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Rationale for adjunctive therapies targeting inflammation pathobiology based phenotypes in pediatric sepsis; from Meningococcemia/atypical Hemolytic Uremic Syndrome, to H1N1 Influenza/Methicillin Resistant Staphylococcus Aureus, to Critical Pertussis, to Epstein Barr Virus Lymphoproliferative Disease, to Ebola and other Viral Hemorrhagic Fevers

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Abstract

Adjunctive therapies have been proposed for use in at least 5 inflammation pathobiology phenotypes in pediatric sepsis-induced multiple organ failure (MOF). Here, we provide host-pathogen interaction prototypes to facilitate understanding of the rationale for personalized therapy in these phenotypes. Meningococcemic sepsis and Shiga-like toxin-associated atypical Hemolytic Uremic Syndrome sepsis result in thrombocytopenia and MOF due to endothelial dysfunction and formation of small vessel thromboses that can respond to plasma exchange and C5a monoclonal antibody therapy. H1N1 Influenza A sepsis is associated with immune paralysis that can result in opportunistic secondary infection with invasive methicillin resistant *Staphylococcus aureus* (MRSA) and MOF that can respond to Granulocyte Macrophage Colony Stimulating Factor therapy. Hyperleukocytosis-associated MOF is associated with critical *Bordetella Pertussis* pulmonary hypertension and cardiovascular collapse which can respond to leukoreduction therapy. Epstein Barr Virus lymphoproliferative disease-associated sequential MOF has high levels of soluble-Fas Ligand that cause liver failure, and can respond to anti-CD20 monoclonal antibody therapy. Viral hemorrhagic fevers such as Ebola or Dengue can lead to macrophage activation syndrome characterized by hyperferritinemia, hepatobiliary dysfunction and disseminated intravascular coagulation (DIC)-related MOF that can theoretically respond to anti-inflammatory therapies. We discuss the literature on adjunctive anti-inflammatory and immune modulation therapies that, in addition to traditional organ support and infection source

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control, might be part of a personalized precision medicine approach to reverse of each of these inflammatory pathobiology phenotypes.

Keywords

Thrombocytopenia associated MOF; Immune paralysis; Hyperleukocytosis; Sequential MOF; Macrophage Activation Syndrome

Introduction

Adjunctive therapies are considered by clinicians for use in the management of children with sepsis inflammation pathobiology phenotypes and multiple organ failure (MOF). Here, we examine host-pathogen interaction models (or prototypes) that provide the rationale for proven, experimental, or proposed inflammation pathobiology phenotype-targeted therapies in pediatric sepsis-induced MOF. A few general themes for the management of pediatric MOF are always pertinent, including the search for and removal of sources of ongoing infection and inflammation, and support of cardiovascular and other organ functions. In addition to this general approach, one can also use clinical criteria and confirmatory tests to identify one or more of five inflammation phenotypes that can be targeted with pathobiology-based adjunctive therapies (Table 1).

Thrombocytopenia associated MOF (TAMOF) centers on endothelial dysfunction, impairment of metalloproteinase activity of ADAMTS13, and consumptive coagulopathy that results in microvascular impairment and organ injury. We review two host-pathogen interaction models for TAMOF: 1) *Neisseria meningitides* induced *purpura fulminans*, associated with complement dysfunction, endothelial injury, production of von Willebrand factor (vWF) ultra large multimers and intravascular coagulation, (Figure 1a); and 2) Atypical hemolytic uremic syndrome (aHUS) as a result of genetic polymorphisms in ADAMTS13 activity or inhibitory complement regulation (Figure 1b). In addition to microbiological source control, both types are amenable to therapeutic plasma exchange, which removes thrombogenic ultra large vWF multimers and restores ADAMTS13 activity. aHUS is also amenable to biologic terminal complement inhibitors such as Eculizumab.

There are also host genetic risk factors for the development of TAMOF including ADAMTS13 deficiency syndrome (Upshaw-Schulman Syndrome, also known as congenital thrombotic thrombocytopenic purpura), and deficiency in complement H[1,2]. Environmental risk factors include elaboration of inflammatory cytokines resulting in direct endothelial activation or injury, liver failure, and inhibitory ADAMTS13 antibodies as seen in acquired thrombotic microangiopathy (TMA)[3–5]. Free hemoglobin resulting from red blood cell hemolysis in TAMOF is a driver for further endothelial and other organ injury. Other sources of pathologic free hemoglobin include any aged blood cells prone to hemolysis upon transfusion, cardiopulmonary bypass, extracorporeal membrane oxygenation (ECMO), or continuous renal replacement therapy (CRRT)[6–8].

There are a number of TAMOF-directed adjunctive therapeutic options. In a small, single center prospective randomized clinical study, plasma exchange therapy (1.5 volumes on day

1 followed by 1 volume on days number 2 through end, with end being determined by return of organ function and platelet count) was associated with removal of ultra large vWF multimers, restoration of ADAMTS13 functional activity, and improvement in end-organ functional markers[9,10]. Meta-analyses indicate a mortality benefit with use of plasma exchange therapy in adults[11–13]. Although the data for use of plasma exchange therapy in critically adult and pediatric ICU populations for the indication of sepsis and septic shock is mixed, there is potential benefit in the TAMOF syndrome, based upon biologic plausibility and a track record of efficacy of its use in microangiopathies[14,15]. The decision to provide plasma exchange should be made based on the clinical condition, including degree of coagulopathy and platelet count. In addition, plasma exchange should be considered in the setting of severe neurologic disease. Evidence for activation of the complement system should also prompt a consideration for plasma exchange therapy. Eculizumab, a C5 terminal complement cleavage inhibitory monoclonal antibody, can be considered in the setting of TAMOF. This therapy has been most extensively studied in the setting of aHUS associated with an ineffective inhibitory complement response, where eculizumab has been shown to improve renal function, need for renal support, and improve quality of life among adult patients[16]. Eculizumab has been approved for use in pediatric aHUS[17]. Moreover, earlier administration of the antibody in an aHUS disease course is associated with improved renal recovery[18,19].

Immunoparalysis associated MOF centers on impairment of both innate and adaptive immune function with resulting inability for the host to contain a primary or secondary infection. The host pathogen example we use for this phenotype of MOF is H1N1 Influenza A infection with impairment of monocyte function and contraction of adaptive immune populations (Figure 2). In addition to antiviral and antibacterial therapy to control pathogen burden and removal (when appropriate) of pharmacologic sources of immune suppression, providing granulocyte macrophage colony stimulating factor (GM CSF) has been successfully used to reverse innate immune paralysis. Programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) blockade as well as provision of recombinant lymphocyte survival factors such as IL-7 are being evaluated as methods to restore adaptive immune function.

Immunoparalysis associated MOF is associated with decreased *ex vivo* TNF-alpha response < 200 pg/mL and decreased expression of the MHC-II molecule HLA-DR <8000 molecules or <30% of control level beyond 3 days of illness[20–22]. Among patients with severe sepsis, lymphopenia is an independent predictor of mortality[23]. A phenotype of prolonged lymphocyte depletion was described among spleen and other lymphoid tissue samples from pediatric and adult patients who died of sepsis associated MOF[20,24]. Poor outcomes associated with the immunoparalysis phenotype include increased association of ventilator associated pneumonia, reactivation of latent herpesvirus family infections, and secondary opportunistic infections, upon which multi-organ failure may be superimposed[25,26].

An examination of the clinical features associated with mortality during the 2009–2010 H1N1 influenza pandemic highlighted the relationship between viral immune suppression and the development of opportunistic methicillin resistant staphylococcus aureus infection (MRSA). In a retrospective observational cohort of 838 patients <21 years admitted to

pediatric ICUs across the United States, overall mortality was 8.9%[27]. Leukopenia and neutropenia were associated with mortality (RR 1.8, 95% CI 1.2–2.9; RR 2.8, 95% CI 1.5–5.5, respectively). Interestingly, bacterial pneumonia (co-infection) with MRSA, but not methicillin sensitive staphylococcus aureus (MSSA), was associated with death (RR 3.2, 95% CI 1.8–5.9). In a multivariate model, among the 251 previously healthy children enrolled in the study, only presumed MRSA infection was significantly associated with mortality (RR 8, 95% CI 3.9–20.6). Whole blood TNF alpha hyporeponsiveness to endotoxin was highly and independently associated with both mortality and length of ICU stay.

The potential treatments for immune paralysis depend on the background immune status of the host. For chronically immune suppressed patients such as transplant recipients, withholding or reducing immune suppression is indicated in the setting of severe sepsis associated MODS.[28] Among patients with immunoparalysis associated MOF who had a pre-intervention RR of death of 11 [95% CI 1.4–89], Hall et al showed that treatment with low-dose GM-CSF at 125 micrograms/kg per day for seven days given over a minimum of 12 hours as an infusion prevented secondary infection, death, and restored *ex vivo* TNF response to LPS[29]. Similarly, in randomized double-blinded study of adults undergoing general surgical procedures with immunosuppression defined by HLA-DR expression <10,000 molecules on the surface of monocytes, administration of a single dose of GM-CSF, but not influenza vaccine, was associated with restored HLA-DR expression, improved WBC count and less severe delirium compared to placebo[30]. Therefore, the immune paralysis phenotype of MODS can be considered a reversible condition with appropriate modulation of the host immune response.[25]

Recent mouse model data has shown that PD-1 and PD-L1 blockade results in improved survival in a model of fungal sepsis[31] and ex-vivo data has implicated PD-1 blockade as a potential therapy in patients with severe sepsis and the immune paralysis phenotype.[32] Clinical trials are currently enrolling adult patients with severe sepsis to test the role of PD-1-PD-L1 axis blockade using BMS-936559 (anti-PD-L1) in sepsis survival and organ function (https://clinicaltrials.gov/ct2/show/NCT02576457).

Critical pertussis associated MOF (Figure 3). results in impairment of the innate immune response, and a poor adaptive immune response. In addition, both epithelial targeting and direct effects of pertussis toxin on leukocyte populations causes robust margination of neutrophils and lymphocytes from the tissues to the circulation and the infected epithelial surfaces. The result is a unique pathophysiology of hyperleukocytosis in very young infants. The high viscosity and endothelial dysregulation with cellular plugging of pulmonary arterioles and venules results in pulmonary hypertension and cardiopulmonary collapse. The critical pertussis syndrome is associated with bacterial superinfection, and broad spectrum antimicrobial source control should be initiated in patients with this MOF phenotype. In addition, leukoreduction therapy by therapeutic apheresis, organ support (potentially with the use of ECMO), and a trial of inhaled nitric oxide therapy are reasonable but unproven approaches to treatment.

The risk factors associated with mortality among <120 day old infants include lower birth weight, younger gestational age, younger age at time of onset, higher peak WBC and lymphocyte count >30,000 cells per microliter. In multivariate analysis, WBC count and birth weight were the only pre-ICU admission factors that were associated with death[33]. Among patients admitted to the ICU, 43% go on to require endotracheal intubation and mechanical ventilation[34]. A median WBC count > 27,000 mm^3 was significantly associated with need for mechanical ventilation, the presence of pulmonary arterial hypertension and death. Despite the brisk leukocytosis observed among infants, there is evidence that pertussis toxin has direct immune inhibitory effects on macrophage and neutrophil activation putting the infants at risk for co-infection (Figure 3).

Therapies for critical pertussis include treatment of primary infection, prevention of secondary infection, ventilatory support, and leukoreduction to minimize the pathologic effects of hyperleukocytosis. Unfortunately, limited data exists regarding whether the timing of *Bordetella pertussis*-directed antimicrobial therapy impacts the important clinical outcomes of hospitalization days, need for mechanical ventilation, or death[35]. In addition to macrolide therapy, broad spectrum antimicrobial therapy is employed for critically ill pertussis patients with severe sepsis or septic shock with special consideration for the possibility of bacterial respiratory superinfection. Rowlands and colleagues proposed an algorithm for use of leukoreduction therapy in critical pertussis based on white blood cell counts > 50,000 mm^3 and presence of cardiopulmonary compromise[36,37]. Additional therapies specific to pertussis pathophysiology as a toxin-driven disease include anti-Pertussis toxin-enriched intravenous immune globulin (P-IVIG). Although standard IVIG therapy has been shown to be ineffective in changing the severity of pertussis disease or in decreasing symptoms, preliminary trials have indicated that targeting the toxin has symptomatic benefit as well as a reduction in the degree of lymphocytosis without significant adverse events[38,39]. Further study is needed to determine the efficacy of P-IVIG or a combination of leukoreduction and anti-toxin therapy in the critical pertussis cohort, specifically.

Sequential MOF **(SMOF)** is a sequential respiratory and then hepato-renal failure syndrome that is driven by perturbation in the immune system's ability to perform activation-induced cell death (AICD) that leads to lymphoproliferation, and to perform DNA viral infection killing that leads to uncontrolled viremia (Figure 4). This phenotype is most commonly found among patients with organ transplantation who are maintained on T cell immunosuppressant therapy and have Epstein Barr Virus (EBV) or other DNA viral infection. In this condition, EBV infection of B cells provides a pool for viral proliferation, resulting in post-transplant lymphoproliferative disorder (PTLD), and impairment of the normal Fas-Fas ligand (Fas-FasL) mediated apoptotic pathways. Soluble FasL interferes with the T and NK cells ability to cull infected B cells, and also leads to direct hepatocyte injury through FasL-Fas interaction. Expanded innate populations further damage organs by inflammatory cytokine and direct effects. The treatment of sequential MODS includes withdrawal of immune suppression as able and B cell reduction therapy with rituximab (anti-CD20 antibody) in addition to antiviral therapy.

The mortality has been approximately 50% once the diagnosis is made. The clinical SMOF phenotype was correlated with both high serum IL-10 concentrations and soluble Fas (s-Fas) and soluble Fas-Ligand (s-FasL), suggesting perturbation of Fas-FasL mediated activation induced cell death pro-apoptotic pathway (Figure 4)[40,41]. The at-risk host population for SMOF includes recipients of transplanted organs or tissues requiring immunosuppression, similar to the risk factor profile for PTLD. Conversely, SMOF has also been observed in viral disease without transplantation in certain host genetic backgrounds, such as X-linked lymphoproliferative disease (XLP-1). XLP1 patients with mutations in the SLAM-associated protein (SAP) are susceptible to EBV infection due to a signal transduction defect in T cells and NK cells, rendering ineffective cytotoxic control of EBV-infected cell proliferation[42]. This differs from X-linked inhibitor of apoptosis (XIAP) mutations in BIRC4 (XLP-2), in which patients are susceptible to EBV infection, but present with splenomegaly and recurrent episodes of hemophagocytic syndrome, and inflammatory bowel disease[43]. In addition, mutations in this apoptotic signaling pathway have been found in approximately 75% of individuals with the autoimmune lymphoproliferative syndrome (ALPS), in which immune contraction due to AICD is impaired.

Similar to other MOF phenotypes, therapy for SMOF depends on the ascertained etiology. For cases involving clinical SMOF, the magnitude of DNA viremia is important to consider. For PTLD-associated SMOF, the mainstay of therapy has been reduction of immune suppression to allow for cytotoxic T and NK cell control of the causative DNA virus. Reduction in immune suppression is balanced by risk of rejection in the setting of solid or hematopoietic organ transplantation. The use of rituximab has emerged as an additional method that reduces the B cell, and in turn, the DNA viral reservoir[44]. The therapeutic principle is that CD20 on mature B cells is engaged by the monoclonal antibody, which in turn has direct signaling effects on the B cell (apoptosis, B cell receptor down regulation) and activation of cytotoxic response (NK cell and T cells via direct engagement and antibody dependent cytotoxicity)[45]. Based on soluble Fas levels in adult patients with diffuse large B cell lymphomas, the measured Fas-FasL axis is related to rituximab treatment response[46]. Antiviral therapy remains critical to controlling DNA viremia and expansion of the infected pool. The observation that NK cell cytotoxic activity is impaired in the setting of pediatric PTLD, and is associated with increased PD-1 expression may result in new attention to monitoring NK cell numbers and functional capacity in the host response[47].

Severe sepsis associated Macrophage Activation Syndrome (MAS) is exemplified by the host pathogen interaction of filoviruses (i.e. Ebola virus) or other viral families associated with hemorrhagic fevers (Figure 5). The hallmark of severe sepsis associated MAS is high ferritin, hepatobiliary dysfunction and DIC. In Ebola hemorrhagic fever (EHF), viral infection activates innate immune cells, impairs innate cells ability to present antigen and co-stimulate T cells, and results in adaptive and NK cell immune paralysis. The infection of vascular endothelium and macrophage production of pro-coagulant inflammatory mediators leads to DIC. Pathogen associated molecular patterns and damage associated molecular patterns (such as hemoglobin – haptoglobin complexes, and putatively, ferritin) act to damage tissues and activate macrophages as well as lead to direct cellular injury. The treatments for EHF specifically may include immune serum in the form of chimeric

monoclonal antibodies (i.e. ZMapp) to control viremia. In general terms, inflammation reduction therapy, such as steroids, IVIG and plasma exchange, is useful in severe sepsis associated MAS. Anti-cytokine therapy such as anakinra has also been shown to be beneficial as treatment of hyperinflammation in severe sepsis associated MAS, and other cytokine blocking therapies are being evaluated[48]. The pathophysiology of hemorrhagic fever driven MAS is based on unchecked and unabated inflammation and hemolysis driven myeloid innate immune cell activation (Figure 5).

Viral hemorrhagic fever syndromes, such as those associated with the filoviruses (Ebola, Marburg), some flaviviruses (Dengue) and some arenaviruses (Lassa), have been reported as examples of viral disease associated MAS. Ebola has the clinical features of macrophage activation syndrome with associated cytopenias, disseminated intravascular coagulation, ongoing capillary leak syndrome, and hepatic dysfunction[49,50]. Of note, a recent examination of 55 biomarkers in Ebola hemorrhagic fever (EHF) patients indicated that elevations in serum thrombomodulin and serum ferritin were both associated with hemorrhage and mortality[51,52]. Elevation of serum ferritin has been shown to correlate with mortality among pediatric patients with MAS in multiple settings[53,54].

Treatment for severe sepsis associated MAS are varied but fall into two broad categories: 1) control of source of inflammation and 2) modulation of immune functional and inflammation pathways. With regard to EHF, treatment with multiple chimeric monoclonal antibodies directed against viral components (ZMapp) has been evaluated in nonhuman primates and in a clinical trial during the 2014 Sierra Leone outbreak, with inconclusive results. While there may have been potential clinical benefit, there was an absence of statistical significance, requiring further study[55,56]. With regard to anti-inflammatory and immune modulatory therapies for MAS, there are several potential approaches. One of the most studied anti-cytokine therapies has been IL-1 blockade with recombinant IL-1RA (anakinra), which has been associated with a two-fold decrease in mortality in adults with severe sepsis associated MAS.[48,57]. Anti-IL-6 monoclonal antibody tocilizumab has shown efficacy in the treatment of systemic onset juvenile idiopathic arthritis induced MAS, and in cytokine release syndrome after chimeric antigen receptor (CAR) T cell treatment for blood cancers[58,59]. Anti-IL-18 therapies, which may be promising for MAS, are currently under development. Unrelated to biologic therapies, a study of Turkish children with severe sepsis and features of MAS compared the hemophagocytic lymphohistiocytosis (HLH)-94 chemotherapy protocol with a regimen of plasma exchange and IVIG or methylprednisolone and found the HLH-94 protocol had an associated survival of only 50% whereas the IVIG and steroid regimens had a survival of 100%, p=0.002 when combined with plasma exchange therapy, supporting an anti-inflammatory approach for MAS patients[60,61].

Conclusions

We have described 5 inflammation pathobiology driven phenotypes of pediatric sepsis induced MOF, using specific host-pathogen interaction models to illustrate therapeutic opportunity for personalized precision medicine approaches that have the potential to improve outcomes in selected children. Further evaluation is necessary.

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Text Abbreviations

MOF	Multiple Organ Failure
MRSA	methicillin resistant *Staphylococcus aureus*
DIC	disseminated intravascular coagulation
ADAMTS13	thrombospondin type 1 motif, member 13
TAMOF	*Thrombocytopenia associated MOF*
vWF	von Willebrand factor
ADAMTS13	a thrombospondin type 1 motif, member 13
aHUS	Atypical hemolytic uremic syndrome
TTP	thrombotic thrombocytopenic purpura
TMA	thrombotic microangiopathy
ECMO	extracorporeal membrane oxygenation
CRRT	continuous renal replacement therapy
GM-CSF	granulocyte macrophage colony stimulating factor
PD-1	Programmed cell death protein 1
PD-L1	programmed cell death ligand 1
P-IVIG	anti-Pertussis toxin-enriched intravenous immune globulin
SMOF	Sequential MOF
AICD	activation induced cell death
EBV	Epstein Barr Virus
Fas-L	Fas ligand
PTLD	post-transplant lymphoproliferative disorder
s-Fas	soluble Fas
s-FasL	soluble Fas-Ligand
XLP-1	X-linked lymphoproliferative disease
SAP	SLAM-associated protein

XIAP	X-linked inhibitor of apoptosis
XLP-2	BIRC4
ALPS	autoimmune lymphoproliferative syndrome
MAS	Macrophage Activation Syndrome
EHF	Ebola hemorrhagic fever
CAR	chimeric antigen receptor
HLH	hemophagocytic lymphohistiocytosis

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Key Points

- Adjunctive therapies are considered by clinicians for use in the management of children with sepsis inflammation pathobiology phenotypes and multiple organ failure (MOF).
- A few general themes for the management of pediatric MOF are always pertinent, including the search for and removal of sources of ongoing infection and inflammation, and support of cardiovascular and other organ functions.
- One can also use clinical criteria and confirmatory tests to identify one or more of five inflammation phenotypes that can be targeted with pathobiology-based adjunctive therapies

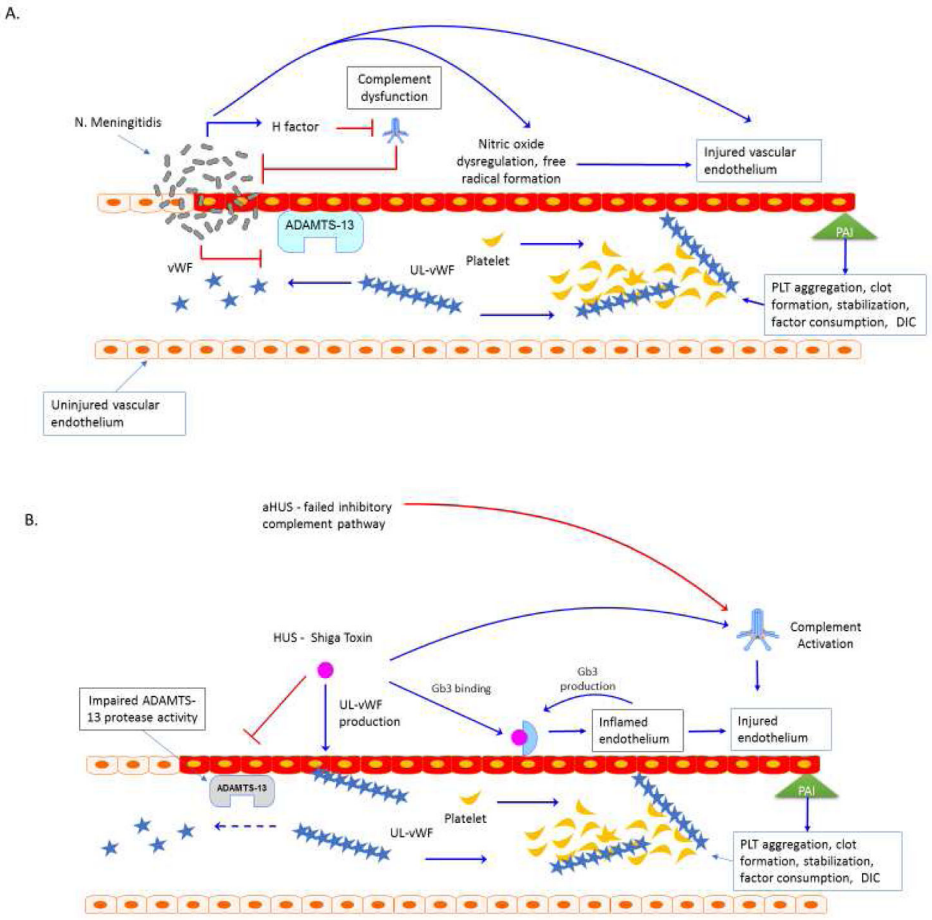

Figure 1. Thrombocytopenia Associated MOF. A. Purpura fulminans

Neisseria meningitidis can adhere to and form colonies on the vascular endothelium via pilin[62]. Meningococcal virulence factor such as factor H binding protein can down regulate complement response and allow bacterial proliferation[63]. Bacterial-endothelial interactions result in endothelial inflammation via NFkB responses and can further promote endothelial infection, capillary leakage and translocation of bacteria across the vessel[64–66]. Invasive meningococcal disease is associated with decreased activity of the metalloproteinase ADAMST13 and increased activity of von Willebrand factor (vWF) [67, 68]. Ultra-large vWF (UL vWF) multimers contribute to platelet (PLT) aggregation and intravascular thrombosis[68,69]. Endothelial inflammation contributes to platelet dysfunction via *N. meningitidis*-derived nitric oxide with impaired vascular homeostasis and NO mediated impairment ADP-mediated platelet aggregation[70]. Inflammatory cytokines change the coagulation profile towards pro-coagulation with a reduction of activated protein C (APC) and anti-thrombin III (ATIII) and upregulation of prothrombin and the anti-fibrinolytic plasminogen activator inhibitor-1 (PAI-1)[71]. The end result of the interaction of multiple 'inflammatory' pathways in the organ is micro-thrombosis, tissue ischemia, oxidative insult, ischemia and cell death. Plasm exchange therapy can reverse this process. **B. Atypical Hemolytic Uremic Syndromes.** Sterile microangiopathies such as thrombotic thrombocytopenia purpura (TTP) are associated with inhibition of ADAMST13 activity via

the presence of auto-inhibitors, whereas atypical HUS or “congenital TTP” has been associated with ADAMST13 and inhibitory complement gene mutations[72,73]. Upon infection with Shiga toxin (ST)-producing pathogens, ST has direct effects on the vascular endothelium and results in increased release of ultra-large vWF multimers and to directly inhibit ADAMST13 activity level, further promoting thrombosis formation[73–74]. ST mediated aHUS causes a pro-inflammatory endothelial state by promoting leukocyte adhesion and by producing endothelial derived cytokines such as IL-8, similar to the vascular pathophysiology observed in purpura fulminans. Exposure of human endothelial cells to the pro-inflammatory cytokines TNF-al and IL-1b increase expression of globotriaosylceramide (Gb3) on endothelial cells and result in further susceptibility to ST by a feed-forward mechanism[75]. Shiga-toxin causes production of Complement 3a (C3a), with loss of thombomodulin (TM), changes in cell surface adhesion molecules and a propensity toward clot production[2, 76–78]. In aHUS, mutations in the alternative complement regulatory pathway, especially mutations in the complement factor H, impair control of Complement 3b (C3b) on the cell surface by inability to recognize sialic acid and helps to explain the thrombogenic potential in these patients[2, 79]. Mutations have recently been described in multiple complement regulatory genes in adult patients with aHUS, with 12% of patients having compound mutations[80]. In addition to plasma exchange therapy, the anti-C5a monoclonal antibody eculizumab is FDA approved for this process.

Abbreviations: Thombocytopenia Associated Multiple Organ Failure (TA-MOF); Nuclear factor kappa b (NFkb); A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMST13); von Willebrand factor (vWF), Platelet (PLT); Nitric Oxide (NO); Activated protein C (APC); Anti-thrombin III (ATIII); plasminogen activator inhibitor-1 (PAI-1); thrombotic thrombocytopenia purpura (TTP); hemolytic uremic syndrome (HUS); atypical HUS (aHUS); shiga toxin (ST); tumor necrosis factor alpha (TNF-a); interleukin 1 beta (IL-1b); globotriaosylceramide (Gb3); complement 3a (C3a); thrombomodulin (TM), complement 3b (C3b).

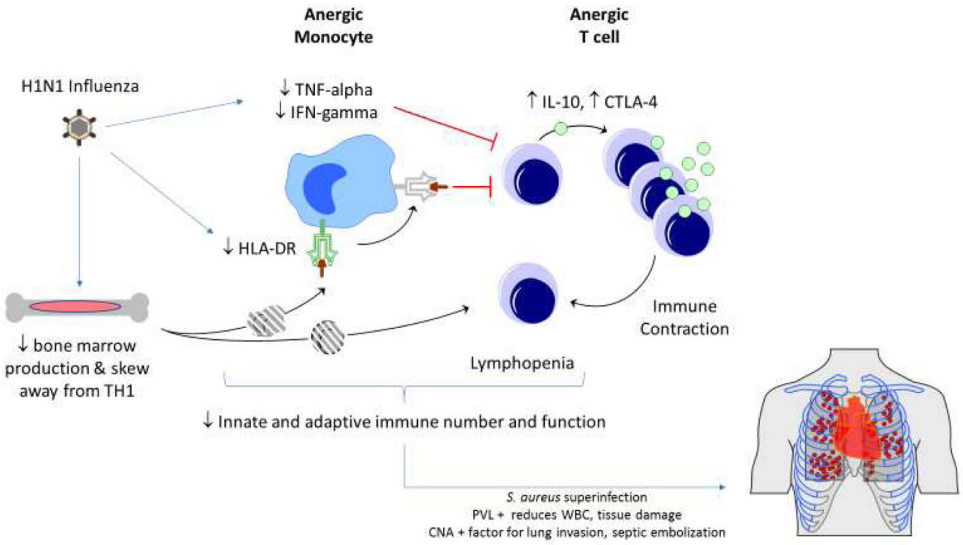

Figure 2. Immune Paralysis MOF

H1N1 influenza has adaptive and innate immune suppressive effects and is associated with the development of MRSA superinfection and death[27] in children, and altered bone marrow microenvironment and with reduced leukocyte output in an animal model, with reduced human leukocyte antigen (HLA)-DR expression and reduction in proinflammatory tumor necrosis factor alpha (TNF-a) and interferon gamma (IFN-g) responses[22, 80–85]. Alterations in innate immune cell function impact polarization and homeostasis of T cell populations, as T cells interacting with anergic monocytes have increases in CTLA-4 mediated negative co-stimulation and anti-inflammatory IL-10 responses[86]. Altered adaptive immune homeostasis favors contraction of immune populations and lymphopenia. Combined adaptive and innate leukopenia and decreased immune function predisposes to secondary infection with methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA elaboration of Panton-Valentine Leukocidin (PVL) further reduces innate immune cell number and leads to cytotoxic lung and soft tissue damage[87, 88]. *S. aureus* collagen adhesin (CNA) is a virulence factor in invasive pulmonary disease, and may contribute to septic embolization of the pathogen[88]. Low dose GM-CSF administration can be given to reverse immune paralysis. Abbreviations: Human Leukocyte Antigen DR (HLA-DR); tumor necrosis factor alpha (TNF-a); interferon gamma (IFN-g); Cytotoxic T lymphocyte antigen 4 (CTLA4); Interleukin 10 (IL-10); Methicillin Resistant *Stapholococcus aureus* (MRSA); Panton-Valentine Leukocidin (PVL); collagen adhesin (CNA).

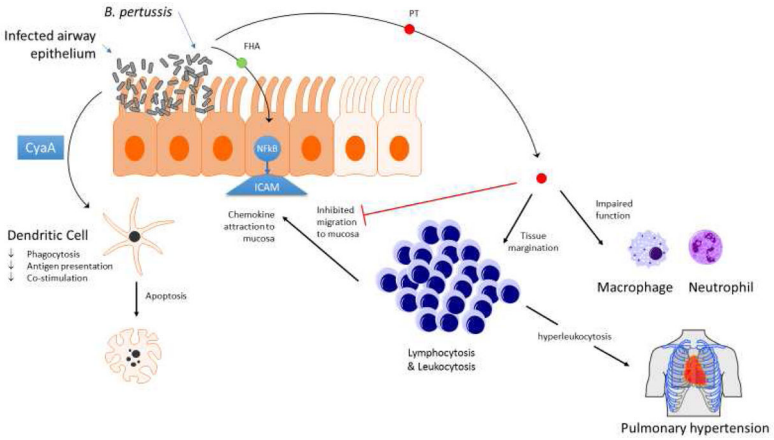

Figure 3. Hyperleukocytosis – Critical Pertussis MOF

Bordetella pertussis infects the airway epithelium inducing inflammation and altered mucosal immune response. Bacterial production of the virulence factor CyaA has several deleterious effects on CD11b+ dendritic cells, including decreased ability to perform phagocytosis, decreased capacity to present antigen to T cells, and decreased expression of co-stimulatory molecules. CyaA leads to dendritic cell apoptosis[89]. The virulence factor filamentous hemagglutinin (FHA) induces NF-kB mediated up-regulation of epithelial ICAM-1 at sites of bacterial invasion[89,93]. This, in part, results in the histopathological findings typical of pertussis infection including leukocyte activation and infiltration[90]. Leukocytosis and lymphocytosis and subsequent plugging of pulmonary arterioles and venules is specific to early infancy, predominantly due to margination of cells from tissues[90–92]. Pertussis toxin (PT) decreases the ability of leukocytes to exit the blood vessel via impairment of CD62L expression and LFA-1[92]. Pertussis toxin has a further role in impairing macrophage and neutrophil activation and function via alterations in toll like receptor (TLR)-4 signaling, whereas prolonged exposure of innate cells to FHA modulates NFkB responses downward[93, 94]. Complement mediated opsonization and phagocytosis and intracellular killing of *B. pertussis* is impaired. Another virulence factor, ACT, leads to innate immune cell apoptosis[94]. In addition to leukocyte plugging and high blood viscosity, perturbations in nitric oxide mediated regulation of pulmonary vascular tone contribute to pulmonary hypertension[90–92]. Infants have responded to extracorporeal leukocyte reduction therapies as well as inhaled nitric oxide and ECMO for reversal of cardiovascular collapse. Abbreviations: filamentous hemagglutinin (FHA); intercellular adhesion molecule 1 (ICAM-1); pertussis toxin (PT); toll-like receptor 4 (TLR-4).

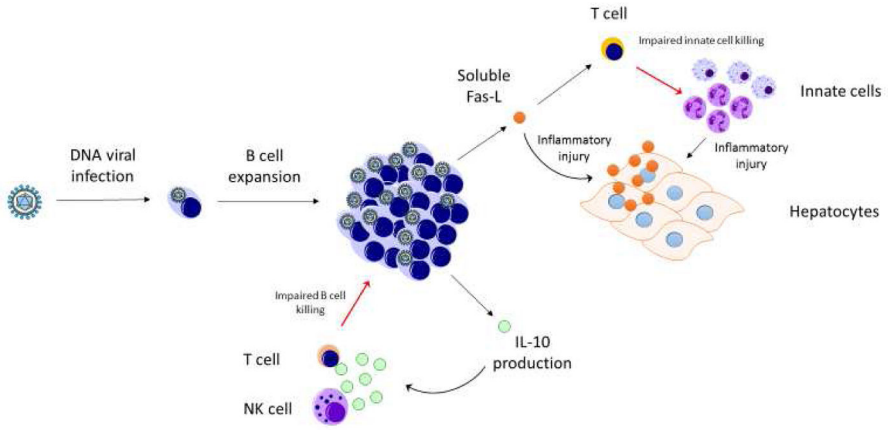

Figure 4. Sequential MOF

The host-pathogen relationship model in sequential MOF is Epstein Barr Virus (EBV) infection of host B cells, such as occurs in the transplant recipients. Virulence factors such as viral-encoded IL-10 modulate the host immune antigen presenting, NK cell, and T cell antiviral response and contribute to viral survival and lymphoproliferation[95, 96]. B cells, among other cell types, have been hypothesized to be a source for soluble Fas ligand (s-FasL)[40]. Membrane bound FasL on T and NK cells interacts with Fas on target cells and results in cellular apoptosis required to cull immune populations in activation induced cell death (AICD)[97]. The production of large amounts of s-FasL by B cells may interfere with this pathway, impairing cytotoxic culling of B cells that are the cellular pool required for EBV infection. Production of s-FasL impacts T cells ability to kill innate immune cells, which leads to inflammatory injury at end organs including hepatocytes. Furthermore, engagement of Fas on hepatocytes by anti-Fas antibody results in direct hepatic injury and sFasL >200 pg/mL in patients was associated with hepatocyte destruction and mortality[40, 98–100]. Immune suppressant withdrawal allows recovery of NK function and FDA approved use of the anti CD20 monoclonal antibody Rituximab removes the reservoir of infection.

Abbreviations: Epstein Barr Virus (EBV); Fas ligand (FasL); soluble Fas ligand (sFasL); activation induced cell death (AICD).

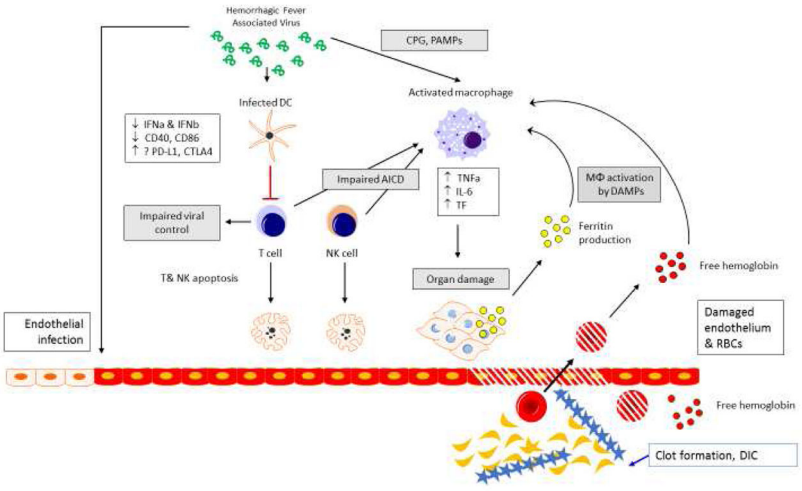

Figure 5. Severe Sepsis Associated Macrophage Activation Syndrome

Severe sepsis associated MAS can be modeled after the host pathogen interaction of the hemorrhagic fever viruses, including the filoviral family member Ebola. The unchecked inflammatory response is driven by myeloid lineage cells including monocytes and macrophages, and functionally impaired T and NK cell responses. T cells are skewed away from robust antiviral responses and towards regulatory T cell responses, in part due to lack of co-stimulation from professional antigen presenting cells and potentially due to negative costimulation and induction of apoptosis[101–103]. Macrophages are activated toward production of IL-6, TNF-a, and tissue factor (TF). IL-6 and TNF-a contribute to the cytokine storm phenotype, and TF promotes endothelial dysfunction and consumptive coagulopathy[104]. Danger associated molecular pattern (DAMP) and pathogen associated molecular pattern (PAMP) pattern recognition receptor (PRR) engagement through production of ferritin from either cellular damage or by the reticuloendothelial system, elaboration of free hemoglobin (via formation of hemoglobin-haptoglobin complexes and stimulation of CD163 receptors on monocytes and macrophages), or by toll-like receptor (TLR)-9 stimulation via CpG, may provide a feed forward mechanism by which monocytes and macrophages are further stimulated toward a pro-inflammatory phenotype. The pro-inflammatory cytokines IL-1 and TNF-alpha, which are produced at high levels by monocytes and macrophages, have been shown to have direct injurious effects on end organs and tissues (heart, liver, kidney, etc.)[105, 106]. High ferritin levels were recently associated with mortality among patients with epidemic Ebola hemorrhagic fever[50]. Anti-inflammatory therapies effective in reversing MAS have not been evaluated in Ebola infected patients. Abbreviations: Interleukin 6 (IL-6); tissue factor (TF); Danger associated molecular pattern (DAMP); pathogen associated molecular pattern (PAMP); pattern recognition receptor (PRR); toll-like receptor 9 (TLR-9).

Table 1

Five inflammation pathobiology phenotypes and putative adjunctive therapies

Phenotype	Clinical Criteria	Biomarker/Prototype	Adjunctive Therapy
Thrombocytopenia Associated MOF	Platelets < 100,000/mm^3 Acute Kidney Injury Elevated LDH	ADAMTS 13< 57% Discussed Prototypes = Purpura fulminans/Atypical HUS	a) Plasma Exchange[9,,11–15,61] removes ultra large vWF multimers and restores ADAMTS13 activity b) C5a Antibody[16–19] Inhibits activated complement (FDA approved for aHUS)
Immune paralysis Associated MOF	Persistent or Secondary Infections	Monocyte HLA-DR expression < 30% or 8,000 molecules; Whole blood ex vivo TNF response to LPS < 200 pg/mL; Absolute Lymphocyte Count < 1,000 mm^3 Discussed Prototype = H1N1/ MRSA	GM-CSF[25,29,30] Immune suppressant withdrawal[28] Restores TNF response to endotoxin
Hyperleukocytosis and pulmonary hypertension associated MOF	Age < 6 months Pulmonary HTN	WBC > 50,000 mm^3 Discussed Prototype = Critical Pertussis	Extracorporeal Leukoreduction[36] removes circulating WBC and decreases pulmonary hypertension
Sequential MOF with liver failure	Respiratory distress Followed by Hepatobiliary Dysfunction	s-FasL > 200 pg/mL Discussed Prototype = Epstein Barr Virus Lymphoproliferative Disease	a) Hold immune suppressants b) Give anti CD20 monoclonal antibody[44,45] removes EBV reservoir (FDA approved for PTLD)
Macrophage Activation Syndrome	Hepatobiliary Dysfunction and Disseminated Intravascular Coagulation	Ferritin > 500 ng/mL Discussed Prototype = Viral Hemorrhagic Fevers	IVIG + Steroids + Plasma Exchange[61] Anakinra[48,57] Tocilizumab[58,59] decreases macrophage inflammation