Targeting the “sweet spot” in septic shock – A perspective on the endothelial glycocalyx regulating proteins Heparanase-1 and -2

Thorben Pape\textsuperscript{a1}, Anna Maria Hunkemöller\textsuperscript{b1}, Philipp Kümpers\textsuperscript{b}, Hermann Haller\textsuperscript{a}, Sascha David\textsuperscript{c} and Klaus Stahl\textsuperscript{a,d,*}

\textsuperscript{a} Division of Nephrology and Hypertension, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
\textsuperscript{b} Department of Medicine, Division of General Internal and Emergency Medicine, Nephrology, and Rheumatology, University Hospital Münster, Albert-Schweitzer-Campus 1, 48149 Münster, Germany
\textsuperscript{c} Institute of Intensive Care Medicine, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland
\textsuperscript{d} Division of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

Correspondence to Klaus Stahl: Division of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany. stahl.klaus@mh-hannover.de (K. Stahl)

https://doi.org/10.1016/j.mbplus.2021.100095

Abstract

Sepsis is a life-threatening syndrome caused by a pathological host response to an infection that eventually, if uncontrolled, leads to septic shock and ultimately, death. In sepsis, a massive aggregation of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) cause a cytokine storm. The endothelial glycocalyx (eGC) is a gel-like layer on the luminal side of the endothelium that consists of proteoglycans, glycosaminoglycans (GAG) and plasma proteins. It is synthesized by endothelial cells and plays an active role in the regulation of inflammation, permeability, and coagulation. In sepsis, early and profound injury of the eGC is observed and circulating eGC components correlate directly with clinical severity and outcome. The activity of the heparan sulfate (HS) specific glucuronidase Heparanase-1 (Hpa-1) is elevated in sepsis, resulting in shedding of heparan sulfate (HS), a main GAG of the eGC. HS induces endothelial barrier breakdown and accelerates systemic inflammation. Lipopolysaccharide (LPS), a PAMP mainly found on the surface of gram-negative bacteria, activates TLR-4, which results in cytokine production and further activation of Hpa-1. Hpa-1 shed HS fragments act as DAMPs themselves, leading to a vicious cycle of inflammation and end-organ dysfunction such as septic cardiomyopathy and encephalopathy. Recently, Hpa-1’s natural antagonist, Heparanase-2 (Hpa-2) has been identified. It has no intrinsic enzymatic activity but instead acts by reducing inflammation. Hpa-2 levels are reduced in septic mice and patients, leading to an acquired imbalance of Hpa-1 and Hpa-2 paving the road towards a therapeutic intervention. Recently, the synthetic antimicrobial peptide 19–2.5 was described as a promising therapy protecting the eGC by inhibition of Hpa-1 activity and HS shed fragments in animal studies. However, a recombinant Hpa-2 therapy does not exist to the present time. Therapeutic plasma exchange (TPE), a modality already tested in clinical practice, effectively removes injurious mediators, e.g., Hpa-1, while replacing depleted protective molecules, e.g., Hpa-2. In critically ill patients with septic shock, TPE restores the physiological Hpa-1/Hpa-2 ratio and attenuates eGC breakdown. TPE results in a significant improvement in hemodynamic instability including reduced vasopressor requirement. Although promising, further studies are needed to determine the therapeutic impact of TPE in septic shock.

© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a pathological host response to infection [1–3]. It can result in significantly reduced organ perfusion, namely septic shock [1,2]. The mortality of septic shock remains high at up to 59 % [4]. In 2017, 48.9 million sepsis cases were reported worldwide including 11 million deaths [5]. Additionally, the incidence of sepsis is continuously increasing and currently stands at 11.6 % in Germany [6]. Therefore, the world health organization recognized sepsis as a health priority [7].

The pathophysiology of sepsis is dominated by immune dysfunction, coagulopathy and endothelial dysfunction [8–16]. "Damage-associated molecular patterns" (DAMPs) are released by injured tissue and "pathogen-associated molecular patterns" (PAMPs) expressed on microorganisms. These in turn activate so-called "pattern-recognition receptors" (PRRs) on host cells, such as the Toll-like receptors (TLR), which subsequently induce a cascade of signaling events ultimately leading to a systemic inflammatory response [13,14]. As an example, TLR-4 is activated by lipopolysaccharides (LPS) expressed on gram-negative bacteria [13]. In sepsis, a massive accumulation of PAMPs by microorganisms (i.e. LPS) and DAMPs by damaged tissue overstimulates immune cells which results in an overwhelming systemic release of cytokines such as Interleukin (IL)-6 or tumor necrosis factor α (TNFα) [14–16]. This phenomenon has been named “cytokine storm” – a pathological, overshooting immune response that gives rise to organ dysfunction [13,14].

Also, the inner layer of the vasculature – the endothelium – plays a crucial role in the pathology of sepsis-associated multiple organ dysfunction. Literally all of its physiological functions are severely disturbed during sepsis [10,17,18]. The endothelium does not only function as a physical barrier but is actively involved in the regulation of physiological (i.e. gas exchange, nutrient diffusion etc.) and pathophysiological processes (i.e. inflammation, coagulation and permeability) [12]. Therefore, endothelial activation results in a pro-inflammatory, pro-adhesive, pro-coagulative milieu with a widespread affection of cell–cell-junctions. This ultimately leads to vascular leakage, clinically described as "capillary leakage syndrome" [19,20].

The endothelial glycocalyx (eGC), a gel-like layer on the surface of endothelial cells of all vascular beds, consisting of sugar and protein molecules, serves as a physical barrier for water and colloids but also significantly regulates endothelial function [21]. The eGC is additionally involved in the modulation of signaling processes [22]. This review focuses on the role of the eGC in sepsis with an emphasis on the heparan sulfate (HS) shedding enzyme heparanase-1 (Hpa-1) and its putative natural antagonist heparanase-2 (Hpa-2), in sepsis.

Structure and function of the endothelial glycocalyx

The vascular endothelium is covered by a gel-like layer on its luminal site, the eGC [21]. This carbohydrate-rich layer consists of three major components: 1) membrane-bound proteoglycans (e.g. Syndecan-1) [23], 2) glycosaminoglycans (GAG) bound to proteoglycan core proteins and 3) plasma proteins (i.e. Albumin, antithrombin) [21,24,25]. GAGs are linear polymers of disaccharides with variable length that are modified by e.g., sulfation and (de-)acetylation. Over 50 % of the GAGs consist of heparan sulfate (HS) [26,27]. Other important GAGs are hyaluronic acid (HA), which is linked to the transmembrane receptor cluster of differentiation 44 (CD44) [28,29], chondroitin sulfate (CS), dermatan sulfate and keratine sulfate [21]. The highly sulfated GAG chains form a negatively charged barrier. Proteoglycans consist of a
core protein linked with one or more glycosaminoglycan chains. Glycoproteins, namely proteins from selectin family, integrin family and immunoglobulin superfamily, play a major role in cell recruitment from the bloodstream and in cell signaling [24].

The eGC plays a crucial role in endothelial homeostasis [26,27], is synthesized by endothelial cells themselves [21,30] and is tightly and dynamically regulated by degradation and de novo synthesis [31]. Its negatively charged components prevent extravascular leakage of protein and intravascular fluid [26,27]. In addition, the eGC regulates inflammation and coagulation. It has anti-inflammatory effects by creating an anti-adhesive milieu and inhibiting leucocyte migration [32]. Thinning of the eGC exposes much smaller and hence previously hidden endothelial surface adhesion molecules, including intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). This in turn, allows neutrophils to recognize and adhere to the endothelial surface [33,34]. Platelet aggregation is prevented and important anticoagulant molecules, such as antithrombin, thrombomodulin and tissue factor pathway inhibitor, are stored in the eGC resulting in an anti-coagulant effect [26,27]. Shear stress and blood flow are key regulators of the eGC. Increased shear stress leads to vasodilatation via nitric oxide (NO) release and consequently, to stress reduction [26,27]. Synthesis and maintenance of different eGC components (i.e., hyaluronan) is induced by laminar shear stress via direct mechanotransduction to the endothelium and the shear-responsive transcription factor Krüppel-like factor 2 (KLF2) [35,36].

As the assessment of the eGC is difficult both in vivo and in vitro, different interpretations regarding its thickness exist [21]. Commonly, the thickness of the eGC in vivo is determined by the equivalent of the so called perfused boundary region (PBR) representing the width of red blood cells penetrating into the (damaged) eGC [37] (Fig. 1).

**Role of the endothelial glycocalyx in sepsis**

Early and substantially injury of the eGC has been reported during sepsis [21,27,38,39]. A key clinical problem of septic patients is hemodynamic instability that is driven by an altered vasomotor status with vasoplegia resulting in low perfusion pressure at the organ level. Hypotension per se causes lower shear stress to the eGC which results in its shedding and therefore, reduced eGC thickness [21,35,40,41]. The eGC is further degraded by activated matrix metalloproteinases (MMP) and disintegrin metalloproteases (ADAM), which are all elevated in sepsis [42,43], probably due to activation by pro-inflammatory cytokines (e.g., TNF-α) [27,35]. In

![Fig. 1. Perfused boundary region correlates with endothelial glycocalyx damage. (A) In quiescent endothelium, red blood cells (RBC) flow in laminar flow in the middle of vessels. Some RBCs flow in the perfused boundary region (PBR). The endothelial glycocalyx (eGC), here exemplarily represented by the glycosaminoglycan heparan sulfate (HS) and the proteoglycan syndecan-1, is a complex layer on top of the endothelial surface. Excess heparanase-2 (Hpa-2) may stabilize membrane bound HS. The glycocalyx layer is not perfused by RBCs, leading to a laminar blood flow in healthy vessels. (B) In septic endothelium, heparanase-1 (Hpa-1) sheds HS and therefore, eGC. Additionally, there is an acquired deficiency of protective Hpa-2. Consequently, RBCs can penetrate into outer vessel regions, leading to a higher PBR and ultimately, blood stasis. The PBR therefore, directly correlates with damaged eGC in vivo. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
addition, the endothelium-destabilizing factor angiopoietin-2 (Angpt-2) is a key mediator of eGC degradation and is released from Weibel-Palade bodies during inflammation [44]. Degradation products themselves act as DAMPs and further stimulate immune response [21,27]. Due to shedding of the eGC, embedded endothelial adhesion molecules, such as ICAM-1 and VCAM-1, are exposed and leukocytes can more easily transmigrate [21,38]. Any damage to the eGC impairs the carefully protected sub-glycocalyceal space – a protein-poor space between the glycocalyx and the vascular endothelium – which maintains a fairly high oncotic pressure gradient, thereby uncoupling the oncotic effect of interstitial concentration from the vessel lumen [45,46]. Loss of the eGC not only increases capillary permeability and interstitial edema formation, but also impairs “autotransfusion” capacity (i.e., the result of water moving into the circulation out of the non-circulating glycocalyx, which contributes about 25% of total intravascular volume [47]. Furthermore, shedding of the eGC results in secondary vascular barrier dysfunction and capillary leakage by opening the endothelial cleft and consequently, increased protein-rich fluid extravasation [21,26]. Intravascular coagulation is induced as platelets aggregate at eGC deficient endothelial cell surfaces and anticoagulant activities of the endothelium, that are critically dependent on eGC components as co-factors, are suppressed (antithrombin, thrombomodulin, Tissue Factor Pathway Inhibitor) [21]. Together, early and substantial shedding of the eGC is found in sepsis, which contributes to a pathologic local inflammation, increased permeability and activated coagulation.

Circulating markers of eGC injury correlate with sepsis severity and outcome [27,38,48–52]. As an equivalent of reduced eGC thickness and therefore deeper penetration of the red blood towards the vessel wall, the above introduced “PBR” increases [40,41,53] (Fig. 1) and correlates with outcome in septic patients [54]. Consistently, the blood concentration of eGC’s main proteoglycan, syndecan-1, correlates with the measured PBR in septic patients [53]. Therefore, serum levels of syndecan-1 serve as a global surrogate parameter for eGC damage. Syndecan-1 serum levels are elevated in patients with sepsis compared to healthy individuals or patients after major surgery [55]. Higher Syndecan-1 serum levels also correlate with dosage of vasopressor support, microcirculatory dysfunction (lactate) and parameters of dysregulated coagulation (International normalized ratio (INR), activated partial thromboplastin time (aPTT)) in septic patients [48,49]. Circulating syndecan-1 levels were also reported to be associated with the severity of disseminated intravascular coagulation (DIC) [56].

Importantly, higher circulating heparan sulfate (HS) levels were also associated with higher an increase in the mortality in septic patients [57]. Since HS is the major GAG of the eGC [26,27], its degradation process and functions involving its main regulators, Heparanase-1 (Hpa-1) and Heparanase-2 (Hpa-2), will be discussed in more detail within the scope of this review (Fig. 2).

**Heparan-sulfate and its cleavage enzyme Heparanase-1**

Hpa-1 is a HS-specific glucuronidase [38] that is produced primarily by endothelial cells [58], but also by platelets and neutrophils [59]. Hpa-1 targets are the HS chains covalently linked to core proteins of the ubiquitous and multifunctional HS proteoglycans, present at cell surfaces and extracellular matrix and involved in cell signaling, survival, proliferation, migration, and invasion [60,61]. Hpa-1 is cleaved from its 65 kDa proenzyme to its active 50 kDa form by inflammatory cytokines, i.e. TNF-α [27], reactive oxygen species [60], LPS [22] and Cathepsin L (Ctsl) [62]. Activation of Hpa-1 catalyzes the degradation of the HS layer of the eGC [38,60]. Hpa-1 concentration in patient’s blood was found to be elevated during sepsis [60,63] and seems to be higher in gram-negative compared to gram-positive bacteremia [60].

In contrast, we and others recently also observed reduced Hpa-1 concentrations in severe septic shock [64,65], which might be due to close binding of Hpa-1’s soluble form to the eGC during HS shedding and therefore, results in lower serum concentrations [66]. Apart from these divergent data on Hpa-1 concentration, Hpa-1 activity is found significantly elevated in diverse septic conditions and has been explored in different tissues, most extensively in the pulmonary microcirculation [38,60].

Schmidt and colleagues analyzed the effects of degraded pulmonary endothelial glycocalyx on lung injury and neutrophil adhesion [38]. When examined by intravital microscopy, thickness of the pulmonary eGC is reduced in LPS-treated mice, whereas eGC thickness in cremasteric tissue remains unchanged. On the contrary, Yang and colleagues, found reduced eGC thickness in cremasteric tissue [67]. Schmidt and colleagues found increased adhesion of neutrophils in pulmonary tissue in LPS-treated mice and a higher availability of the adhesion molecules ICAM-1 and VCAM-1, labelled by fluorescent microspheres. Interestingly, this higher expression results primarily from eGC degradation rather than new protein synthesis as total ICAM-1 and VCAM-1 protein remain unchanged in LPS-challenged mice and mouse lung microvascular endothelial cells (MLMVECs) when already increased neutrophil adhesion is found [38]. In mice subjected to cecal ligation and puncture (CLP) – a model of abdominal polymicrobial sepsis – increased lung permeability is accompanied by increased pulmonary Hpa-1 expression [38,68]. It has been proposed that loss of eGC mediated by Hpa-1 contributes to increased perme-
Fig. 2. Heparanase-2 prevents inflammation by stabilizing membrane bound heparan sulfate from heparanse-1 degradation. (A) In quiescent endothelium, the endothelial glycocalyx (eGC), here exemplarily represented by the glycosaminoglycan heparan sulfate (HS) and the proteoglycan syndecan-1, is a complex layer on top of the endothelial surface. Excess heparanase-2 (Hpa-2) stabilizes membrane bound HS and only low concentrations of Heparanase-1 (Hpa-1) are found within the circulation (a broad surrogate of its local concentration). (B) In septic endothelium, lipopolysaccharide (LPS) binds to the receptor cluster of differentiation 14 (CD14) and is via lymphocyte antigen 96, MD2, transferred to toll-like receptor (TLR)-4, which is then activated. Downstream of TLR4 the inflammatory transcription factor NF-κB is activated which leads to production and release of cytokines, e.g. interleukin (IL)-6. Consequentially, the HS specific glucuronidase Hpa-1 is activated and degrades eGC by cleaving membrane bound HS chains. Shed HS fragments circulate and stimulate themselves TLR-4 in turn leading to a vicious cycle of inflammation. Unburied adhesion receptors and disruption of the endothelial cleft lead to endothelial mediated accelerated inflammation and leakage. Hpa-1 up-regulates the expression and activity of the coagulation initiator- tissue factor (TF) and at the time interacts with the tissue factor pathway inhibitor (TFPI) on the cell surface membrane of endothelial cells, leading to dissociation of TFPI and therefore resulting in increased cell surface coagulation activity. Additionally, loss of HS chains enables increased platelet aggregation and mediates loss of antithrombin-III activity. Free circulating HS fragments trigger production of mitochondrial reactive oxygen species (ROS) resulting in (septic) cardiomyopathy. At the same time they competitively inhibit brain derived neurotrophic factor (BDNF), leading to cognitive dysfunction. Hpa-2 prevents membrane bound HS from being shed by Hpa-1. Therefore, LPS cannot be transferred from CD14 to TLR-4 and downstream signaling is inhibited. Circulating HS fragments are likely to be antagonized by Hpa-2 as well. As an acquired deficiency of Hpa-2 is found in sepsis, these protective functions are most likely critically diminished.
ability, inflammation, and neutrophil migration, ultimately leading to acute respiratory distress syndrome (ARDS) in human sepsis [38]. Accordingly, human plasma Hpa-1 activity is increased in both pulmonary and non-pulmonary sepsis and higher Hpa-1 levels are found in human biopsies with diffuse alveolar damage compared to normal lung tissue [38]. Interestingly, it was recently shown that human plasma Hpa-1 activity is also increased in coronavirus disease-2019 (COVID-19) [69].

Experimental inhibition of Hpa-1 is found to be protective for overall degradation of eGC reducing lung permeability and even improving survival in LPS-treated Hpa-1 deficient mice [38]. Heparin and the non-anticoagulant N-desulfated/re-N-acetylated heparin (NAH) fragment serve as an alternative substrate for Hpa-1 and have been extensively studied in murine sepsis models and cell culture experiments [38,70]. NAH prevented thinning of the eGC on human endothelial cells in vitro, which was observed after the addition of serum from sepsis patients – but not after the addition of serum from healthy controls [71]. However, the 65-kDa proenzyme of Hpa-1 has also been reported to be protective [72], which may be explained by nonenzymatic effects [73].

Acute kidney injury (AKI) is a common complication in sepsis [74]. Lygizos and colleagues analyzed Hpa-1 in kidneys of septic mice four hours after CLP surgery [39]. A higher glomerular Hpa-1 expression and activity were accompanied by loss of glomerular filtration as indicated by elevated blood urea nitrogen (BUN). At the same time, HS degradation activity in the urine was found elevated. Consequently, antagonizing Hpa-1 could prevent experimental septic AKI. These data together suggest a novel mechanism in the pathogenesis of sepsis-associated AKI. Interestingly, in contrast to the pulmonary microvasculature, neutrophil adhesion and endothelial permeability remain unchanged in kidneys of septic mice, suggesting a different mechanism of injury compared to ARDS [39].

Hpa-1 is also involved in coagulopathy. Hpa-1 overexpressing mice generate a larger thrombus size within a shorter period of time compared to control mice in arterial injury and stent occlusion models, supporting an important pro-coagulant effect of Hpa-1 [75]. Mechanistically, Hpa-1 up-regulates the expression of the blood coagulation initiator tissue factor (TF) [76] and at the same time interacts with the tissue factor pathway inhibitor (TFPI) on the cell surface membrane of endothelial cells, leading to dissociation of TFPI and therefore, resulting in increased cell surface coagulation activity [77]. Additionally, Hpa-1 directly enhances TF activity [78]. Hpa-1 is found to increase the thrombin-antithrombin level as well as D-Dimers in Hpa-1-treated mice to levels comparable to LPS-treated mice [79,80]. Injection of Hpa-1 following previous LPS administration enhances laboratory derangement of coagulation factors resembling DIC independently from inflammation. IL-6 is not elevated in Hpa-1-treated mice and thrombin-antithrombin levels and D-Dimers are not elevated in Hpa-1 deficient LPS-treated mice. These data suggest that Hpa-1 might be able to induce activation of the coagulation system independent of an inflammatory response while also enhancing diffuse coagulation activation resulting in DIC [79,80]. More recently, both Hpa-1 expression and activity were found to be elevated in platelets during human sepsis. The active 50 kDa form of Hpa-1 in platelets, not its 65 kDa proenzyme though, correlated with mortality [81]. Whether increased Hpa-1 expression and activity in thrombocytes of septic patients is mechanistically associated with increased risk of thrombus formation, still needs to be explored.

Intestinal inflammation in sepsis is also associated with Hpa-1 [82,83]. The level of HS degradation activity is elevated in intestinal tissue in CLP-subjected mice and associated with a loss of HS on intestinal villi. Accordingly, both Hpa-1 activity and its expression in intestinal tissue are elevated in sepsis [83]. Like in the lung, leukocyte adhesion and inflammation is increased in intestinal tissue [82]. Accordingly, Hpa-1 inhibitors, such as NAH, antagonize increased intestinal inflammation and permeability [82,83].

These findings suggest that Hpa-1 expression in sepsis is heterogenous and depends on the investigated tissue. Nevertheless, it has an increased activity in most organs thus contributing to multi-organ failure (i.e., ARDS, AKI, intestinal barrier dysfunction) [38,39,79–83].

Mechanistically, Lipopolysaccharide (LPS), found on the outer membrane of gram-negative bacteria, is a key PAMP in sepsis and can act as an Hpa-1 inducer [22,38,60]. LPS activates the toll-like receptor (TLR)-4 which downstream leads to cleavage and activation of the inflammatory transcription factor “nuclear factor ‘kappa-light-chain-enhancer’ of activated B-cells” (NF-κB). NF-κB activates cytokine production and release of e.g., Interleukin (IL)-6 [60]. IL-6 then activates Hpa-1, which results in cleavage of HS fragments from the eGC [60]. Additionally, Hpa-1 was demonstrated to further stimulate the pro-inflammatory response via TLRs mediated by non-catalytic mechanisms [84]. Hpa-1’s catalytic activity is reported to promote LPS response on TLR-4 receptors thereby, fulfilling a direct positive feedback loop in macrophages and endothelial cells [22,85]. Moreover, Hpa-1 shedding of the HS layer leads to higher accessibility of the TLR-4 receptors on the naked endothelial surface. Accessibility of further receptors that are involved in leukocyte adhesion or coagulation, which then further augments endothelial mediated inflammation and coagulation, is generally increased by Hpa-1 [21,38]. In addition, the endothelium destabilizing factor Angiopoietin-2 (Angpt-2) induces Hpa-1 activity after being released from
endothelial cells upon stimulation [44]. Circulating HS fragments are elevated in humans with sepsis (in gram negative sepsis higher than in gram positive etiology) and associated with increased mortality [27,63,86]. HS, when anchored within the eGC scaffold, modulates TLR-4 signaling and downstream inflammatory response [22]. In contrast, Hpa-1 shed, circulating HS fragments are reported to further act as DAMPs themselves and further stimulate TLR-4 signaling. TLR-4 induces Hpa-1 activation, leading to a vicious cycle of inflammation [22,57,60,63,87,88]. Additionally, HS fragments are known to stabilize the pro-inflammatory cytokine interferon gamma [89].

Levels of circulating HS fragments have been found to be involved in various mechanisms of organ dysfunction in sepsis [63,87,90,91]. For instance, HS fragments can increase the production of mitochondrial reactive oxygen species (ROS) in murine cardiomyocytes [90]. In contrast, the expression of receptors regulating mitochondrial coactivator-1 (PGC-1α), is reduced by HS [90]. The lower expression of PPAR and PGC-1α can be antagonized by inhibition of TLR-4 suggesting HS fragments to be involved in myocardial contractile dysfunction and cardiomyopathy [63,90]. Hipsensteel and colleagues analyzed the impact of HS fragments on the hippocampus in mice and humans [87]. Cognitive impairment is a well-known early complication of sepsis, termed septic encephalopathy [92]. HS fragments lead to hippocampal pathology in terms of both, an acute dysfunction of the hippocampal blood brain barrier, and a later loss of hippocampal volume in sepsis survivors [87]. Sulfated and charged HS fragments are released by Hpa-1 dependent shedding of the eGC, which then competitively inhibit brain-derived neurotrophic factor (BDNF)-mediated hippocampal long term potentiation resulting in cognitive dysfunction [87]. In contrast to LPS, in microglia, HS fragments and Hpa-1 reportedly exert anti-inflammatory properties [93]; however, this effect has not been described for any other tissue. In summary, circulating HS fragments act as DAMPs further enhancing dysregulation of the host response in sepsis.

**Role of Heparanase-2 as an opposing regulator of Heparanase-1**

Hpa-2 is a molecule with an overall sequence identity of 40% and a sequence resemblance of 59% to Hpa-1 [94,95]. Unlike Hpa-1, the physiological role of Hpa-2 remains largely obscure. Genetic studies revealed that mutations in Hpa-2 gene – leading to a truncated Hpa-2 protein – cause the urofacial syndrome (UFS), a rare autosomal recessive disease with severe dysfunctional urination and peripheral neuropathies [96,97]. Homozygous Hpa-2 mutant mice suffer from growth retardation and poor weight gain after birth, ultimately, leading to death [97]. Given the lethal phenotype of homozygous Hpa-2 mutant mice, it is conceivable that Hpa-2 plays a significant role in the development and function of multiple organs other than the bladder [97]. Conditional and tissue specific knockouts may help to better understand its physiology in the future. Hpa-2 is spliced alternatively and has three known isoforms (Hpa-2a, Hpa-2b, Hpa-2c). Only Hpa-2c (in this review referred to as Hpa-2) is secreted and has a higher affinity to HS and heparin than Hpa-1 [95,97]. In contrast to Hpa-1, Hpa-2 undergoes no proteolytic processing and therefore lacks enzymatic activity [95,97]. Hpa-2 is known to be involved in diverse cancer pathologies, i.e. in head, neck, colorectal and ovarian cancer [95,97]. So far, however, it remains unknown if this is due to inhibition of Hpa-1 activity or by mechanisms independent of a putative interaction with Hpa-1. For example, Hpa-2 might inhibit signaling of cleaved eGC components. Direct inhibition of inflammatory cytokines that downstream lead to eGC breakdown, seems also possible. The exact mechanism of Hpa-2 remains to be elucidated. It has been reported that Hpa-2 is secreted by endothelial cells into the circulation of humans and its levels are closely associated with syndecan-1 and -4 proteoglycans on the cell surface [22,95,98]. However, transcriptional and post-transcriptional regulation of Hpa-2, especially in respect to endothelial cell biology and disease states associated other than cancer, are incompletely understood and need further investigation – especially in systemic inflammatory syndromes such as sepsis.

Only recently, our own group discovered that administration of exogenous Hpa-2 can reduce Interleukin (IL)-6 production in LPS stimulated endothelial cells [22]. Moreover, Hpa-2 can antagonize the LPS-dependent increase of endogenous Hpa-1 activity [22]. Further downstream, Hpa-2 reduces LPS-induced activation of NF-κB signaling in endothelial cells. Simultaneous Hpa-1 inhibition shows no additive effect suggesting that both molecules act via the same molecular pathway [22]. Besides an anti-inflammatory phenotype, also a broad anti-permeability effect has been observed in Hpa-2 overexpressing endothelial cells [22]. LPS binds to CD14, a TLR-4 co-receptor [99] and is subsequently transferred to TLR-4 by lymphocyte antigen 96 (MD2), an accessory molecule, which recruits two adapter proteins, MyD88 and TIR-domain-containing adapter-inducing interferon-β (TRIF) which further leads to induction of further intracellular signaling. Cytokine synthesis and release (e.g. IL-6) as well as subsequent Hpa-1 activation are then initiated [22,100–102]. Hpa-2 counteracts this response by inhibiting TLR-4 stimulation, more precisely the activation of MyD88 and TRIF-mediated pathways [22]. Binding of LPS to
CD14 itself is not inhibited by Hpa-2; rather Hpa-2 prevents LPS transfer from CD14 to the TLR-4/MD2 complex [22]. Importantly, Hpa-2 stabilizes membrane bound HS [22]. It appears to bind to its cleavage sites thereby preventing Hpa-1 mediated shedding (in Hpa-2 overexpressing endothelial cells [22]). As membrane bound HS itself may inhibit LPS transfer from CD14 to TLR-4, Hpa-2 augments this inhibition and the endothelial surface adhesion molecules to be expressed after eGC shedding may still be covered by HS chains [22,38].

Heterogeneous findings concerning Hpa-1 in sepsis exist, all demonstrating elevated Hpa-1 activity but different Hpa-1 concentrations [63–65]. In contrast, a substantial acquired Hpa-2 deficiency has been demonstrated in both murine sepsis models (LPS and CLP) as well as septic patients [22,65]. Recently, our group was able to show that Hpa-2 is also decreased in critically ill patients with COVID-19, a disease that can be complicated by overwhelming systemic inflammation and endotheliopathy [103–105].

In conclusion, the ratio between injurious Hpa-1 and protective Hpa-2 appears crucial for local HS turnover and eGC degradation [22,65]. A concurrent increase in Hpa-1 and a reduction in Hpa-2, resulting in an imbalanced Hpa-1/Hpa-2 ratio, may play a crucial role in the pathological host response in sepsis [22,65,103].

**Therapeutic considerations in sepsis**

As specific treatment strategies modulating the dysregulated host response in sepsis are lacking, clinical management concepts are limited to infection control, volume supplementation, vasopressors and organ supportive therapies [106,107]. This also applies to strategies what would specifically target the eGC [27]. In the following we would like to highlight potential therapeutic concepts that aim at restoring the Hpa-1/Hpa-2 ratio to attenuate sepsis associated eGC injury.

As Hpa-1 activity is highly upregulated in sepsis, a major therapeutic target would be the direct inhibition of Hpa-1 activity as initially described by Schmidt and colleagues in preclinical in vitro studies and septic mouse models [38]. The broadly used anticoagulant “heparin” can also function as a natural Hpa-1 antagonist [38]. Hpa-1 inhibition by heparin prevents endotoxinemia-associated eGC loss and neutrophil adhesion. Furthermore, Hpa-1 inhibition attenuates sepsis-induced acute lung injury and mortality in mice. Those effects could be achieved by administration of heparin even three hours after intraperitoneal LPS administration [38]. Likewise, Yini and colleagues showed in experiments with beagle dogs, that unfractionated heparin (UFH) reduces eGC shedding [108]. Low molecular weight heparin (LMWH) was also able to reduce eGC shedding in inflammation in rats [109]. In septic patients, however, application of high doses of heparin will remain highly controversial due to its anticoagulant effect in patients that are at risk of diffuse and potential life threatening bleeding related to the frequent existence of a disseminated consumption of coagulation factors [56]. Non-anticoagulant heparins (e.g., NAH) as well as Heparin-mimetics (i.e. Roneoparstat, Necuparanib, PG545) have been demonstrated to effectively inhibit Hpa-1 without anti-coagulant side effects in preclinical investigations [38,39,56,100–112]. Most studies of NAH and its inhibiting effect on Hpa-1 focus on tumor pathophysiology though [110,111]. Other Heparin-mimetics (i.e., Muparfostat, Roneoparstat, Necuparanib, PG545) that inhibit Hpa-1’s enzymatic activity are being evaluated in phase I clinical trials for various types of cancer and appear to be well tolerated [113]. Nevertheless, keeping in mind the unphysiological volume of distribution and the unpredictable pharmacokinetics and -dynamics in sepsis patients, those results are hardly transferable from oncology phase I studies with hemodynamically stable patients to the ICU area.

Recently, Martin and colleagues described the protective effect of the synthetic peptide 19–2.5 on the eGC by inhibition of both Hpa-1 activity and Hpa-1 shed HS fragments in mice subjected to CLP [86]. Peptide 19–2.5 is a synthetic antimicrobial peptide that has been primarily investigated for its potential to antagonize the most potent bacterial toxins from both gram-negative (e.g. LPS) and gram-positive bacteria (e.g. lipoproteins/peptides (LP)) [114]. Treatment of CLP-subjected mice with peptide 19–2.5 lowers Hpa-1 plasma levels, circulating HS-fragments, and reduces Hpa-1 activity. Additionally, these mice show lower Hpa-1 expression at the transcriptional level (mRNA) in heart, liver, lung, kidney and spleen [86]. Further studies have investigated the anti-inflammatory effects of peptide 19–2.5 [63,90,115]. Isothermal titration experiments with peptide 19–2.5 show that it has a low immunogenic potential. LPS-induced cytokine induction in human mononuclear cells in the presence of common antibiotics such as tetracycline, ciprofloxacin and amoxicillin show that classical anti-infective agents had no influence on the inhibiting action of peptide 19–2.5. Peptide 19–2.5 intercalates into the membrane and inhibits LPS to bind to cell receptors such as the receptor complex TLR4/MD2 [115]. Although promising, clinical studies are needed to evaluate the potential of this novel drug in patients.

Recent resolution of the crystal structure of the Hpa-1 protein will hopefully enable the development of specific high affinity Hpa-1-inhibiting small molecules in the future [116,117].

An acquired deficiency of the natural Hpa-1 antagonist Hpa-2 has been demonstrated in both preclinical and clinical investigations in sepsis [22,65]. Supplementation with exogenous Hpa-2 attenuates Hpa-1 induced eGC breakdown and...
subsequent inflammation in septic mice (see above) [22]. However, a recombinant Hpa-2 substitution to directly restore protective Hpa-2 levels and therefore re-balance the Hpa-1/Hpa-2 ratio is not available at present.

In the absence of a specific Hpa-1 inhibitory therapy available for clinical use in sepsis, it appears reasonable to explore the potential of already existing adjunctive therapeutic modalities targeting both excessive cleaved HS fragments and the dysbalanced eGC regulating Hpa-1/2 ratio.

Therapeutic plasma exchange (TPE) has been investigated as an adjunctive extracorporeal sepsis treatment and has recently gained much attention. TPE removes excessive injurious molecules, i.e. pro-inflammatory, pro-coagulant and endothelium destabilizing proteins [118,119]. Simultaneously, deficient but protective proteins, i.e. anticoagulant and endothelium-stabilizing proteins as well as immunoglobulins, are replaced [118–120]. Given the potential of removing of excessive injurious mediators while at the same time replacing consumed protective factors [121], TPE might represent a therapeutic option until specific host response modulating therapies are available.

This begs the question whether it is possible to transfer this concept of adjunctive TPE to the pathophysiological field of the eGC?

In a prospective predefined sub-study of a randomized clinical trial it was demonstrated that early septic patients have only mildly elevated cytokine levels but significantly increased syndecan-1 levels as a surrogate parameter of (early) eGC damage [122]. In fact, directly after transfusion of fresh frozen donor plasma (FFP) to critical ill septic patients, syndecan-1 blood concentrations can be significantly decreased [122]. Recently, our group demonstrated in patients with early and severe septic shock, that TPE is able to reduce blood concentrations of products of eGC shedding including HS (reduced by about a third after one TPE in septic patients). At the same time, TPE attenuates acquired Hpa-2 deficiency by effectively replacing Hpa-2 blood concentration [65]. A planned German multicenter randomized controlled trial will investigate the impact of TPE in septic shock (EXCHANGE 2 study). A pre-defined sub-analysis of this trial might also help to further clarify the potential of TPE as an eGC protective therapeutic option.

In the future, it may be important to combine different therapeutic strategies to prevent degradation of eGC in sepsis. Specific Hpa-1 inhibitor therapies are needed to effectively counteract upregulated injurious Hpa-1 [116,117]. At the same time, TPE may replenish protective Hpa-2 while at the same time removing injurious eGC shedding products [65]. Together, eGC shedding in sepsis may be protected effectively by simultaneously combining different therapeutic strategies in modulating diverse eGC regulating molecules and effectors. Further studies are needed to further evaluate the potential of therapeutic efforts in modulating eGC in sepsis.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Authors contributions**

TP, AMH and KS did literature research and wrote the initial manuscript. TP, AMH, PK, SD and KS co-wrote the manuscript. TP, AMH, PK, HH, SD and KS discussed data. All authors read and approved the final manuscript.

**DECLARATION OF COMPETING INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1 These authors contributed equally to this work.

**References**

[1] Singer, M., Deutschman, C.S., Seymour, C.W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G. R., Chiche, J.-D., Coopersmith, C.M., Hotchkiss, R.S., Levy, M.M., Marshall, J.C., Martin, G.S., Opal, S.M., Rubenfeld, G.D., van der Poll, T., Vincent, J.-L., Angus, D.C., (2016). The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*, 315, 801–810. https://doi.org/10.1001/jama.2016.0287.

[2] Angus, D.C., van der Poll, T., (2013). Sepsis and septic shock. *N. Engl. J. Med.*, 369 (9), 840–851. https://doi.org/10.1056/NEJMra1208623.

[3] Thompson, K., Venkatesh, B., Finfer, S., (2019). Sepsis and septic shock: current approaches to management. *Int. Med. J.*, 49 (2), 160–170. https://doi.org/10.1111/imj.14199.
parameters and endothelial glycocalyx dimensions in resuscitated sepsis. Crit. Care., 23, 260. https://doi.org/10.1186/s13054-019-2542-2.

[54] Beurskens, D.M.H., Bol, M.E., Delhaas, T., van de Poll, M. C.G., Reutelingsperger, C.P.M., Nicolaes, G.A.F., Sels, J.-W., (2020). Decreased endothelial glycocalyx thickness is an early predictor of mortality in sepsis. Anaesth. Intensive Care., 48 (3), 221–228. https://doi.org/10.1177/0310057X20916471.

[55] Steppan, J., Hofer, S., Funke, B., Brenner, T., Henrich, M., Martin, E., Weitz, J., Hofmann, U., Weigand, M.A., (2011). Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalyx. J. Surg. Res., 165 (1), 136–141. https://doi.org/10.1016/j.jss.2009.04.034.

[56] Ikeda, M., Matsumoto, H., Ogura, H., Hirose, T., Shimizu, K., Yamamoto, K., Maruyama, I., Shimazu, T., (2018). Circulating syndecan-1 predicts the development of disseminated intravascular coagulation in patients with sepsis. J. Crit. Care., 43, 48–53. https://doi.org/10.1016/j.jcc.2017.07.049.

[57] Nelson, A., Berkestedt, I., Bodelsson, M., (2014). Circulating glycosaminoglycan species in septic shock. Acta Anaesthesiol. Scand., 58 (1), 36–43. https://doi.org/10.1111/aas.12233.

[58] Godder, K., Vlodavsky, I., Eldor, A., Weksler, B.B., Haimovitz-Freidman, A., Fuks, Z., (1991). Heparanase activity in cultured endothelial cells. J. Cell. Physiol., 148 (2), 274–280. https://doi.org/10.1002/jcp.1041480213.

[59] Ishai-Michaeli, R., Eldor, A., Vlodavsky, I., (1990). Heparanase activity expressed by platelets, neutrophils, and lymphoma cells releases active fibroblast growth factor from extracellular matrix. Cell Regul., 1 (1), 833–842. https://doi.org/10.1091/mbc.1.1.833.

[60] Martin, L., Koczera, P., Zechendorf, E., Schuerholz, T., (2016). The endothelial glycocalyx: new diagnostic and therapeutic approaches in sepsis. Biomed Res. Int., 2016, 1–8. https://doi.org/10.1155/2016/3758278.

[61] Cassinelli, G., Torri, G., Naggi, A., (2020). Non-anticoagulant heparins as heparanase inhibitors. Adv. Exp. Med. Biol., 1221, 493–522. https://doi.org/10.1007/978-3-030-34521-1_20.

[62] Rabellino, T.J., de Zeeuw, D., (2015). The glycocalyx–linking albuminuria with renal and cardiovascular disease. Nat. Rev. Nephrol., 11 (11), 667–676. https://doi.org/10.1038/nrneph.2015.162.

[63] Martin, L., Schmitz, S., De Santis, R., Doemming, S., Haase, H., Hoeger, J., Heinbockel, L., Brandenburg, K., Marx, G., Schuerholz, T., van Griensven, M., (2015). Peptide 19–2.5 inhibits heparan sulfate-triggered inflammation in murine cardiomyocytes stimulated with human serum sepsis. PLoS ONE, 10 (5), e0127584. https://doi.org/10.1371/journal.pone.0127584.

[64] Matan, M., King, D., Peled, E., Ackerman, S., Bar-Lavi, Y., Brenner, B., Nadir, Y., (2018). Heparanase level and procoagulant activity are reduced in severe sepsis. Eur. J. Haematol., 100 (2), 182–188. https://doi.org/10.1111/ejh.12997.

[65] Stahl, K., Hillebrand, U.C., Kiyan, Y., Seeliger, B., Schmidt, J.J., Schenk, H., Pape, T., Schmidt, B.M.W., Welle, T., Hoener, M.M., Sauer, A., Wygrecka, M., Bode, C., Wedemeyer, H., Haller, H., David, S., (2021). Effects of therapeutic plasma exchange on the endothelial glycocalyx in septic shock. Intensive Care Med. Exp., 9, 57. https://doi.org/10.1186/s40635-021-00417-4.

[66] Shu, J., Santulli, G., (2019). Heparanase in health and disease: the neglected housekeeper of the cell? Atherosclerosis., 283, 124–126. https://doi.org/10.1016/j.atherosclerosis.2019.01.017.

[67] Yang, X., Meegan, J.E., Jannaway, M., Coleman, D.C., Yuan, S.Y., (2018). A disintegrin and metalloproteinase 15-mediated glycocalyx shedding contributes to vascular leakage during inflammation. Cardiovasc. Res., 114, 1752–1763. https://doi.org/10.1093/cvr/cvy167.

[68] Han, S., Lee, S.-J., Kim, K.E., Lee, H.S., Oh, N., Park, I., Ko, E., Oh, S.J., Lee, Y.-S., Kim, D., Lee, S., Lee, D.H., Lee, K.-H., Chae, S.Y., Lee, J.-H., Kim, S.-J., Kim, H.-C., Kim, S., Kim, S.H., Kim, C., Nakaoka, Y., He, Y., Augustin, H.G., Hu, J., Song, P.H., Kim, Y.-I., Kim, P., Kim, I., Koh, G.Y., (2016). Amelioration of sepsis by TIE2 activation-inhibited vascular protection. Sci. Transl. Med., 8, 335ra55. https://doi.org/10.1126/scitranslmed.aad9520.

[69] Buijssers, B., Yanginlar, C., de Nooijer, A., Grondman, I., Maciej-Hulme, M.L., Jonkman, I., Janssen, N.A.F., Rother, N., de Graaf, M., Pickkers, P., Kox, M., Joosten, L.A.B., Nijenhuis, T., Netea, M.G., Hilbrands, L., van de Veerdonk, F.L., Duivenvoorden, R., de Mast, R., van der Vlag, J., (2020). Increased plasma heparanase activity in COVID-19 Patients. Front. Immunol., 11, https://doi.org/10.3389/fimmu.2020.575047. 575047.

[70] Wiesinger, A., Peters, W., Chappell, D., Kentence, D., Reuter, S., Ravenstäd, H., Oberleithner, H., Kümper, P., Sokolov, I., (2013). Nanomechanics of the endothelial glycocalyx in experimental sepsis. PLoS ONE, 8 (11), e80905. https://doi.org/10.1371/journal.pone.0080905.

[71] Drost, C.C., Rosas, A., Kusche-Vihorg, K., Van Slyke, P., Kim, H., Hoang, V.C., Maynes, J.T., Wemmann, D.O., Ravenstäd, H., Linke, W., Lukasz, A., Hesse, B., Kümper, P., (2019). Tie2 activation promotes protection and reconstitution of the endothelial glycocalyx in human sepsis. Thromb. Haemost., 119 (11), 1827–1838. https://doi.org/10.1055/s-0039-1695768.

[72] Bashenko, Y., Ilan, N., Krausz, M.M., Vladovskiy, I., Hirsh, M.I., (2007). Heparanase pretreatment attenuates endotoxin-induced acute lung injury in rats. Shock., 28, 207–212. https://doi.org/10.1097/shk.0b013e31803118d4.

[73] Fux, L., Ilan, N., Sanderson, R.D., Vladovskiy, I., (2009). Heparanase: busy at the cell surface. Trends Biochem. Sci., 34 (10), 511–519. https://doi.org/10.1016/j.tibs.2009.06.005.

[74] Gómez, H., Kellum, J.A., (2016). Sepsis-induced acute kidney injury. Curr. Opin. Crit. Care., 22 (6), 546–553. https://doi.org/10.1097/MCC.0000000000000356.

[75] Baker, A.B., Gibson, W.J., Kolachalama, V.B., Golomb, M., Indolfi, L., Spruill, C., Zcharia, E., Vladovskiy, I., Edelman, E.R., (2012). Heparanase regulates thrombosis in vascular injury and stent-induced flow disturbance. J. Am. Coll. Cardiol., 59 (17), 1551–1560. https://doi.org/10.1016/j.jacc.2011.11.057.

[76] Nadir, Y., Brenner, B., Zetser, A., Ilan, N., Shafat, I., Zcharia, E., Golshmidt, O., Vladovskiy, I., (2006). Heparanase induces tissue factor expression in vascular endothelial and cancer cells. J. Thromb. Haemost., 4, 2443–2451. https://doi.org/10.1111/j.1538-7836.2006.02212.x.
Tiralongo, Beacham, I.R., Blanchard, H., von Itzstein, M., (2015). Functional and structural characterization of a heparanase. Nat. Chem. Biol., 11 (12), 955–957. https://doi.org/10.1038/nchembio.1956.

[117] Wu, L., Jiang, J., Jin, Y., Kallemeijn, W.W., Kuo, C.-L., Artola, M., Dai, W., van Elk, C., van Eijk, M., van der Marel, G.A., Coddé, J.D.C., Florea, B.I., Aerts, J.M.F.G., Overkleeft, H.S., Davies, G.J., (2017). Activity-based probes for functional interrogation of retaining β-glucuronidases. Nat. Chem. Biol., 13 (8), 867–873. https://doi.org/10.1038/nchembio.2395.

[118] Knaup, H., Stahl, K., Schmidt, B.M.W., Idowu, T.O., Busch, M., Wiesner, O., Welte, T., Haller, H., Kielstein, J. T., Hoeper, M.M., David, S., (2018). Early therapeutic plasma exchange in septic shock: A prospective open-label nonrandomized pilot study focusing on safety, hemodynamics, vascular barrier function, and biologic markers. Crit. Care., 22, 1–11. https://doi.org/10.1186/s13054-018-2220-9.

[119] Stahl, K., Schmidt, J.J., Seeliger, B., Schmidt, B.M.W., Welte, T., Haller, H., Hoeper, M.M., Buddle, U., Bode, C., David, (2020). Effect of therapeutic plasma exchange on endothelial activation and coagulation-related parameters in septic shock. Crit. Care., 24, 71. https://doi.org/10.1186/s13054-020-2799-5.

[120] Stahl, K., Bikker, R., Seeliger, B., Schmidt, J.J., Schenk, H., Schmidt, B.M.W., Welte, T., Haller, H., Hoeper, M.M., Brand, K., David, S., (2021). Effect of therapeutic plasma exchange on immunoglobulin deficiency in early and severe septic shock. J. Intensive Care Med., 36 (12), 1491–1497. https://doi.org/10.1177/0885066620965169.

[121] David, S., Stahl, K., (2019). To remove and replace—a role for plasma exchange in counterbalancing the host response in sepsis. Crit. Care., 23, 14. https://doi.org/10.1186/s13054-018-2289-1.

[122] Straat, M., Müller, M.CA., Meijers, J.CM., Arbous, M.S., Spoelstra-de Man, A.ME., Beurskens, C.JP., Vroom, M. B., Juffermans, N.P., (2015). Effect of transfusion of fresh frozen plasma on parameters of endothelial condition and inflammatory status in non-bleeding critically ill patients: a prospective substudy of a randomized trial. Crit. Care., 19 (1) https://doi.org/10.1186/s13054-015-0826-6.