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Immune dysfunction following severe trauma: A systems failure from the central nervous system to mitochondria

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When a traumatic injury exceeds the body's internal tolerances, the innate immune and inflammatory systems are rapidly activated, and if not contained early, increase morbidity and mortality. Early deaths after hospital admission are mostly from central nervous system (CNS) trauma, hemorrhage and circulatory collapse (30%), and later deaths from hyperinflammation, immunosuppression, infection, sepsis, acute respiratory distress, and multiple organ failure (20%). The molecular drivers of secondary injury include damage associated molecular patterns (DAMPs), pathogen associated molecular patterns (PAMPs) and other immune-modifying agents that activate the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic stress response. Despite a number of drugs targeting specific anti-inflammatory and immune pathways showing promise in animal models, the majority have failed to translate. Reasons for failure include difficulty to replicate the heterogeneity of humans, poorly designed trials, inappropriate use of specific pathogen-free (SPF) animals, ignoring sex-specific differences, and the flawed practice of single-nodal targeting. Systems interconnectedness is a major overlooked factor. We argue that if the CNS is protected early after major trauma and control of cardiovascular function is maintained, the endothelial-glycocalyx will be protected, sufficient oxygen will be delivered, mitochondrial energetics will be maintained, inflammation will be resolved and immune dysfunction will be minimized. The current challenge is to develop new systems-based drugs that target the CNS coupling of whole-body function.

KEYWORDS

trauma, hemorrhage, immune, inflammation, mitochondria, system, ALM, cytokines

Introduction

Globally, over one billion people sustain traumatic injuries, and over six million die annually (1). Mortality is twofold higher in low- and middle-income countries compared to high-income countries, and up to 5-times higher in resource-limited rural and remote regions (1, 2). In patients who survive the first few hours of hospital admission, complications can occur at different times (**Figure 1**) (3). The first window is 3–6 to 24 h where CNS dysfunction (∼50% of early deaths) and circulatory collapse (usually manifesting as shock) lead to early deaths (40% of early deaths) (**Figure 1**) (4–6). The second window occurs over the next few weeks and typically involves infectious complications with a prolonged indolent form of multiple organ failure, immunosuppression and sepsis, referred to as Persistent Inflammation, Immunosuppression and Catabolism Syndrome (PIICS) (∼20% of deaths) (**Figure 1**) (4, 7, 8). Sepsis develops in ∼10% of these patients and multiple organ dysfunction syndrome (MODS) in around 70% (3, 9, 10). Despite decades of research, little progress has been made in the development of effective drugs to treat the polytrauma patient (2, 11, 12). The lack of progress in drug development may reflect the way we think about the problem (13, 14). In this review, our aim is to discuss the inflammatory and immune mechanisms that are believed to be responsible for early and late secondary injuries and death following traumatic injury, and possible ways to reduce morbidity and mortality from a systems-based perspective. Before doing so, we will briefly discuss the physiological importance of the system.

Challenging the steady-state and evolutionary internal tolerances

After a traumatic injury, defined as one or more sudden injuries requiring immediate medical attention, the body activates a series of defense mechanisms to restore homeostatic balance. The concept of homeostatic balance was introduced into medicine in 1916 by Cannon (15). Cannon's genius was to combine the ideas of Pfluger's "*natural adjustments*" (1877), Bernard's concept of "*milieu intérieur*" (1878), and Richet's "*living beings were stable but modifiable*" (1900) into a unified scheme (16). Cannon proposed that every living organism was in a *dynamic state of constancy*, with its constituent parts and processes being *actively* maintained in balance despite external fluctuations (15). The system is not an equilibrium system as it requires a continual flow of matter, energy and exchange with the environment (16, 17). In the mid-1930s, Cannon's concept was refined to include negative and positive feedback circuits (18), and the system's steady-state was now viewed as the net sum of negative and positive feedback mechanisms that operate within a range of tolerances, which differ from person to person, and from species to species. The system has evolved such that small injury perturbations are self-limiting and quickly resolved. However, when the trauma overwhelms the system, it triggers a CNS stress response that typically involves excessive sympathetic and neuroendocrine outflows from the brain's central control, hyperinflammation, immune dysregulation, coagulopathy, endothelial activation and metabolic dysfunction (13, 19, 20). *If homeostatic balance is not restored early, secondary injury processes will amplify and may become life-threatening* (14).

Early and Late Deaths after Major Traumatic Injury

Early deaths: 3-6 to 24 h	Late deaths: 1 to 7-14 days
(~30% of deaths after admission)	**(~20% of deaths after admission)**
• Of 30% early deaths, ~50% are from complex CNS, ~40% from cardiovascular, shock, and ~10% other. • Unresponsive to fluids and support. • Lactate and creatine kinase rises. • CNS swelling in non-TBI patients. • Profound cardiac and vascular failure.	• Prolonged indolent form of multiple organ failure different from the past. • Immunosuppression. • Multiple episodes of sepsis. • Excessive or dysfunctional immune response to trauma.

FIGURE 1
After hospital admission, complications occur at different times and depend upon type and severity of injury. These have been characterized as early and late Deaths. Modified after Rauf et al. (3) and Brohi et al. (4). Patients who died at the point-of-injury or in the first few hours upon arrival to hospital have not been included. CNS, central nervous system; TBI, traumatic brain injury.

First line-of-defense: The innate immune system

> When I first put forward the biological theory of inflammation 8 years ago, I expressed the idea that this reaction is affected by the intermediation of a physiological continuity between "the cells of the connective tissue, those of the endothelial wall and the leucocytes, which form a complete chain and play the principal part in the inflammation of vertebrates." The connective tissue cells which are first attacked, would, I thought, transmit the action to the vascular wall, the cells of which would contract to facilitate the passage of the white corpuscles.
>
> Metchnikoff (21) p. 191.

Any trauma to the body inflicts a barrier breach in three-dimensional space and one in time. Damage signals from cellular, vascular and nerve injury are sent around the body, and to the CNS *via* nerve afferents and resident damage control mechanisms to begin the process of tissue repair and remodeling (14, 22). Recovery begins by rapidly closing the breach, activating immuno-inflammatory processes, removing damaged cells and killing any invading microbes (**Figure 2**).

Immune defense occurs in two parts: First, there is a local frontline defense from patrolling resident immune cells in tissues, and second, from deployment of additional leukocyte subsets from the circulation. Early defense includes activation of tissue resident macrophages, dendritic cells (DCs), neutrophils (PMNs), mast cells, a subset of memory B cells, natural killer (NK) cells, complement (22–24) and recently characterized resident T cells, referred to as innate lymphoid cells (ILCs), which are believed to interact with other resident cells, and trigger the early adaptive immune response and recruitment of cells from the circulation to repair and restore tissue function (25–29) (**Figure 2**).

This diverse group of resident innate cells have evolved different pattern-recognition receptors that detect and respond to changes in the local environment, including damage associated molecular patterns (DAMPS), pathogen associated molecular patterns (PAMPs) and other immune-modifying triggers (**Figure 2**) (30–32). DAMPs are released from damaged, stressed or dying cells, including extracellular and cell membrane, cytosolic, cytoskeleton, nuclear mitochondrial, endothelial and blood components (30, 33), while PAMPs are signature proteins, lipoproteins, nucleic acids and saccharides located on the cell surface or released from invading pathogens. Together, they activate the body's early immune and inflammatory systems to dial in the right response to repair and restore function (**Figure 2**). Early post-traumatic DAMP markers include high mobility group box protein 1 (HMGB1), mitochondrial DNA (mtDNA), S100, cell fragments, and many other molecules from injured or dying cells and proteoglycans and glycoproteins from endothelial-glycocalyx shedding (34). Importantly, DAMPs and PAMPs are not mutually exclusive and may share co-receptors and accessory molecules, and form partnerships to coordinate the right response (35).

A 2011 landmark study of Xiao and collaborators shed light on the early activation patterns of the immune system following severe blunt trauma and *burn injuries*. The group reported there was ∼80% activation of the leukocyte transcriptome in the circulation, which they termed a genomic storm (36). This storm developed within 4–12 h and lasted days to weeks. Importantly, in Xiao's study, what separated patients who developed secondary complications was not the magnitude of the storm, rather the time to resolve it (36). Prolonged resolution times led to worse outcomes. Moreover, both pro-inflammatory and anti-inflammatory pathways were activated early, which challenges the older two-hit and other sequential pro-inflammatory and compensatory anti-inflammatory models of trauma (37). On a cautionary note, although transcriptomic analysis establishes early temporal patterns of change, it provides little or no knowledge into the molecular mechanisms. Future studies should include proteomic and pathway-level analysis to establish the different roles of the early innate (and adaptive systems) to amplify inflammation after severe trauma.

Early drivers of inflammation and immune dysfunction

Inflammation is universal, beneficial and restorative. However, after major trauma, it can be lethal. As mentioned earlier, the massive release of DAMPs can overwhelm the system and trigger a hyperinflammatory state that, if not resolved in a timely manner, can lead to immune dysfunction, immunosuppression, infection, sepsis and MODS (4, 13, 19, 38–41). The disruption can lead to pathological interactions between monocyte, macrophage, NK and DCs, T cell dysfunction, and the development of persistent lymphopenia (8–10, 34, 38, 40, 42–45). Persistent lymphopenia carries a high mortality. Brohi's group recently reported a 45% mortality rate in trauma patients when the lymphocyte count was $\leq 0.5 \times 10^9$/L at 48 h after hospital admission (38). In addition, the type of trauma determines a patient's susceptibility to persistent lymphopenia and infection, with traumatic brain injury (TBI) patients having disproportionally worse outcomes compared to those with burns, polytrauma or major surgery (43). A recent study of Campbell et al. reported that 37% of TBI patients were lymphopenic on hospital admission, and its persistence was associated with increased risk of mortality and pneumonia (46). Wang further reported that up to 83% of severe TBI patients contracted a respiratory infection within 3 days following injury (43, 47).

The mechanisms responsible for persistent lymphopenia and immunosuppression are not well understood (38, 48). The difficulty is that immunosuppression is a highly heterogeneous response involving differential T cell loss, T-cell exhaustion, T-helper 1 (Th1) depression, receptor shedding, and expansion of myeloid-derived suppressor cells (MDSCs) that have suppressive activity (44, 49). Separating the relative contributions of different immune cell subsets to post-traumatic immunosuppression has been a challenge. In a ground-breaking study, Mansen and colleagues examined early changes in circulating lymphocytes and showed that trauma patients who developed MODS within 24 h had nearly 2-fold higher $CD56^{dim}$ NK cells, 80% lower gamma delta (γδ)-low T cells and 4-fold higher IFN-γ upon hospital admission, compared to patients who did not (38). $CD56^{dim}$ NK cells are potent mediators of natural and antibody-dependent cytotoxicity and only weakly secrete cytokines (50). Moreover, the group showed that the patients who developed MODS also developed lymphopenia within 24 h of injury, which if persisted to 48 h led to high mortality (38). The association between lymphopenia, MODS and decreased frequencies and functional responses of innate T cells in trauma patients suggests that early immuno-inflammatory events may "predetermine" late secondary complications. The rise in NK cells and early fall in γδ-low T cells seen in patients who developed MODS may be clinically significant and predict risk for late complications, however, further studies are required (38, 48).

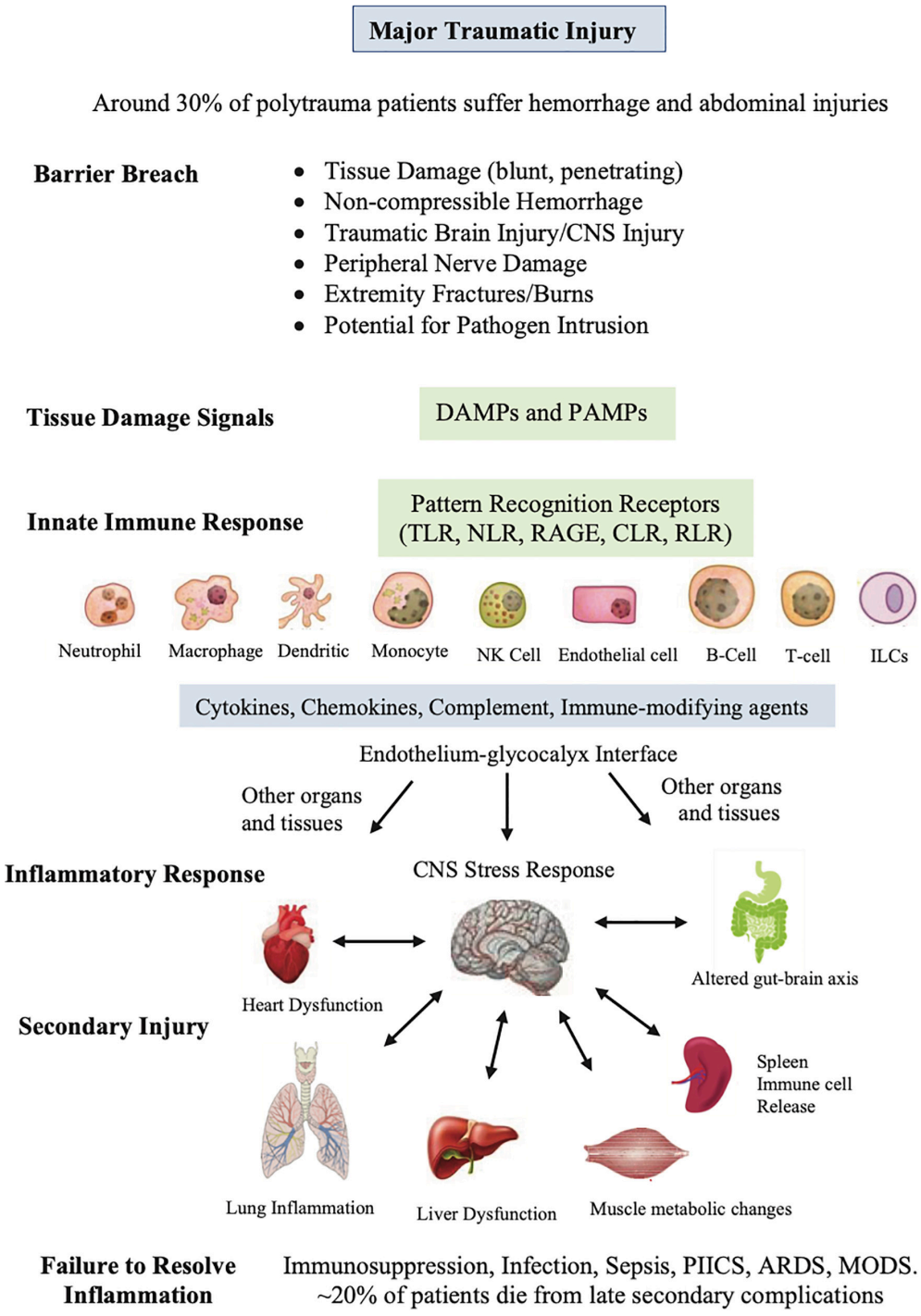

FIGURE 2
Sequence of events that occur after major traumatic injury. This diverse group of innate cells resident in the tissues detect and respond to changes in the local environment, including damage associated molecular patterns (DAMPs), pathogen associated molecular patterns (PAMPs), neural signals, and other immune-modifying triggers. The pattern recognition receptors on these cells include Toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic-acid-inducible gene-I (RIG-I)-like receptors (RLRs), and receptor for advanced glycation end products (RAGE). PAMPs can be derived from viruses, opportunistic bacteria, fungi, and protozoa and helminths. The innate cells orchestrate an immune response to response to the barrier breach by releasing different inflammatory factors. If dysregulated, the response can lead to secondary injury to the CNS and major organs of the body. The spleen has been included as it is reservoir of platelets, peripheral macrophages, undifferentiated monocytes and other immune cells. ILCs, innate lymphoid cells; NK, natural killer cells, PIICS; Persistent Inflammation, Immunosuppression and Catabolism Syndrome, ARDS; acute respiratory distress syndrome, MODS; multiple organ dysfunction.

TABLE 1 Possible mechanisms for T-cell apoptosis and immunosuppression after major trauma.

Pathway	Mechanisms	Comment	References
Cell-autonomous T cell death (ACAD)	• Intrinsic "caspase" pathway • Independent of death signals • Regulated by declining Bcl-2 at level of mitochondria • Cytochrome C released • Activates caspases • T cells undergo apoptosis without TCR restimulation	Toward the end of the immune response, activated lymphocytes not restimulated can die by permeabilizing the mitochondrial membrane. Bcl-2 is an anti-apoptotic protein that blocks the release of cytochrome c from mitochondria.	(53–55)
Stress-induced activation-induced cell death (AICD)	• Extrinsic "caspase" pathway • Death receptors: TNFR1, Fas, DR3, DR6, Trail-R1. • Glucocorticoid receptors (GRs) may also be involved • Receptor-driven apoptosis • Bax, bak, and BH3 domain • Activate caspases 8 and 3	A death receptor-mediated apoptosis pathway. The ligands for death receptors form a family of related cytokines collectively named as the TNF family.	(42, 52, 53, 56)
Monocyte-T cell interaction	• Extrinsic pathway • Inflammasome activation in monocytes sense DAMPs • IL-1β induced Fas-mediated monocyte driven T cell death *via* apoptosis	Monocytes sense injury-released DNA (DAMPs) *via* the AIM2 inflammasome and induce the extrinsic cell death of T cells.	(57)

Another early driver of immune complications is HMGB1, which is a major DAMP that induces inflammation *via* TNF-α, IL-6, and IL-1β that in turn stimulate pattern recognition receptors TLR4 and RAGE on immune cells (**Figure 2**) (44, 51). In a rat polytrauma model (femoral osteotomy, blunt chest contusion and burn injury), Muire and colleagues showed that HMGB1 was an early contributor to the onset of lymphopenia and the loss of $CD4^+$, $CD8^+$, and γδ-T cells (34). Interestingly, the decrease in T cells was partly attenuated when HMGB1 was neutralized immediately post-trauma, however, the γδ-T cell population was not affected (51). The authors proposed that diminished levels of surface expression of RAGE and TLR4 on T cells, *via* ectodomain shedding, may be responsible for suppression *in vivo* (51). HMGB1 has also been shown to activate MDSCs after trauma and cancer (44), and is a late mediator of sepsis (44, 51), which further highlights the complexity of the system.

Apoptosis is believed to play a central role in persistent lymphopenia (52–58). Three main mechanisms for inducing lymphocyte apoptosis include: (1) cell-autonomous T-cell death (ACAD), (2) stress-related activation-induced cell death (AICD), and (3) newly discovered inflammasome-dependent monocyte activation (52–58) (**Table 1**). Persistently elevated plasma interleukin (IL)-10 levels have further been correlated with monocyte deactivation, reduced T cell activation and secondary infectious complications (8, 39, 40, 42, 59). Platelets also modulate T cell subsets *via* PAR4 that may link the innate and adaptive systems *via* pro-inflammatory cytokines (58). The interconnectedness of the T cell subsets and potential drivers of immunosuppression requires further research. Interestingly, post-injury immunosuppression shares many similarities with non-traumatic, sepsis-induced immunosuppression (41, 57).

Central nervous system and organ interconnectedness: A major overlooked factor

> The defense of the organism against deleterious agencies, which is at first confined to the phagocytic mechanisms and the somatic system of nerves, by and by spreads to and is undertaken by the psychical nervous apparatus . . . One function of these psychical cells has been to develop a complete science for the defense of the organism against hostile influences.
>
> Metchnikoff (21) p. 195.

Metchnikoff had it right over 130 years ago. Activation of the "psychical cells" of the CNS following severe trauma results are important, and involve the release of norepinephrine, epinephrine and hormones (ACTH and glucocorticoids) from the adrenal medulla into the circulation and from the postganglionic nerve endings innervating the heart, and other organs of the body (14, 60–65). Traditionally, this is known as the whole-body stress response which dates back to Cannon (20, 66). The link between CNS injury, the immune system and immunosuppression is less well known. Yang and colleagues recently showed in a rat model of TBI that activation of sympathetic nervous system upregulated the expression of programmed cell death-1 (PD-1) on $CD4^+$ and $CD8^+$ T cells, and subsequently contributed to immunosuppression (43). The group speculated that immunosuppression may be partly mediated by stress hormones targeting β-adrenergic receptors (β-AR) on T cells (and indirectly B cells), because propranolol, a β-AR blocker, restored dysfunction *in vitro,* although they acknowledge it was more complex in the intact animal (43).

CNS modulation of the immune system occurs *via* the central hypothalamic-pituitary-adrenal (HPA) axis and the brainstem's nucleus tractus solitarius (NTS) (67–70). After major trauma, the CNS balance switches to a sympathetic dominance and suppression of the parasympathetic system that normally counters inflammation *via* activation of the parasympathetic vagal cholinergic neurons and splanchnic/splenic nerves, known as the inflammatory reflex (71–73). The shift in CNS balance also impacts other organs,

such as the heart and vasculature, and the gut microbiome *via* the gut-brain axis, which can alter blood flow to the gut wall and cause ischemia and increased permeability, where bacteria and/or their active metabolic products (lipopolysaccharides, cytokines, neuropeptides, and protein messengers) can enter the blood stream or lymph vessels and increase PAMPs and a patient's susceptibility to infection (74, 75). Together, all these factors *may contribute to predetermining the extent and resolvability of the immuno-inflammatory response* after major trauma.

Another unappreciated fact in the polytrauma patient is that many undergo a second trauma from the corrective surgery itself (20). At all times, from the prehospital setting to after major surgery, the brain remains "wide awake" to changes in circulating DAMPs and PAMPs, inflammatory cytokines and immune cells circulating in the body (20, 76). Even the anesthetized brain remains "awake" because the blood brain barrier (BBB) is disrupted from the trauma and changes in cerebral blood flow and shear stress, which is part of the injury phenotype (77), and this is further amplified in the patient with a TBI (20, 74, 78, 79). Following any major trauma, the brain loses its "immune privilege" as it is no longer "separated" from the rest of the body (77). This is a research area in its infancy. We recently showed in a rat model of a laparotomy, designed to simulate a penetrating wound, that profound changes in gene expression occurred in brain, heart and other organs (80). Abdominal trauma was associated with 10–20-fold increases in plasma corticosterone, pro-inflammatory cytokines, endothelial injury markers, neutrophils (6 h), lactate (3 days), and coagulopathy (80). Lymphocytes decreased by ∼70% at 6 h and 3 days, and IL-10 dramatically increased from undetectable baseline levels to 483 pg/ml after 6 h and again at 3 days (1,149 pg/ml). Cortical excitability was high over 3 days with 30-fold increases in M1 muscarinic receptor expression and α-1A-adrenergic expression, and similar in heart with 8-fold increases in β-1-adrenergic receptor expression, and up to 6-fold increases in M2 and M1 muscarinic receptors after 6 h despite no changes in hemodymanics (80). These "silent" changes are remarkable given that there was only one incision, with no further injury to brain or heart. Unfortunately, we did not examine changes in the different T-cell subsets to further understand changes in immune activation.

Systems hypothesis of trauma

> Except on few occasions, the patient appears to die from the body's response to infection rather than from it.
>
> William Osler (81)

Osler's point cannot be overemphasized. It is not the infection that kills you it's the body's response to the trauma. The shift in homeostatic balance toward extreme limits and death led us to develop the Systems Hypothesis of Trauma (SHOT) (82) (**Figure 3**), which has undergone a number of iterations to include hemorrhagic trauma and the trauma of surgery (12, 13, 16, 20). SHOT has three pillars of protection.

1. CNS-cardiovascular coupling (Central Controller)
2. Endothelial glycocalyx material exchange (Systemic Integrator)
3. Mitochondrial integrity (Systemic Regulator)

First pillar: Central nervous system-cardiovascular coupling

If the CNS can be protected early after trauma and the "hyperdrive" response can be suppressed, we argue that the immuno-inflammatory storms and lymphopenia will be reduced (20). According to SHOT, shifting autonomic balance toward parasympathetic outflows in the first minutes to hours after trauma would assist to maintain ventriculo-arterial (VA) coupling close to unity. VA coupling is a metric rarely discussed or measured in major trauma patients. It is the ratio of arterial elastance (Ea) to left-ventricular (LV) elastance (Ees) and can be measured from routine echocardiography (83–88). When the ratio is close to unity, the efficiency of the system is considered optimal. If the ratio is excessively high or low, the heart as a pump and vascular load become uncoupled with adverse downstream clinical outcomes (86, 89, 90). *The clinical advantage of VA coupling over ejection fraction (EF) or cardiac output (CO) is that it provides arterial load properties in addition to LV function* (86, 87). If the proximal arterial vessels become stiff, as a result of the CNS stress response, it increases load on the pump (91), whereas if the heart becomes stiff it cannot relax optimally to fill and eject blood into the conduit vessels (87). If both occur, they lead to VA uncoupling, tissue hypoperfusion, mitochondrial damage (92, 93) and subsequent immuno-inflammatory dysfunction (**Figure 3**). In the case of a high VA coupling ratio, vasodilator therapies can lower Ea and reduce the Ea/Ees ratio toward 1.0, and in the case of a low ratio, inotropes can increases Ees to improve VA coupling (92).

We predict further that maintaining VA coupling would improve immune function by reducing gut-brain axis dysfunction and preventing the gut wall from becoming ischemic and leaky which exacerbates immuno-inflammatory conditions, coagulopathy, immunosuppression, infection and sepsis (75). Howard and colleagues reported in trauma patients rapid changes in the microbiome during resuscitation and stabilization (94), although further studies are required to understand the role of the gut in exacerbating

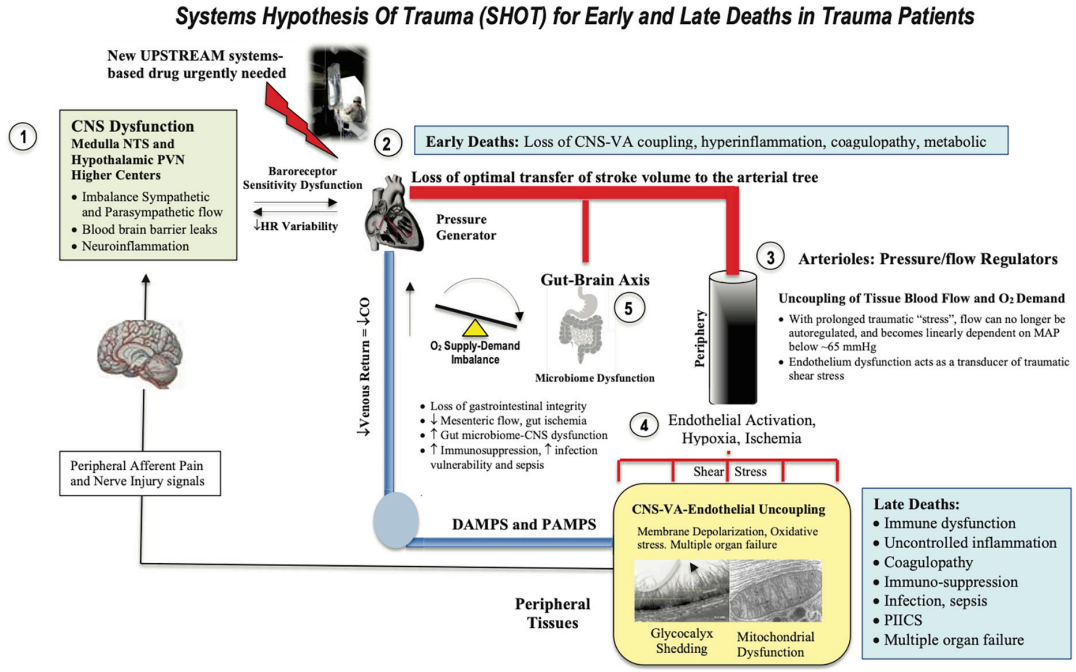

FIGURE 3
Systems Hypothesis Of Trauma (SHOT) showing key inter-connected sites of uncoupling during early and late deaths: (1) brain, (2) heart, (3) vasculature, (4) endothelial-glycocalyx-mitochondrial unit, and (5) gut barrier breaches. Our hypothesis is that if central and local control of cardiac output and ventriculo-arterial (VA) coupling are improved, endothelial and microvascular function will be improved and tissue O_2 delivery will be maintained. An uncoupling is reflected by an increase in stress hormones, sympathetic discharge, loss of baroreceptor sensitivity, reduced heart rate variability (183, 184), unresolved inflammation, immune dysfunction, coagulopathy and mitochondrial dysfunction. New drugs are required to prevent inflammatory hyperdrive and support a VA-coupled, high flow, vasodilatory system with endothelial-glycocalyx protection and tissue oxygenation (185). HR, heart rate; ATP, adenosine triphosphate; CNS, central nervous system; NTS, nucleus tractus solitarius; PVN, paraventricular nucleus of the hypothalamus; MAP, mean arterial pressure. PIICS, Persistent Inflammation, Immunosuppression and Catabolism Syndrome, see text for description of early and late deaths.

systemic inflammation and infectious complications after major trauma.

Second pillar: The endothelial glycocalyx

The second pillar of SHOT is to maintain the health of the endothelium (95). The endothelium is located at the nexus of the blood and tissues and controls the transfer of O_2, metabolic fuels, hormones, immune cells and factors, inflammatory regulators and fluids (96–102). Trauma-induced damage to this organ is termed the endotheliopathy of trauma (EoT), which is characterized by endothelial activation, vasoactivity, loss of barrier function, leukocyte adhesion, coagulopathy, inflammation and organ dysfunction (103–110). In addition, the endothelium, like the BBB, is highly sensitive to changes in blood flow and shear stress, which can alter vascular tone, tissue perfusion, exchange and permeability (111).

The endothelial surface area (SA) has been estimated to be 3,000–7,000 m^2 (98, 112). However, this estimate ignores the SA of the glycocalyx mesh that is synthesized and secreted by the endothelium and anchored to its cellular lining. As mentioned, the function of the endothelial glycocalyx is dynamic and diverse and it also acts as a vascular filter overlying the endothelial cell-cell junctions as it contains a large volume of non-circulating plasma (1–1.7 L) (113–115). We have estimated for the first time the SA of glycocalyx and found it was at least 10-fold higher than the endothelium ($SA_{glycocalyx}$ = 46,120 m^2) (see **Figure 4**). This is equivalent to a SA of over ~200 tennis courts or 8 USA football fields, and given its central role has major implications to immune function and secondary injury progression post-trauma.

When damaged by inflammatory mechanisms, the endothelium can rapidly shed its glycocalyx "fuzz" *via* sheddases, and release nanoscale bioactives and DAMPs, such as thrombomodulin, syndecan-1, heparan sulfate, hyaluronic acid, and other proteoglycans and glycoproteins, into the circulation (96, 104, 107, 116–119). This degradation is believed to perpetuate immuno-inflammation and coagulopathy (13, 120–125), immunosuppression (102, 126, 127) and mitochondrial dysfunction (115, 128, 129). SHOT predicts if VA coupling is close to unity and tissue perfusion and O_2 exchange

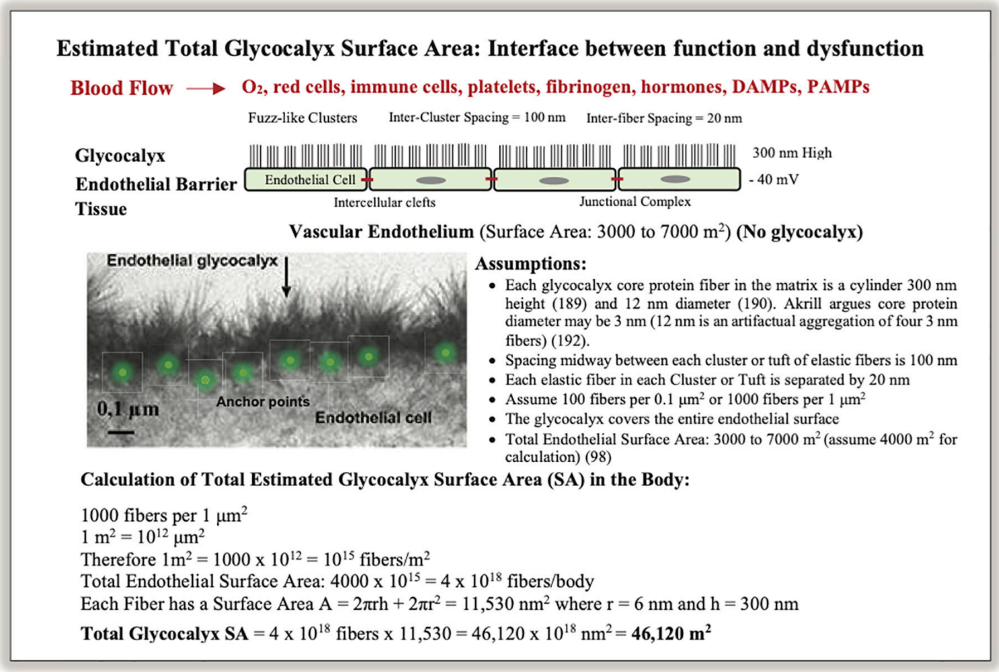

FIGURE 4
A schematic of the vascular endothelium and calculation of total glycocalyx surface area (SA) in humans. Photo insert was modified from Chappell et al. (186). Glycocalyx fibers appear in clusters composed of proteoglycans, glycosaminoglycans, and glycoproteins, which are anchored into endothelial cells by core-proteins (113, 115, 187, 188). Together they form a dynamic structure that participates in shear stress regulation, barrier protection, vascular permeability, inflammation, coagulopathy, fibrinolysis, mechanotransduction, immune function and cytokine signaling (97, 101, 104, 116). The glycocalyx is difficult to characterize because of its fragility and instability, and its structural dimensions critically depend on the method of ultrastructural visualization (97, 116, 189–193). The SA calculation should be viewed as approximate. A glycocalyx SA of 44,120 m^2 for material exchange equates to ~200 tennis courts or over 8 US football fields (see text).

can be maintained, damage to the endothelium-glycocalyx will be minimized and these secondary injury processes reduced (**Figure 3**). Remarkably, if adequate tissue perfusion can be restored, the damaged glycocalyx has the capacity to repair itself (130, 131). Timing of repair appears to depend upon the duration and extent of hypoperfusion and ischemia, and the type and severity of trauma (130, 131).

Third pillar: Mitochondrial integrity

Maintaining the functional integrity of mitochondria post-trauma is essential for a good outcome. Mitochondria are sensor organelles of ancient bacterial origins involved in ATP production, substrate regulation, immune cell signaling, calcium homeostasis, endoplasmic reticulum communication, and cell death regulation (32, 132–135). After severe trauma, prolonged hypoperfusion leads to mitochondrial damage. However, before damage occurs there is a switch from aerobic mitochondrial oxidative and to anaerobic glycolytic metabolism, which can only be sustained for short periods of time (136). Damage occurs from depletion of local glycogen stores, depolarization of the sarcolemma membrane, increased lactate, reduced pH, increases in cell Ca^{2+} loading, a fall in ATP phosphorylation and redox potentials, increased reactive oxygen species, reduced inner mitochondrial membrane proton pumping, opening of the permeability transition pore, collapse of mitochondrial membrane potential and finally the release of cytochrome C, and other DAMPs (32, 134, 135, 137–142). DAMPs from damaged mitochondria exacerbate CNS injury, cardiovascular dysfunction and secondary injury (143–145). According to SHOT, improving CNS protection and CNS-cardiovascular-endothelium coupling will improve tissue perfusion and protect mitochondrial integrity (146) (**Figure 3**).

Other unifying models of traumatic injury

In 2017, Johansson and colleagues introduced a model of SHock-INduced Endotheliopathy (SHINE) to better understand the underlying pathophysiological mechanisms for critically ill patients (147). Like SHOT, they propose that shock-induced sympatho-adrenal hyperactivation is a critical driver of endothelial cell and glycocalyx damage, hypoperfusion, and

subsequent hemostatic aberrations and multiorgan dysfunction (110). More recently, Henriksen reported that patients with identical trauma severity developed *significantly different degrees of endothelial dysfunction*, as measured by syndecan-1, and proposed a minimum of four shock-induced endotheliopathy phenotypes (148) with the differences most likely driven by a genetic component (148). Moreover, they introduced a new research tool in trauma by using metabolic systems biology, which should be encouraged. A major difference between SHOT and SHINE is the functional linkage between CNS and VA coupling, which is testable. SHINE does not include this key linkage, which describes the coupling of cardiac and arterial vascular reactivity to optimally propel blood to deliver sufficient oxygen from the lungs to tissue mitochondria and prevent and/or reduce ongoing immuno-inflammatory dysfunction (discussed above).

Urgent need for systems-based therapies: Heterogeneity vs. homogeneity in research

How do we switch the injury phenotype of a polytrauma patient to a survival one? Why are there no effective drugs to treat immune dysregulation in the early hours to days following major trauma or in the critically ill patient? We argue the main reasons for lack of progress in drug development include:

1. Failure to replicate the heterogeneity of humans.
2. Poorly designed trials lacking diversity.
3. Inappropriate use of pathogen-free animals.
4. Ignoring sex-specific differences.
5. The flawed practice of single-nodal targeting.

The heterogeneity of the human condition is a major variable when conducting animal experiments to solve a medical problem (149). Preclinical models typically use animals from relatively homogeneous breeding colonies whereas humans are genetically, epigenetically, biologically and physiologically heterogeneous (149, 150). Large animals, such as pigs and sheep, do have some advantages with similar physiologies and/or anatomies as humans, however, they are more costly than using rodents (149, 150). A second confounding variable are poorly designed human trials that are either not sufficiently powered or recruit patients who do not adequately represent the wider population for which the drug therapy is intended (151–153).

Similar problems apply to preclinical models that use specific pathogen-free (SPF) animals. SPF animals were introduced in the early 1960s to minimize disease or infection as an unwanted variable in experimental design (151, 154). However, SPF animals have different gut microbiota that can profoundly influence basic physiology, stress behaviors and the immuno-inflammatory response to trauma (151–153, 155). Beura et al. showed that SPF adult mice, for example, have "immature" immune systems that were more prone to infection than conventionally bred mice (156). SPF animals may be useful for studying specific questions in biochemical mechanisms, but they do not mimic the patient following trauma (152). The current consensus is that conventionally bred animals are the animals of choice if translation of a new drug therapy is the end-game (151). In addition, the mouse model may be problematic for trauma studies because unlike rats, guinea-pigs, pigs, sheep, dogs, and humans, mice can enter a dormant state, called torpor, when subjected to traumatic stress (157, 158). Torpor itself can profoundly change the animal's immune system by reducing the numbers of circulating leukocytes, lowering complement levels, and changing the animal's response to infection (159).

The other important variable in preclinical and clinical studies is sex. An increasing number of animal and human studies show sex-specific differences in pathophysiological responses to polytrauma, hemorrhagic shock, TBI and burns (160–162). Chaudry and colleagues have been emphasizing the importance of sex in biochemical research for over two decades. They showed that administration of female sex hormone 17β-estradiol in males and ovariectomized females after trauma-hemorrhage prevented the suppression of immune response (163, 164). On the basis of accumulated data, greater inclusion of females in preclinical modeling and translation has been earmarked by the National Institutes of Health (NIH), European Commission, US Department of Defense and FDA (151–153).

Lastly, the practice of single-nodal targeting is another factor for why there are no effective systems-acting drugs for the polytrauma patient. Past drug development efforts have focused more on alleviating symptoms rather than addressing an underlying problem. The current practice of treat-as-you-go using sequential, single-target therapies leads to what US surgeon William C. Shoemaker considered: "an uncoordinated and sometimes contradictory therapeutic outcome" (165). Targeting individual pro-inflammatory cytokines, or any single step along a signaling pathway, ignores the critical importance of the system. Single-nodal thinking rarely solves a medical problem unless the site is believed to be a central hub or upstream intersection point. The IL-1 receptor has been proposed to be such a target, and while *anakinra* (IL-1 antagonist) has an excellent safety record, further trials are required to demonstrate its clinical efficacy after trauma or infection (166, 167). Reductionism in scientific discovery is important in breaking a system into its constituent parts, however, *it does not do away with the system* (151–153). This flawed way of thinking, we believe, is a major contributor for the high failure rate of translating promising new drugs in clinical trials (168). Choosing the right model and experimental design, a systems approach is much more likely to increase animal-to-human translational success to improve trauma care.

Adenosine, lidocaine and magnesium (ALM): Toward a systems-based drug therapy

> If you control hemorrhage and infection, the patient will do the recovery, since every cell in his body is working hard in that direction already. But you must understand what those cells are doing so that you can help them.
>
> Walter B. Cannon [Moore, (169) p. 816].

We have been developing a small-volume intravenous (IV) ALM fluid therapy to treat polytrauma for civilian and military use (12, 16). In different animal models, ALM confers a survival advantage after hemorrhagic shock (12, 16, 170, 171), traumatic injury (170–174), sepsis (175, 176) and endotoxin insult (177). The ALM survival phenotype is not replicated with individual actives adenosine, lidocaine or magnesium (12, 16). ALM confers its benefit by shifting CNS function from sympathetic to parasympathetic dominance (178), blunting inflammation (172), correcting coagulopathy (179), maintaining VA coupling, improving tissue blood flow, lowering energy demand and protecting mitochondria (178). Studies carried out by US Army Institute of Surgical Research also showed that ALM therapy restored 97% of endothelial glycocalyx after severe hemorrhagic shock (180). Currently, we don't know how and when the "switch" from an injury phenotype to a survival phenotype occurs, however, we suspect it is early because the same 5 h therapy confers dual protection against trauma and infection (12, 14, 16). It is possible ALM may act in the first minutes to hours after administration to assist the body to develop a "normal" immune response with timely resolution of the immuno-inflammatory genomic storms. While the preclinical ALM data appear promising, translation to humans remains challenging given the failure rate of translating new drugs into humans exceeds 95% (181), and of those that do obtain FDA approval, around 30% show postmarket safety concerns (182). Understanding the underlying mechanisms of action of ALM is vital for safe translation.

Conclusion

Trauma is a leading cause of death and disability worldwide. Currently there are no effective drug therapies to reduce hyperinflammation and immune dysfunction, immunosuppression, infection and MODS following major trauma. The present treat-as-you-go approaches fail to appreciate that immuno-inflammatory complications are a systems failure, and not a single nodal failure. New therapies are required to target the CNS control of cardiovascular function, endothelial-glycocalyx shedding, tissue O_2 supply and its mitochondrial circuitry in both homeostatic and pathophysiological processes to prevent those complications.

Author contributions

GD: concept. GD, JM, and HL: data collection, data analyses, interpretation, and manuscript preparation and editing. All authors contributed equally to the design, implementation, literature analysis and writing of the manuscript.

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Conflict of interest

GD is the sole inventor of the ALM concept for cardiac surgery, trauma and sepsis.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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