half-life (up to 29 h) [81], further expands the potential of intracellular protein therapy for sepsis.

The brain in sepsis and long-term consequences for survivors

Scenes from the sepsis battlefield in intensive care units (ICUs) are especially harrowing when patients display delirium and fall into coma. These signs of acute brain dysfunction are linked to microvascular injury that may contribute to alterations in cerebral blood flow and impairment of the blood brain barrier. In brain endothelial cells, transforming growth factor β-activated kinase 1 controls fever and lethargy mediated by IL-1β [82]. The measure of impaired endothelial function, known as the reactive hyperemia index, correlates with the number of days with delirium or coma [83]. Likewise, disruption of the integrity of brain white matter analyzed by magnetic resonance imaging correlated with duration of delirium in the ICU and persisted for at least 3 months, leading to lower cognitive scores at 3 and 12 months [84]. A groundbreaking study of 1194 sepsis survivors revealed moderate to severe cognitive impairment as compared with non-sepsis patients [85]. This outcome has profound consequences for patients, their families and societal resources for long-term care, adding immeasurably to the high cost of sepsis.

Late-life sepsis

Increased susceptibility to sepsis is observed in physiologic and accelerated aging, as indicated by the highest incidence and mortality above 65 years of age [49]. In a study monitoring accelerated aging in neonatal progeroid syndrome, four of five patients followed for 1–7 years died due to sepsis or aspiration pneumonia [86]. Accelerated aging is caused by mutations in nuclear lamins. Therefore, laminopathies, as well as defective lamin processing associated with vascular aging in the normal population, are correlated with transcriptional dysregulation, oxidative stress, inflammatory signaling, vascular smooth muscle apoptosis and accelerated atherosclerosis [87]. Innate immunity adaptor sterile alpha- and armadillo-motif-containing protein protects lamins from inflammation-induced apoptotic degradation [88].

Sepsis resolution

The emerging concept of sepsis resolution by lipid mediators, such as resolvin D1, is linked to regulation of inflammation, reduction of apoptosis and restoration of homeostasis [89]. Notably, resolvin D1 inhibits endoplasmic reticulum stress-induced apoptosis of liver cells by

reducing SREBP-1 expression and caspase 3 activity [90]. Expression of SREBPs, the transcription factors responsible for lipid homeostasis in a model of hyperlidemia, was reduced by NTM [45]. Thus, NTM-regulated expression and action of SREBPs [45], as well as reduction of endotoxin-induced caspase 3/7 activity [60], offer potential mechanisms for cytoprotective effects of NTM in late-stage sepsis, characterized by immunosuppression [76]. Further investigation may provide a missing link between sepsis resolution and transcriptional control.

Animal models of sepsis: 'The best laid schemes of mice and men, go oft astray'

Robert Burns in his ode 'To a Mouse' reflected on the relationship between himself and the rodents living in his field. Mice are widely popular for sepsis studies due to the many genetically modified strains available and their ease of handling. The surgical model of sepsis known as cecal ligation and puncture (CLP) is frequently employed [4,5]. A non-surgical murine model of sepsis evolving from peritonitis avoids surgical wounding and the uncontrolled spillage of cecal contents into the peritoneal cavity that is inherent to CLP [91]. These models have generated significant new findings, such as attenuation of multiple organ injury and improved survival by genetic ablation of NF-κB signaling in endothelial cells [4].

In 2013, the field of experimental sepsis was stunned by a comparison of human and murine transcriptomes obtained from peripheral blood leukocytes after severe injury due to trauma and burns. The conclusion of this study, highlighted in the title, was that 'Genomic responses in mouse models poorly mimic human inflammatory diseases' [92]. The mainstream media rapidly heralded a mass exodus of mice from biomedical research institutions, evoking deep concern among basic and translational sepsis investigators [93]. A reexamination by scientists from Japan of the same datasets came to a diametrically opposed conclusion: that 'Genomic responses in mouse models greatly mimic human inflammatory diseases' [94], quelling the kerfuffle. However, a lack of convincing explanation from the authors of the first report for this scientific dichotomy confirms the wisdom of Robert Burns that 'the best laid schemes of mice and men, go oft astray'.

Non-human primates (baboons and vervets) display exceptionally high intrinsic resistance to endotoxin (>5 mg per kg body weight), whereas human volunteers respond to 2–4 ng per kg body weight, a dose that is one million times lower [57]! This enormous difference in sensitivity to endotoxin, the common virulence factor of Gram-negative bacteria that cause two-thirds of human sepsis cases [22], may explain the therapeutic incongruence between baboons and humans in some recently tested sepsis treatments [32]. In contrast, chimpanzees, like humans, are extremely susceptible to endotoxin, offering a relevant preclinical model for sepsis studies [95].

The Shock Society and the International Sepsis Forum have addressed the challenges of translational research in shock and sepsis [96] and strongly recommended greater standardization of preclinical models. This includes gender and age matching, akin to the measures employed for clinical studies of sepsis, and reflecting its most adverse impact on extreme age groups (neonatal and late-life sepsis). Ultimately, to quote Ian Anderson, 'but a mouse is a mouse for all that'; hence, the verification of preclinical sepsis studies must come from investigations in humans.

The rising tide and cost of sepsis

Sepsis is a major public health problem in the United States (US) and worldwide, affecting up to 19 million patients per year [49]. Approximately 21 000 patients were diagnosed with sepsis in US hospitals each week in 2011 [97], and approximately 20–25% of those with severe sepsis were facing death within 28 days [49]. Thus, the number of sepsis victims approaches that of acute myocardial infarction and exceeds deaths from stroke. An analysis of 192 980 patients with severe sepsis in the US found that sepsis arose from a medical condition in 71% and a surgical condition in 29% [98]. Cumulatively, severe sepsis undermines advances in the medical and surgical management of diseases and counteracts the trend for increased life expectancy in the US and worldwide.

Sepsis is responsible for the most expensive hospital stays in the US, often costing more than $500 000 per hospitalization. In 2011, the estimated annual cost of sepsis in the US exceeded $20 billion [97]. This mounting cost, corresponding to two-thirds of the annual US National Institutes of Health budget, did not include expenses for long-term care of sepsis survivors who suffer life-long developmental abnormalities (neonates) and incapacitating cognitive decline (older patients) [85,99]. Both extreme age-groups have an unacceptably high mortality (up to 45%), and the incidence of severe sepsis in newborns doubled between 1995 and 2005. Thus, sepsis is one of the most challenging problems to prevent and treat in modern hospitals.

Conclusion

Over the centuries, the specter of plagues manifested by sepsis has been an inseparable part of human existence. Human immune defenses are continually challenged by microbiomes from within and without human body. Recent impressive advances in understanding the relationship between human and microbial genomes bring us closer to the development of new preventive vaccines and therapeutic countermeasures to be deployed for containment of sepsis. They are needed in the face of (i) widespread emergence of microbes that escape antimicrobial therapy (e.g. MRSA), (ii) drift from immunization-acquired immunity (e.g. influenza viruses), and (iii) unsuc-

Table 1 Compilation of antisepsis regimens used currently as preventive (A) and proven therapeutic measures (B). In addition, some experimental therapeutic measures (C) and proof-of-concept measures awaiting preclinical testing (D) are listed (for details see text and references cited)

Cause of sepsis	Regimen	Outcome	Ref.
(A) Preventive measures			
Streptococcus pneumoniae	Vaccination	Reduced incidence	[41]
Neisseria meningitides	Vaccination	Reduced incidence	[42]
Haemophilus influenzae	Vaccination	Reduced incidence	[37]
(B) Proven therapeutic measures			
Undetermined (blood culture negative)	Empiric antimicrobial therapy + fluid resuscitation/ vasopressors + respiratory therapy	Increased survival	[13]
Bacterial	Pathogen-directed antimicrobial therapy + fluid resuscitation/ vasopressors + respiratory therapy	Increased survival	[13]
Fungal	Pathogen-directed antimicrobial therapy + fluid resuscitation/ vasopressors + respiratory therapy	Increased survival	[13]
Viral	Pathogen-directed antimicrobial therapy + fluid resuscitation/ vasopressors + respiratory therapy	Increased survival	[13]
(C) Experimental therapeutic measures			
Viral (Ebola virus)	Survivors' blood plasma/monoclonal Ab ZMapp + fluid resuscitation/vasopressors + respiratory therapy	Increased survival	[16]
Polymicrobial peritonitis (mice)	Recombinant SLIT protein	Increased survival	[5]
Polymicrobial peritonitis (mice)	Pepducin (protease-activated receptor 1 peptide agonist)	Increased survival	[73]
Polymicrobial peritonitis (mice)	Imatinib (Abl-related gene kinase inhibitor)	Reduction of edema in multiple organs	[74]
Ventilator-induced lung injury (mice)	Imatinib (Abl-related gene kinase inhibitor)	Increased edema and inflammation in lungs	[75]
Bacillus anthracis spores (mice)	Pathogen-directed antimicrobial therapy + nuclear transport modifier (cSN50)	Increased survival	[54]
(D) Proof-of-concept measures awaiting preclinical testing			
–	Intracellular protein therapy with recombinant cell-penetrating SOCS-3	–	[80] [81]
–	Intracellular protein therapy with recombinant cell-penetrating CRADD	–	[35]

cessful outcomes of over 100 trials in sepsis [32]. Table 1 summarizes the proven and experimental antisepsis regimens discussed above. The prospect of new pan-specific vaccines that surpass antigenic drift and vigilant precautions to reduce both hospital- and community-acquired infections, combined with new cytoprotective measures for microvascular endothelium, suggest that the estimated rise of sepsis cases to 2 million in the US by 2020 [100] can be reversed. Hopefully, the same will apply to stemming the rise of sepsis worldwide.

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Disclosure of Conflict of Interests

J. Hawiger reports financial support from AGH Therapeutics Inc. outside the submitted work. In addition, R. A. Veach, J. Hawiger and J. Zienkiewicz have multiple issued and pending patents relating to cell-penetrating NTM peptides and their use for anti-inflammatory therapy. All rights are assigned to Vanderbilt University.

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