Role of Damage-Associated Molecular Patterns and Uncontrolled Inflammation in Pediatric Sepsis-Induced Multiple Organ Dysfunction Syndrome

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Abstract

The incidence of multiple organ dysfunction syndrome (MODS) in sepsis varies from 17 to 73% and furthermore, increases the risk of death by 60% when controlled for the number of dysfunctional organs. Several MODS phenotypes exist, each unique in presentation and pathophysiology. Common to the phenotypes is the stimulation of the immune response by pathogen-associated molecular patterns (PAMPs), or danger-associated molecular patterns (DAMPs) causing an unremitting inflammation. Two of the MODS phenotypes are discussed in detail, thrombocytopenia-associated multiple organ failure (TAMOF) and the hyperinflammatory phenotype–macrophage activating syndrome (MAS) and hemophagocytic lymphohistiocytosis (HLH). In the end, we will briefly review the role of mitochondrial dysfunction as a significant contributor to the pathogenesis of MODS.

Keywords

- pediatric severe sepsis
- multiple organ dysfunction syndrome
- pathogen-associated molecular patterns
- danger-associated molecular patterns
- macrophage activation syndrome
- hemophagocytic lymphohistiocytosis

Introduction

The term multiple organ failure (MOF) or multiple organ dysfunction syndrome (MODS) was first defined by the 1991 Consensus Conference of the American College of Chest Physicians and the Society of Critical Care Medicine, as "the presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention."[1] Two decades later, these terms are still used interchangeably but the definition has been expanded to include persistent, progressive, or secondary MODS. The incidence of MODS in sepsis varies from 17 to 73% with mortality ranging from 19 to 57%.[2–4] Recent data have suggested that the incidence of MODS is twofold greater among patients with comorbid conditions,

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and furthermore, increases the risk of death by 60% when controlled for the number of dysfunctional organs.[5]

Sepsis is a systemically dysregulated host immune response, along with activation of the complement system, coagulation cascades, and the neuroendocrine system.[6] The prevailing consensus has been that unremitting inflammation serves as the basis of sepsis-induced MODS in children. We have previously documented the presence of increased thrombosis, inflammation, and unresolving infection in autopsy specimens of septic children where MODS was listed as a cause of death.[7] Subsequently, we have also demonstrated that the phenotype of MODS in septic children can be categorized as one of the following: (1) thrombocytopenia-associated MOF (TAMOF) which encompasses a spectrum of mixed thrombotic microangiopathies and coagulopathies including thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), and disseminated intravascular coagulation (DIC).[8–10] (2) pathologic immune activation (macrophage activating syndrome and hemophagocytic lymphohistiocytosis [HLH]) as a hyperinflammation phenotype; (3) immune paralysis defined as lymphoid depletion and prolonged monocyte deactivation (monocyte HLA-DR expression $< 30\%$ or ex vivo TNF response to lipopolysaccharide [LPS] challenge < 200 pg/mL at > 5 days). This phenotype has been associated with secondary bacterial, fungal, or herpes virus family infection.[11] Hotchkiss et al have demonstrated apoptosis-induced loss of cells of the innate and adaptive immune system including CD4 and CD8 T, B, and dendritic cells[12,13]–leading to a severe debilitation of the host's ability to combat inflammation;[14] (4) sequential or liver dysfunction–associated MOF initially presents as acute respiratory distress syndrome followed sequentially by sFasL-Fas mediated liver failure and is associated with viral sepsis and lymphoproliferative disease.[15] An exuberant proinflammatory response to the infectious insult is a trigger for subsequent activation of downstream pathways; however, the lack of a precise pathophysiological mechanism has led to the paradigm that metabolic shutdown of the mitochondria may also play a vital role in the pathophysiology of MODS. In this review, first, we highlight the role of danger-associated molecular patterns (DAMPs) in the pathogenesis of MODS and discuss the first two phenotypes of MODS in details. Lastly, we review the role of mitochondria and immunosuppression as it relates to the phenotypes of MOF as mentioned earlier.

DAMPs in Sepsis Associated with MODS

The inflammatory cascade may be activated by a diverse group of molecular motifs found on pathogens, known as pathogen-associated molecular patterns (PAMPs). These molecular motifs are in turn recognized by a surprisingly limited number of highly conserved pattern recognition receptors (PRRs) which include the toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) receptors.[16–18] Perhaps the most well studied PAMP is lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria. LPS, specifically its lipid A component, is a key initiator of endothelial barrier dysfunction during sepsis.

The proinflammatory response in sepsis has been well characterized[19,20] and it is this uncontrolled inflammatory response that is responsible for the clinical manifestations in a critically-ill child. However, numerous therapeutic trials in humans targeting the early cytokine response of sepsis have failed to replicate the success demonstrated by this approach in preclinical animal models of sepsis.[21,22] The failure of this approach spurred the hunt for potential late-acting mediators of sepsis that might serve as novel therapeutic targets for treatment of critically ill patients with sepsis. One such late-acting mediator of sepsis is high mobility group box 1 (HMGB1). HMGB1 contains two internal repeats of positively charged domains ("HMG boxes" known as "A box" and "B box") in the N-terminus, and a continuous stretch of negatively charged (aspartic and glutamic acid) residues in the C-terminus. These HMG boxes bind to chromosomal DNA and help in the determination of nucleosomal structure, stability, and regulation of gene expression.[23]

Wang et al first demonstrated the cytokine-like properties of HMGB1 and established HMGB1 as a prototype for endogenous danger signals or the so-called "Alarmins."[24–26] Alarmins were defined as endogenous intracellular molecules that (1) are released into the extracellular milieu during cell death or secreted by viable immune cells, (2) recruit and/or activate other immune cells, and (3) maintain homeostasis.[25,27] Active secretion of HMGB1 from monocytes/macrophages begins 8 to 12 hours after exposure to the inflammatory stimulus and represents a delayed onset of release as compared with the early proinflammatory mediators. In a murine model of sepsis, circulating HMGB1 levels increase 18 hours after induction of peritonitis and remain elevated for 3 days.[28] The delayed kinetics of HMGB1 release parallels the onset of animal lethality in animal models of sepsis. Furthermore, treatment with neutralizing anti-HMGB1 antibodies can rescue mice from LPS or sepsis-induced lethality,[29] thereby, solidifying its role as a potential therapeutic target. Collectively, these in vivo and in vitro studies support a paradigm in which HMGB1 plays a pathogenic role in sepsis and is a late mediator of systemic inflammation.

Extracellular HMGB1 binds to the pattern recognition receptors, such as the toll-like receptor (TLR) 2, 4 and receptor for advanced glycation end products (RAGE),[30,31] leading to subsequent production of proinflammatory cytokines and chemokines.[32] Elevated plasma HMGB1 levels in humans with sepsis and septic shock also suggest the pathogenic role of extracellular HMGB1.[33,34] Previously, we have demonstrated elevated serum HMGB1 levels in pediatric patients with sepsis, septic shock, and multiorgan failure.[35] Elevated serum HMGB1 concentrations are present in adult septic patients with severe sepsis and multiorgan failure.[33,34,36,37] Furthermore, septic patients who progressed on to develop MOF had significantly higher plasma HMGB1 levels as compared with those that did not. We also determined the performance of plasma HMGB1 as a diagnostic test for predicting MOF in pediatric sepsis, and a plasma HMGB1 concentration of 9.54 ng/mL had a sensitivity of 55.3% and specificity of 90% ($p = 0.0012$).[38]

The immunomodulating role of extracellular mitochondrial DNA (mtDNA) was discovered recently even though the putative bacterial origins of mitochondria have been known for decades. There is abundant mtDNA present in the cell, as a single cell encloses hundreds of mitochondria, and each mitochondrion contains an estimated 2 to 10 copies of its genome.[39] Similar to bacteria, mitochondria possess a double membrane structure and contain 37 genes coding for two ribosomal ribonucleic acids (RNAs), 22 transfer RNAs, and 13 polypeptides.[40,41] Furthermore, mtDNA have hypomethylated CpG motifs that resemble bacterial CpG DNA and activate TLR9, a PRR that detects bacterial and viral DNA.[42] Thus, extracellular mtDNA can activate signaling pathways and promulgate inflammation due to its similarity to bacterial DNA. Zhang et al, in a seminal study, demonstrated that patients admitted with trauma had significant elevations of mtDNA concentrations in the plasma and injured tissues.[43–45] The authors validated their results in a rat model of trauma/hemorrhagic shock and noted that plasma mtDNA levels were elevated for 7 days after injury.[43] The mtDNA is a novel danger-associated molecular pattern that on its release into the extracellular milieu acts via toll-like receptor-9, a pattern recognition receptor of the immune system. The median concentrations of plasma mtDNA were significantly greater in patients with MOF as compared with patients without MOF. In contrast, the presence or absence of MOF did not correlate with plasma concentration of β globin, a gene present in all nucleated cells of the body.[46] Thus, our findings suggest that there is a mechanistic link between inflammation and MOF and further inquiry into the release of DAMPs and its function in the extracellular milieu may lead to new strategies for preventing septic shock and MOF.[46,47]

Sepsis-Induced Thrombocytopenia-Associated Multiple Organ Failure

Thrombocytopenia-associated multiple organ failure (TAMOF) is a clinical phenotype characterized by new onset thrombocytopenia in a setting of evolving multiple organ failure. TAMOF comprises of a spectrum of syndromes associated with disseminated microvascular thromboses, such as DIC and the thrombotic microangiopathies–thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS).[10] The decrease in platelet counts reflects their involvement in forming microvascular thrombosis and the consequent organ ischemia and dysfunction. Patients succumbed with TAMOF have disseminated microvascular thromboses on autopsies with fibrin-rich microthrombi seen in DIC and HUS, and platelet/von Willebrand's factor (VWF)-rich microthrombi seen in TTP.[48,49] The common pathway for the development of TAMOF is uncontrolled systemic inflammation that leads to persistent activation of the thrombotic and coagulation pathways. Tissue factors, VWF/platelet, and complement pathways are dysregulated in DIC, TTP, and HUS respectively. The severity of the coagulopathy depends on the genetic and environment of the host and the trigger (invading pathogens). For examples, patients with genetic mutations of plasminogen activator inhibitor type 1, ADAMTS-13, or complement factors are at higher risk of developing TAMOF during sepsis.

More than 50% of critically ill children in the intensive care unit with signs of TAMOF are not diagnosed with the classically described syndromes, that is, TTP, HUS, and DIC. We have previously described features of TTP pathophysiology in a cohort of critically ill children with TAMOF (defined as platelet count less than 100,000/mm^3 and at least two organs dysfunction) who were not diagnosed with TTP.[9] TTP is diagnosed in patients with ADAMTS-13 activity levels of $< 10\%$ which is caused by either a genetic mutation to *ADAMTS13* gene or the presence of inhibitory autoantibodies immunoglobulin G.[50,51] ADAMTS-13 is a protease that cleaves ultralarge von Willebrand's factor (ULVWF) which are released by activated endothelium and can spontaneously aggregate platelets into smaller and less thrombogenic forms. In our cohort of pediatric TAMOF, these patients had reduced ADAMTS-13 activity levels with a mean of 33.5% (normal ADAMTS-13 activity level is $> 57\%$) and the presence of pathologic prothrombotic ULVWF multimers. In addition, autopsies on these TAMOF patients had VWF-rich microthrombi in the kidney, brain, and lung.[9] We propose that TAMOF patients, who are not diagnosed with overt TTP, HUS, or DIC, have acquired ADAMTS-13 deficiencies with dysregulation in VWF/platelet pathway. Inflammatory mediators that are elevated during sepsis, such as interleukin (IL)-6, granulocyte elastase, plasmin, thrombin, plasma free hemoglobin, and VWF proteolytic fragments can all inhibit ADAMTS-13, and tumor necrosis factor–α, IL-8 and 6 can all induce ULVWF release from the endothelium.[52–57]

Recently, Wong et al reported in a large multicenter study of a pediatric sepsis biomarker risk model that TAMOF is a distinct clinical phenotype of sepsis.[58] Comparing 209 children with TAMOF to 290 nonthombocytopenic MOF, TAMOF patients, even with a significantly lower proportion having comorbidities had significantly higher mortality, severity of illness scores, and rate of complicated course. Similarly, Claushuis et al reported in a recent large adult sepsis study that thrombocytopenia is significantly associated with mortality and a dysregulated host response including increased cytokine levels and endothelial activation, impaired vascular integrity, and increased gene expression of complement signaling.[59]

In summary, critically ill septic patients with new onset thrombocytopenia who are progressing into MOF are in a distinct clinical phenotype. These patients have activated thrombosis, coagulation, and/or complement pathways that may lead to disseminated microvascular thromboses. This easily clinically identifiable clinical phenotype, TAMOF, should trigger the clinician to evaluate the fibrin pathway with a DIC panel, the VWF/platelet pathway with ADAMTS-13 and VWF, and complement pathway. Identify and remove nidus of infection is the key.

Pathologic Immune Activation (Macrophage Activating Syndrome and Hemophagocytic Lymphohistiocytosis)–Hyperinflammation Phenotype

Pathologic immune activation is an immune dysregulation state that is characterized by hyperinflammation. The underlying problem can be caused by a defect in shutting down the

initial systemic inflammatory response syndrome during sepsis, such as a defect in the Fas/Fas ligand (FasL) apoptotic pathway or perforin/granzyme B-mediated cytolysis. Fas is a transmembrane protein within the tumor necrosis factor (TNF) receptor superfamily and it is widely expressed in many cell types. FasL is a membrane protein with homology to TNF-α and its expression is limited to activated lymphocytes and natural killer cells. Both Fas and FasL expression are regulated by a constitutive and an inflammation-inducible pathway. The interaction of FasL to Fas on a target cell will trigger apoptosis of that cell. This Fas/FasL system plays a pivotal role in the regulation of apoptosis in activated immune cells during systemic inflammation. Fas can be cleaved from the cell surface into a soluble form (sFas). The sFas can function as an FasL inhibitor by binding to FasL and preventing it to interact with uncleaved Fas. Indeed, Doughty et al reported in children with sepsis-induced MODS, sFas levels were significantly higher in nonsurvivors and in those with persistent ($>$ 3 days) or sequential MODS (defined as respiratory failure followed by hepatorenal failure).[15] On the other hand, increased soluble FasL was found to be associated with viral infection, lymphoproliferative disease, liver failure, nonsurvivors, and sequential MODS. Upregulating sFas/FasL pathway have been reported in septic acute respiratory distress syndrome,[60] macrophage activating syndrome (MAS),[61] TTP and DIC,[62] and HLH.[63]

HLH is a syndrome characterized by pathologic immune activation with resultant hyperinflammation. HLH is caused by inherited or acquired defects in cytotoxic T-lymphocyte function that leads to the host's inability to shut down the initial systemic inflammatory response syndrome necessary to control invading pathogens or foreign antigens. HLH mortality is over 50% with 94% of deaths occur in the first 8 weeks and typically associated with uncontrolled infections and sepsis-induced MOF.[64] The diagnostic criteria for HLH share similar features to that of sepsis including fever, splenomegaly, cytopenias (thrombocytopenia, neutropenia, anemia), hyperfibrinogenemia, and/or hypertriglyceridemia, hemophagocytosis (in bone marrow or spleen or lymph nodes or liver), low or absent natural killer cell activity, ferritin $>$ 500 ng/mL, and elevated soluble IL-2 receptor α.[65] Thus, it is essential to recognize HLH and devise appropriate therapeutic strategies as HLH survival is dependent on immune modulation and resolution of activating antigen.[66] Initial descriptions of HLH genes were associated with perforin and regulation of granule-dependent lymphocyte activity. Commonly, when the mutations lead to a nonfunctional protein, HLH will present earlier in life with higher severity compared with hypomorphic mutations which lead to partially functioning protein.[67] MAS, similarly, is characterized by hyperinflammation but usually diagnosed in association with autoimmune diseases. MAS shares similar clinical, laboratory, and pathologic features as that of HLH including genetic mutations.[68,69] The muddiness of HLH and MAS distinction is ongoing. However, what might be more useful for the intensivists are to (1) recognize hyperinflammation phenotype in sepsis-induced MOF; (2) identify the trigger of pathologic immune activation; and (3) employ immune modulation strategy to "calm" the pathologic immune activation. Depending on each individual hospital, HLH/MAS specific laboratory tests might not be readily available such as soluble IL-2 receptor α (sCD25), natural killer cell activity, and soluble hemoglobin-haptoglobin scavenger receptor (sCD163). Elevated ferritin level, a widely available hospital clinical test, has become the common biomarker to trigger the investigation of pathologic immune activation and to follow its responsiveness to therapeutic interventions.[70–72] Whether ferritin is an acute phase reactant or plays a role in the pathology of HLH/MAS is still to be determined.

Management strategy for MAS/HLH revolves around immune modulation. For primary HLH, a hematology consult is recommended with application of the HLH-2004 treatment protocol including decadron and etoposide.[66] For secondary HLH/MAS, there is a need for a graded and personalized approach to the diagnosis and therapeutic strategies with consideration to the age, severity, and comorbidities. Innovative approaches are being evaluated including antiinterferon gamma monoclonal antibodies, IL-1 receptor antagonist, methylprednisone, intravenous immunoglobulin (IVIG), and therapeutic plasma exchange (TPE).[64,66,73,74] For example, Demirkol et al reported that sepsis-induced MOF with secondary "hyper-inflammation" phenotype can be managed with IVIG, TPE, and methylprednisone.[74] In another study, Shakoory et al highlighted the importance of identifying sepsis-induced MOF "hyper-inflammation" phenotype for future sepsis therapeutic trials. These investigators reanalyzed the negative 1997 phase III randomized double-blind placebo-controlled multicenter severe sepsis trial of IL-1 receptor antagonist, which is now an effective therapy for MAS. They reported that IL-1 receptor antagonist was associated with significant improvement in survival in septic patients with MAS clinical features such as concurrent hepatobiliary dysfunction/DIC compared with septic patients without MAS features.[73]

Non-Alarming Effects of Mitochondria in Severe Sepsis

Mitochondria are vital organelles that are responsible for providing the majority of energy necessary for normal cellular functioning. In addition to producing ATP, mitochondria are also responsible for heat generation, intracellular calcium regulation, thermoregulation, and production of reactive oxygen species (ROS).[75] Using an elaborate system of transferring electrons from the Krebs cycle to the electron transport chain via reduced nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) an electrical potential is generated that provides the energy to phosphorylate ADP to ATP oxygen species (ROS).[75] Cardiac autopsy specimens in patients who have died from sepsis demonstrate hydropic mitochondria, whereas mitochondrial membrane injury and autophagolysosomes were intact. Similarly, electron microscopy of renal specimens revealed increased tubular injury, including hydropic mitochondria and increased autophagosomes.[76] Thus, it is possible that the observed mitochondrial structural damage along with dysfunction leads to cell death

and organ failure in sepsis. Both apoptotic and necrotic cell death may be a function of mitochondrial loss and damage which therefore may contribute to organ failure. Further injury may occur secondary to activation of the innate immune system pathways as mitochondrial DNA or peptides as well as mitochondrial stimulated ROSs leak into the bloodstream. The proinflammatory response in sepsis leads to the generation of ROS, reactive nitrogen species (RNS), and nitric oxide (NO) that directly inhibits mitochondrial respiration and causes damage to mitochondrial membrane.[77–79] It is well established that NO in nanomolar concentrations can inhibit mitochondrial electron transport by decreasing the activity of cytochrome *c*-oxidase[80] and higher concentrations of NO lead to the generation of peroxynitrite, a potent cell cytotoxic agent.[81] There is minimal data regarding mitochondrial function in human sepsis, and much of the information comes from laboratory studies performed in septic animal models. Skeletal muscle biopsies, in critically-ill septic patients who died, demonstrate a significant reduction in ATP concentrations in patients who died as compared with survivors or controls. Furthermore, complex I activity had a significant inverse correlation with the pressor requirement in these patients.[82]

Boulos et al incubated human umbilical endothelial cells with serum from septic shock patients and observed a significant depression of mitochondrial respiration. Pretreatment with 3-aminobenzamide, a poly-ADP-ribose polymerase-1 (PARP-1) inhibitor attenuated this suppression lending credence to the theory that NO and PARP-1 play an important role in the inhibition of mitochondrial respiration during septic shock.[83] Studies that have examined oxidative phosphorylation capability and oxygen consumption of mitochondria in septic rats have demonstrated disparate results. Some studies have demonstrated no difference in hemodynamic or oxygen delivery variables between endotoxic and septic groups,[84] in contrast, other studies have revealed a significant decrease in respiratory control index and in state three and four respiration in mitochondria obtained from septic or endotoxic models.[85–87] Thus, there is a progressive decrease in the energy supply and reduction in metabolism without impairment in oxygen delivery resulting in "cytopathic hypoxia"[88,89]. The net result of this dysfunction is that it forces the cell to switch into an anaerobic mode.

Mitochondrial derangements in peripheral blood mononuclear cells (PBMCs) have been associated with severity of sepsis, immune suppression, and mortality in adult patients but there is limited information available in children.[90,91] In a small cohort of pediatric septic patients, Weiss et al demonstrated a reduction in mitochondrial bioenergetics reserve as indicated by spare respiratory capacity and an increase in mitochondrial uncoupling.[92] Furthermore, the authors also demonstrated that septic patients that recovered from organ dysfunction exhibited a higher PBMC mitochondrial membrane potential relative to mitochondrial mass as compared with septic patients with prolonged organ dysfunction.

In summary, the available evidence suggests that mitochondrial dysfunction is a significant contributor in the pathogenesis of MODS. Singer propose that as cell death is not a major feature of sepsis, the cells enter a hibernation-like state leading to MODS due to energy failure.[93] The decrease in metabolic activity will reduce the energy requirements and generate a new steady-state, whereby the cell does not function normally but increases the likelihood of survival.

Conclusion

Despite improvements in the outcomes for pediatric septic shock, recent data still points to a high incidence of MODS. Although much has been learned about the pathogenesis of MODS, the mechanistic underpinnings of MODS in pediatric sepsis have been elusive. Strategies to reverse microvascular thrombosis in TAMOF and uncontrolled inflammation in HLH have opened new avenues to develop strategies to treat MODS. Even though there is enough evidence to suggest that mitochondrial dysfunction is associated with MODS, designing a specific therapy is challenging and remains an area open for further research endeavors.

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