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152 Pathophysiology of Neonatal Sepsis

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INTRODUCTION

A successful immune response is critically necessary to eradicate infectious challenges and prevent dissemination of the infection in the host. However, if inflammation is not limited and becomes generalized, it can result in the constellation of signs and symptoms of a systemic inflammatory response syndrome (SIRS). If the infection is not contained, the spread of the pathogen from its local origin through the blood may result in systemic endothelial activation and precipitate sepsis, severe sepsis, and septic shock. Progression of sepsis to shock may lead to multiple organ dysfunction syndrome (MODS) and ultimately death.

Host immunity is divided into innate and adaptive immune systems for purposes of discussion and teaching but there is a great deal of interaction between the two systems. Innate immunity is rapid, largely nonspecific, and composed of barriers, phagocytic cells, the complement system, and other soluble components of inflammation. After breech of a barrier, cellular elements of the innate immune response are the first line of defense against the development and progression of infection. Adaptive immunity, which is antigen specific, is long lived, and often takes several days to develop, provides immunologic specificity and memory. These systems work together to protect the host from pathogenic challenge but may also precipitate host injury through aberrant responses. The outcome of infection is dependent on at least four major factors: (1) the pathogen, (2) the pathogen load, (3) the site of infection, and (4) the host response. Less is known about the host response in neonates compared with adults for a number of reasons, the principal one being a highly variable definition of disease.

Our understanding of the pathophysiology of sepsis is largely from investigations in adult populations, including both humans and animals. There is clear evidence from both preclinical models of sepsis and humans that neonates manifest different host immune responses as compared with adults.[1-4] Even in comparison with children, neonates manifest a unique host immune response to septic shock.[5] Thus neonatal-specific clinical investigations, particularly in very preterm infants, are required to improve both survival and long-term outcomes for these populations. A better understanding of the pathophysiology will uncover new opportunities for interventional studies ultimately aimed at improving outcomes. To this end, in this chapter we explore the pathophysiology of sepsis in the neonate, with special attention paid to the immunobiology of sepsis.

DEFINITION OF SEPSIS

Adult and pediatric intensivists currently use generally accepted definitions for sepsis for goal-based therapeutic interventions.[6-9] These definitions are critical to facilitate epidemiologic studies, to accurately determine disease prevalence, to select patients for clinical trials, and ultimately to improve the delivery of care. The generally accepted pediatric definition for sepsis, established in 2005, was intended for all children (<18 years old), including term neonates (≥37 weeks' completed gestation).[6] Preterm neonates (<37 weeks' completed gestation) were specifically excluded from the pediatric generally accepted definitions, and neonatal-perinatal subspecialists were not represented among the pediatric consensus experts. To investigate whether the pediatric generally accepted definitions for SIRS and sepsis applied to term infants, Hofer and colleagues[10] retrospectively examined 476 term neonates and found that the generally accepted definitions applied to only 53% of cases of culture-positive early-onset sepsis. Neonatal sepsis has been inconsistently defined on the basis of a variety of clinical and laboratory criteria, which makes the study of this condition very difficult.[11] Diagnostic challenges and uncertain disease epidemiology necessarily result from a variable definition of disease. The lack of a generally accepted definition for neonatal sepsis remains a significant hindrance towards improving outcomes and accurately describing disease pathophysiology. Thus working definitions for the sepsis continuum, specific for preterm and term neonates, are needed to provide a uniform basis for clinicians and researchers to study and diagnose severe sepsis. The addition of immune biomarker–based staging of disease to clinical sign staging is highly likely to increase the accuracy of patient classification for future multicenter clinical trials that will test novel interventions.

EPIDEMIOLOGY AND RISK FACTORS FOR DEVELOPMENT OF NEONATAL SEPSIS

Sepsis or serious infection within the first 4 weeks of life kills more than 1 million newborns globally every year.[12,13] The incidence of neonatal sepsis is variable (from less than 1% to more than 35% of live births) on the basis of gestational age and time of onset (early-onset sepsis [<72 hours after birth] or late-onset sepsis [≥72 hours after birth]).[14-20] Preterm neonates have the greatest sepsis incidence and mortality rates among all age-groups[21-26] (Figure 152-1).

Risk factors for developing sepsis in neonates, particularly the very premature, have been well described.[14,15,27-33] Prematurity, low birth weight (especially infants weighing less than 1,000 g), male sex, a maternal vaginal culture positive for group B streptococcus (GBS), prolonged rupture of membranes, maternal intrapartum fever, and chorioamnionitis are strongly associated with an increased risk for early-onset sepsis.[30] Chorioamnionitis is associated with the greatest risk for subsequent clinical or culture-proven sepsis.[29] Recent studies demonstrate the risk for sepsis in newborn infants born to women with clinical chorioamnionitis is strongly dependent on gestational age, with minimal risk in neonates aged 35 weeks or older and greater risk with increasing degrees of prematurity.[34-41] The risk for neonatal sepsis conferred by maternal GBS colonization[29] is significantly reduced with adequate intrapartum antibiotic prophylaxis.[42]

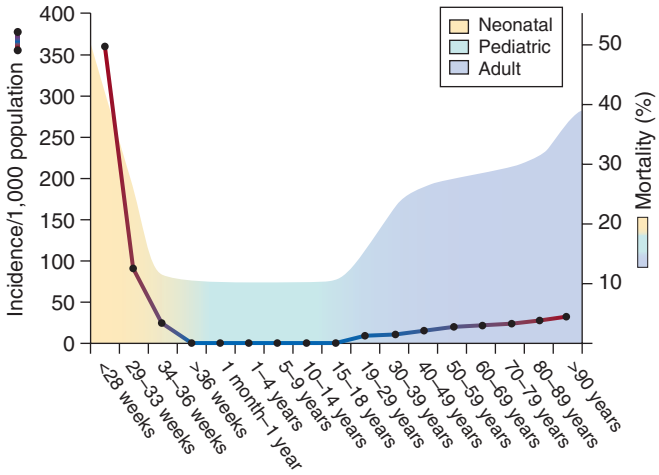

Figure 152-1 Sepsis incidence and mortality in humans across developmental age-groups.

Despite the efficacy of this intervention, the incidence of invasive GBS disease in African American neonates is still more than twice that in white babies,[43] and the incidence of *Escherichia coli* sepsis may be rising in very-low-birth-weight (VLBW) neonates.[44] Vaginal delivery in the presence of maternal active primary herpes simplex virus significantly increases the risk for a neonatal herpes simplex virus infection, which has a fulminant course and high mortality.[45-47] Preexisting maternal immunodeficiency or sepsis also increases the risk for sepsis in the neonate.[48] In addition, care practices after birth, such as intubation, mechanical ventilation, and placement of central venous lines, increase the risk for the development of sepsis.[49]

MICROBIOLOGY OF SEPSIS IN NEONATES

A number of pathogens have been associated with sepsis in the neonatal period. The predominant cause is bacterial; however, certain viral infections are associated with a fulminant course and significant mortality.[50-52] In a large (n = 104,676), multicenter study of VLBW infants (<1500 g), gram-positive organisms accounted for 34% of pathogens causing early-onset sepsis and 61% of those causing late-onset sepsis. In contrast, gram-negative organisms were responsible for 58% of early-onset sepsis and 26% of late-onset sepsis.[53] *Candida* species accounted for 3% of cases of early-onset sepsis and 11% of cases of late-onset sepsis. Infection by gram-negative organisms, particularly *Pseudomonas* species, carries a higher risk for fulminant course and death than infection by other pathogen groups.[14,15,49,54-56] Gram-positive causes of sepsis are dominated by GBS and coagulase-negative staphylococci (CoNS).[15,57] Although the high mortality rate for GBS has been well described (especially among infants born prematurely), mortality rates associated with CoNS are significantly lower.[15,16] Fungi may also be associated with fulminant neonatal sepsis and predominantly affect VLBW infants.[15,58,59] Independent predictors of in-hospital neonatal mortality after late-onset sepsis were *Pseudomonas* infection (adjusted odds ratio [OR], 14.31; 95% confidence interval [CI], 3.87% to 53.0%) and fungemia (OR, 5.69; 95% CI, 2.48% to 13.01%).[60] The limited sensitivity of current methods to identify causative organisms is partially due to an inability to take a large sample of blood from newborn infants with suspected sepsis.[61] Blood culture–negative ("clinical") sepsis is estimated to occur at a nearly 10-fold greater rate than blood culture–positive sepsis.[62] In some of these infants, sepsis may also be due to novel viral pathogens associated with sepsis-like syndromes (e.g., echovirus, enterovirus, parechovirus, Coxsackie virus, adenovirus, parainfluenza virus, rhinovirus, and coronavirus).[50,63-65]

THE ROLE OF BARRIER DEFENSES IN NEONATAL SEPSIS

Physical barriers, including skin and mucosal surfaces, are the first point of contact between the host and potential pathogens. Thus a successful immune defense in addition to epithelial barrier function is critical to prevent the development of local infection. Multiple immune elements are present to prevent attachment and propagation of pathogens while simultaneously permitting the presence of commensal organisms required for homeostasis. Vernix enhances skin barrier function in late-preterm and term neonates. Vernix is a complex material comprising water (80.5%), lipids (10.3%), and proteins (9.1%) produced by fetal sebaceous glands during the last trimester[66] and is largely absent in preterm neonates born before 28 weeks' gestation. Vernix provides a barrier to water loss, improves temperature control, and serves as a shield containing antioxidants and innate immune factors such as antimicrobial proteins and peptides (APPs).[67] The APPs on the surface of the newborn's skin (and replete in the amniotic fluid[68-70]) are capable of killing/inactivating common neonatal pathogens, including GBS, *E. coli*, and *Candida* species.[71] Erythema toxicum is an immune-mediated manifestation that results from bacterial colonization of the skin occurring shortly after birth.[72,73] This common cutaneous immune response is less common in preterm infants than in term infants, highlighting the impact of developmental age on host immune capabilities.[74] In contrast to the moist mucosal surfaces of the respiratory and gastrointestinal (GI) tracts, the skin is arid, which further reduces the chances for microbial invasion.

The outermost layer of the skin, the stratum corneum, prevents microbial invasion, maintains temperature, and reduces the risk for dehydration through prevention of transcutaneous water loss.[75] The immature and incompletely developed stratum corneum of preterm newborns takes at least 1 to 2 weeks after birth to become fully functional[76] and may take up to 8 weeks in the extremely preterm neonate, significantly increasing the risk for barrier dysfunction.[77] Disruption of the cutaneous barrier by trauma (e.g., placement of an intravenous catheter or heel stick) or chemical burn allows microorganisms to enter the subcutaneous tissue, increasing the likelihood of their establishing a local infection (Figure 152-2). The likelihood of a microbial breach of the cutaneous barrier rises in the presence of intravenous catheters, which are essential for critical care. Emollients, aimed at enhancing the barrier function of preterm newborn skin, increase the risk for nosocomial infection and their use is not recommended.[78]

Mucosal barriers contain multiple components that serve to prevent infection, including acidic pH, mucus, cilia, proteolytic enzymes, APPs, opsonins such as surfactant proteins, sentinel immune cells such as macrophages, dendritic cells, polymorphonuclear neutrophils (PMNs), and T cells, as well as commensal organisms.[79] Like the skin, the GI mucosa is quickly colonized after birth and contains a significant repository of microorganisms.[80-82] GI barrier integrity, paramount for prevention of spread of microorganisms out of the intestinal compartment, is dependent on the interaction between commensal organisms and host epithelium. Interleukin (IL)-17, produced by type 3 intestinal innate lymphoid cells in the presence of the microbiota, drives granulocytosis and may protect the neonatal host from infectious challenge.[83] A loss of intestinal barrier integrity likely plays a role in the development of necrotizing enterocolitis (NEC) and late-onset sepsis.[84,85] Prolonged antibiotic treatment, hypoxia, and remote infection are factors known to

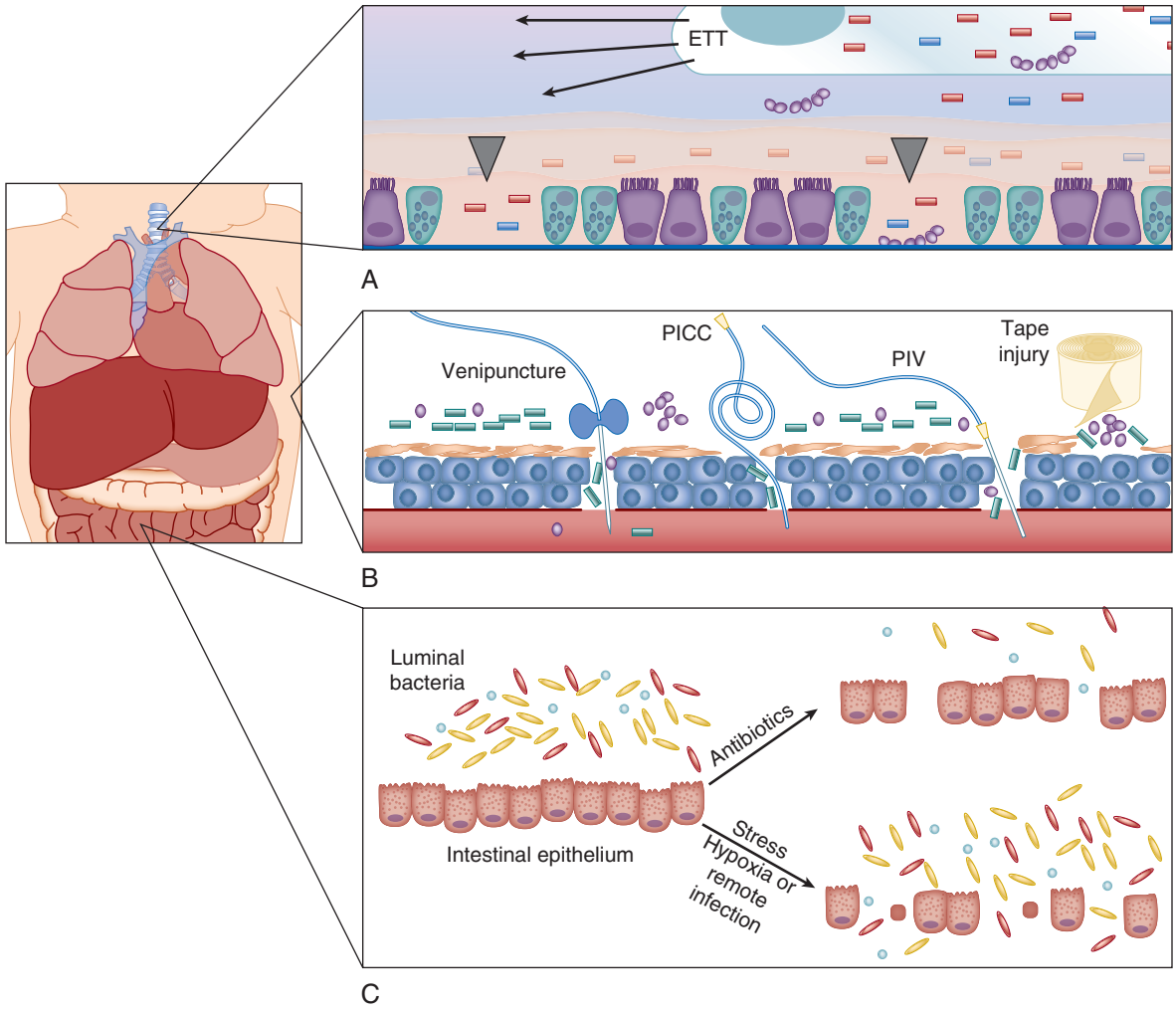

Figure 152-2 Physical barriers. A, Respiratory mucosa. A foreign body (an endotracheal tube, ETT) and/or positive pressure can irritate and injure the respiratory epithelium (ciliated cells; *gray arrowheads* denote denuded areas). Increased numbers of goblet cells *(blue cells with inclusions)* with decreased mucociliary clearance of the airway further increase the likelihood of infection (bacteria represented by *purple spherical chains and blue/pink rods*). **B,** Skin. Disruptions associated with trauma (venipuncture or heel stick), a peripherally inserted central catheter (PICC), a peripheral intravenous line (PIV), or tape-related abrasions compromise the skin barrier (bacteria represented by *clusters of purple spheres and green rods*). **C,** Gastrointestinal mucosa. Luminal bacteria (microbiota) are a valuable component of the mucosal barrier. The interaction between intestinal bacteria and intestinal epithelium is necessary for homeostasis and normal function of repair mechanisms. Disruption of this interaction, through the use of antibiotics or via stress to the organism (e.g., hypoxia or remote infection such as sepsis or pneumonia), results in loss of homeostasis and degradation of the intestinal boundaries with subsequent microbial translocation.

disrupt or injure the neonatal intestinal barrier (see Figure 152-2).[86-88] Under these circumstances, the gut may become the *motor of systemic inflammation*.[89] Mechanistically, Paneth cells and intestinal lymphoid cells may release excessive amounts of IL-17, which, in turn, plays a critical role in the development of SIRS.[90] Many interventions aimed at reducing the frequency of sepsis in neonates via enhancement of mucosal barrier integrity have been evaluated. Neither probiotics nor glutamine supplementation has reduced the incidence of neonatal sepsis.[91] In contrast, human milk feeding is associated with a reduction in the risk for sepsis[92] and NEC[93,94] and is strongly encouraged, especially in preterm infants.

Respiratory mucosa is defended in utero by amniotic fluid and pulmonary APPs, surfactant proteins A and D, alveolar macrophages, and PMNs, among other immune elements. The surface and submucosal gland epithelium of the conducting airways is a constitutive primary participant in innate immunity through the production of mucus and mucociliary clearance of pathogens and debris.[95] Premature neonates have relatively more goblet cells than do maturer neonates, leading to a decrease in mucociliary clearance. Respiratory mucosal function can be impaired by surfactant and saliva deficiency, altered mucus production, and mechanical ventilation. Ventilation is associated with decreased mucociliary clearance, airway irritation, and parenchymal lung injury (see Figure 152-2). Intubation is also associated with the progressive accumulation of colonizing bacteria and bacterial endotoxin in respiratory fluids, with concomitant mobilization of endotoxin-modulating APPs to the airway.[96] Neonates with surfactant deficiency lack APPs such as surfactant proteins A and D, which are also absent in commercially available surfactant preparations.[97] There is an age-dependent maturation in the ability of respiratory epithelium to elaborate APPs (cathelicidin and β-defensins), such that the respiratory epithelium of preterm newborns mounts a deficient APP response.[98]

These deficiencies as well as those related to cellular function in combination with invasive procedures lead to a reduction in respiratory barrier function that increases the risk for sepsis.

MOLECULAR EVENTS DURING EARLY INFECTION

PATHOGEN RECOGNITION

Once the local barrier function has been compromised, pathogen recognition by local immune sentinel cells is the first step towards the development of an immune response (Figure 152-3). Elegant sensing mechanisms have evolved to facilitate detection of potentially pathogenic microorganisms. Multiple classes of pathogen recognition receptors (PRRs) have been discovered that serve as detectors of pathogen-associated molecular patterns (PAMPs), including cell wall and membrane components, flagellum, nucleic acids, and carbohydrates.[99] A litany of PRR classes have been discovered, including the Toll-like receptors (TLRs), NOD-like receptors (NLRs), retinoic acid–inducible protein I like receptors (RLRs), peptidoglycan recognition proteins, β_2-integrins, and C-type lectin receptors. The TLRs, β_2-integrins, and C-type lectin receptors detect pathogens both on the cell surface and in endosomes, whereas RLRs and NLRs detect pathogens only intracellularly. The discovery that TLR4 was integral for a robust lipopolysaccharide (LPS)-mediated inflammatory response after gram-negative sepsis may be why TLRs have been more thoroughly investigated in the setting of sepsis than other PRRs.[100] Each of the 10 known TLRs in humans, present on and within multiple cell types, recognizes extracellular and intracellular pathogens via specific PAMPs.[101,102] Multiple TLRs may be activated in concert by intact or partial microorganisms and in turn activate multiple second-messenger pathways simultaneously.[102,103]

LPS is the prototypic mediator of systemic inflammation and generates many of the clinical findings of sepsis and septic shock, including MODS and death.[104] LPS signals through TLR4 in conjunction with the adaptor proteins CD14 and myeloid differentiation factor 2.[105] In adults a reduction in mortality and improvement in hemodynamics were demonstrated when the level of serum LPS was reduced.[106] The level of LPS is elevated in blood from infected neonates and those with NEC even in the absence of gram-negative bacteremia.[84] High levels of circulating endotoxin found during sepsis and NEC are associated with multiorgan failure, thrombocytopenia, neutropenia, and death.[84] Administration of anti-LPS antibodies to a small number of

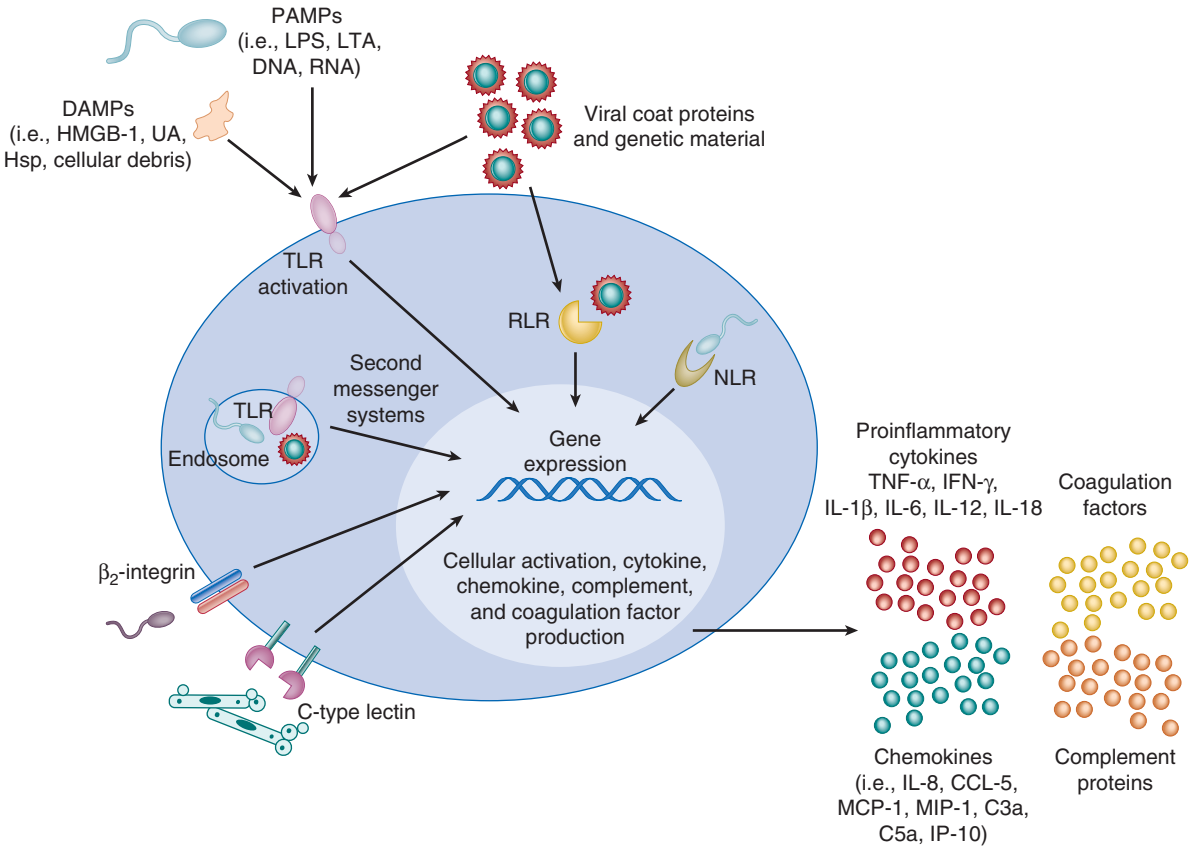

Figure 152-3 Activation of sentinel immune cells. Sentinel cells (e.g., monocytes, macrophages) sense pathogens via pathogen-associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs) binding to pathogen recognition receptors. Pathogen recognition receptors include Toll-like receptors, retinoic acid–inducible protein I like receptors, NOD-like receptors, C-type lectin receptors, and β_2-integrins. PAMPs include lipopolysaccharide (LPS), lipoteichoic acid (LTA), DNA, and RNA. DAMPs can also be sensed through Toll-like receptors and include uric acid (UA), heat shock proteins (Hsp), and high-mobility group box 1 (HMGB-1). Signaling occurs through a series of second messengers and results in transcription and translation of cytokines and chemokines that amplify the immune response. *IFN,* Interferon; *IL,* interleukin; *MCP,* monocyte chemoattractant protein; *MIP,* macrophage inflammatory protein; *NLR,* NOD-like receptors; *RLR,* retinoic acid–inducible protein I like receptor; *TNF,* tumor necrosis factor; *TLR,* Toll-like receptor. (From Wynn JL, Wong HR: Pathophysiology and treatment of septic shock in neonates. *Clin Perinatol* 37(2):439–479, 2010.)

neonates with sepsis (n = 16) with serum endotoxin present reduced the time to recovery but not mortality as compared with the values in placebo-treated neonates.[107] Reduction of serum LPS levels by exchange transfusion in infected neonates (n = 10) was associated with improved survival.[108]

Bacterial cell wall components (such as lipoteichoic acid) signal primarily through TLR1, TLR2, and TLR6, flagellin signals through TLR5, and CpG double-stranded DNA signals through TLR9. Common viral PAMPs such as double-stranded RNA or single-stranded RNA signal through TLR3, and TLR7 and TLR8, respectively. Agonist-TLR binding results in a signaling cascade of intracellular second-messenger proteins ultimately leading to production of cytokines and chemokines, as well as activation of other antimicrobial effector mechanisms.[101] Signaling through TLRs typically leads to the production of nuclear factor κB (NF-κB)-dependent inflammatory cytokines and chemokines, whereas signaling through Toll/IL-1 receptor–domain-containing adapter inducing interferon (IFN)-β (TRIF) induces production of type I IFNs, as well as NF-κB-related inflammatory cytokines. In neonates of all gestational ages, up-regulation of TLR2 and TLR4 messenger RNA (mRNA) occurs during gram-positive and gram-negative infection, respectively.[109] Dysregulation or overexpression of TLR4 is involved in the development of NEC in experimental animal models,[110] implicating the importance of TLRs in the initial immune response to pathogens and their role in neonatal sepsis.

NOD-LIKE RECEPTORS, RETINOIC ACID–INDUCIBLE PROTEIN I-LIKE RECEPTORS, C-TYPE LECTIN RECEPTORS, AND β2-INTEGRINS

Other important intracellular PRRs include NLRs and RLRs. For NLRs, multiple cytosolic proteins are able to act as PAMP sensors (e.g., NLRP1, NLRP3, and NLRC4) and coalesce with adaptor proteins and procaspase 1 to form a multimeric protein complex termed the *inflammasome.*[111] The formation of the inflammasome results in the conversion of procaspase to active caspase 1, which cleaves the inactive precursor proteins IL-1β and IL-18 to their active forms.[111] RLRs are cytoplasmic RNA helicases that, like TLR3, sense double-stranded RNA of viral origin and induce type I IFN production and NF-κB activation.[102] To date, the impact of RLR and NLR signaling has not been specifically examined in neonates with sepsis.

In addition to its roles in leukocyte function (adhesion, phagocytosis, migration, and activation) and complement binding, complement receptor 3 (CR3, also known as *MAC-1* and *CD11b-CD18*) functions as a pathogen sensor on the surface of phagocytes. CR3 binds LPS, as well as a broad range of other microbial products, in cooperation with or independently of CD14, leading to up-regulation of inducible nitric oxide (NO) synthase and NO production.[112] Diminished expression of L-selectin and CR3 on stimulated neonatal PMNs impairs activation and accumulation at sites of inflammation.[99,113,114] Decreased expression of L-selectin and CR3 persists for at least the first month of life in term infants, possibly contributing to an increased risk for infection.[115] The expression of CR3 (CD11b) may be reduced further in preterm neonates as compared with term neonates.[116] In umbilical cord blood from neonates of less than 30 weeks' gestation, PMN CR3 content was similar to levels found in patients with type 1 leukocyte adhesion deficiency (failure to express CD18).[113,114] Thus decreased leukocyte CR3 surface expression increases the likelihood of suboptimal pathogen detection and cellular activation, particularly in the preterm neonate.

C-type lectin receptors are PRRs that recognize bacterial, viral, fungal, and parasitic carbohydrate moieties. C-type lectin receptors may be expressed on the cell surface (e.g., macrophage mannose receptor, mincle receptor, dectin 1, and dectin 2) or secreted as soluble proteins (e.g., mannose-binding lectin [MBL], (which is also named *mannan-binding protein* or *mannan-binding lectin*) as one of the acute-phase reactants. Once bound to its carbohydrate ligand, MBL initiates activation of complement via the lectin pathway to promote opsonization and phagocytic clearance of pathogens. Plasma MBL concentrations are low at birth (especially in preterm infants) but rise steadily throughout infancy and childhood.[117] Low levels of MBL are associated with the increased incidence of sepsis in neonates.[118-120] In addition to decreased concentrations at birth, certain genetic polymorphisms of MBL (namely, *MBL2*), have been associated with an increased risk for infection in some,[121] but not all, studies.[122-124] M-ficolin activates the complement system in a manner similar to MBL and its level is elevated in neonates with sepsis.[125]

THE ROLE OF INFLAMMATION

PRR stimulation results in rapid inflammatory mediator transcription and translation directed at cellular activation and clearance of pathogenic organisms[126] (see Figure 152-3). During sepsis and septic shock, multiple proinflammatory cytokines have been identified, including IL-1β, IL-6, IL-8 (CXCL8), IL-12, IL-18, IFN-γ, and tumor necrosis factor (TNF)-α.[127] Compared with adults with sepsis, neonates with sepsis produce less IL-1β, TNF-α, IFN-γ, and IL-12.[128-133] The decreased cytokine production is due in part to decreased production of important intracellular mediators of TLR signaling, including myeloid differentiation factor 88, IFN regulatory factor 5, and p38, which exhibit gestational age–specific decrements.[134] Recent studies have demonstrated impaired inflammasome activation and mature IL-1β production by neonatal mononuclear cells.[121,135] In a comprehensive study (>140 analytes) of serum from neonates evaluated for late-onset sepsis, IL-18 emerged as a predictive biomarker to differentiate infected neonates from uninfected neonates.[136] IL-18 reduces PMN apoptosis,[137] drives IFN-γ production,[138] and induces production of TNF-α, IL-1β, and CXCL8.[139] IL-18 primes PMNs for degranulation with production of reactive oxygen intermediates on subsequent stimulation.[140] Dysregulation of many of these functions linked to IL-18 are seen in sepsis and septic shock. Increased IL-18 levels have been demonstrated in premature neonates with brain injury[141] and also in an experimental model of NEC,[142-144] highlighting activation pathways common with those in ischemia and inflammation. Excessive levels of IL-1β, TNF-α, IL-6, CXCL8, IL-10, and IL-18, such as those seen with advanced-stage NEC, severe sepsis, or septic shock, correlate with poor survival.[84,145-148] Altered cytokine levels (increased IL-10 and IL-6 levels and decreased CCL5 levels) may identify those neonates at highest risk for the development of sepsis-associated disseminated intravascular coagulation (DIC).[149]

Proinflammatory cytokine production leads to activation of endothelial cells, including increased expression of cell adhesion molecules that facilitate leukocyte recruitment and diapedesis (Figure 152-4). Up-regulation of cell adhesion molecules (soluble intercellular adhesion molecule, vascular cell adhesion molecule, L-selectin, P-selectin, E-selectins, and CD11b-CD18) during sepsis facilitates rolling and extravascular migration of leukocytes.[150-153] Decreased production of L-selectin and expression of C3 in PMNs and monocytes derived from neonates may impair accumulation at sites of inflammation.[113,114]

Chemokine gradients produced by endothelial cells and local macrophages are necessary for effective and specific leukocyte attraction and accumulation (see Figure 152-4). Without adequate leukocyte recruitment, there is increased risk for propagation from a localized to a systemic infection. Although poor cellular chemotaxis in the neonate has been observed, it is not likely a result of reduced serum concentrations of chemokines as baseline levels are similar in preterm and term neonates as compared with adults.[154] Suboptimal cellular chemotaxis may be related to other mechanisms, such as poor complement receptor

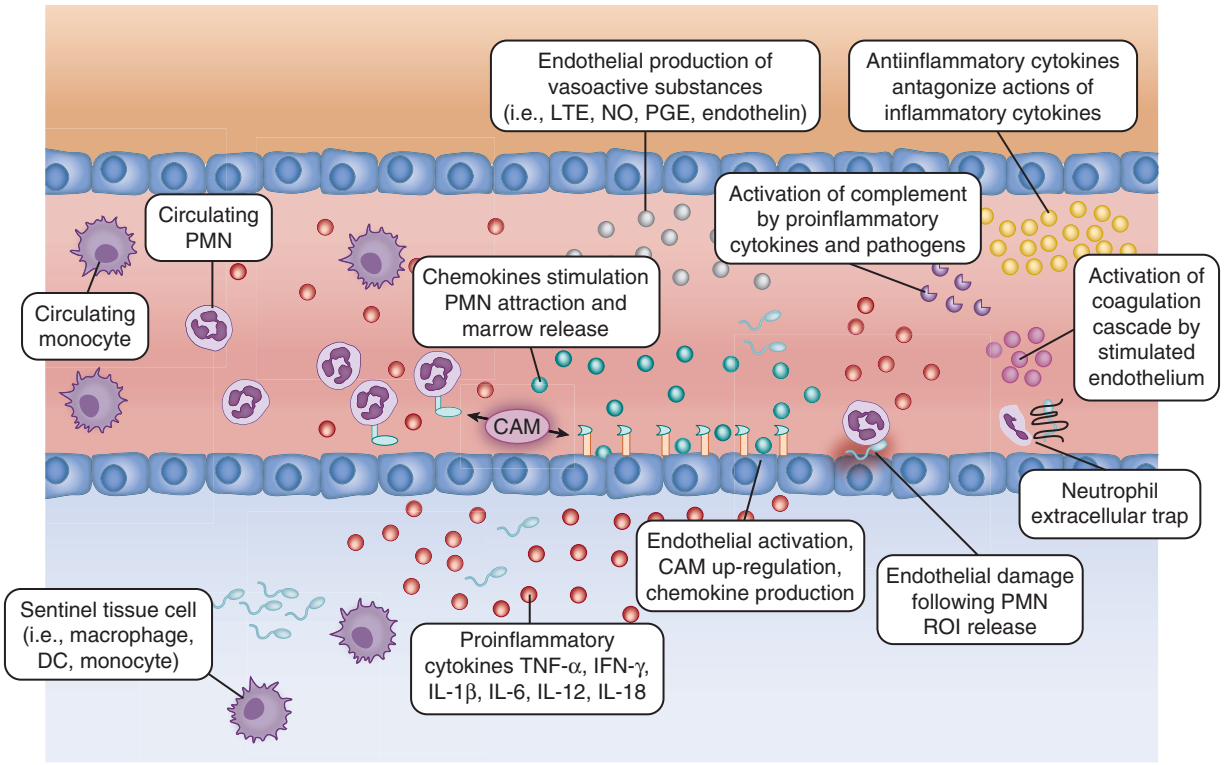

Figure 152-4 Cellular recruitment and endothelial activation following pathogen detection. Pathogen-stimulated tissue/blood monocytes, dendritic cells, and macrophages release proinflammatory cytokines that activate the surrounding endothelium. Endothelial activation results in up-regulation of cell adhesion molecules, production of chemokines and vasoactive substances, activation of complement, and development of a procoagulant state. Recruitment of polymorphonuclear neutrophils (PMNs) occurs along the chemokine gradient surrounding the area of inflammation. Antiinflammatory cytokines counter the actions of proinflammatory cytokines to prevent excessive cellular activation and recruitment that can result in tissue damage and systemic inflammation. Endothelium can be damaged when PMNs release reactive oxygen intermediates or from neutrophil extracellular traps. *CAM,* Cell adhesion molecule; *DC,* dendritic cell; *IFN,* Interferon; *IL,* interleukin; *LTE,* leukotriene; *NO,* nitric oxide; *PGE,* prostaglandin E; *ROI,* reactive oxygen intermediate; *TNF,* tumor necrosis factor. (From Wynn JL, Wong HR: Pathophysiology and treatment of septic shock in neonates. *Clin Perinatol* 37(2):439–479, 2010.)

up-regulation after stimulation,[99] deficiencies in another downstream signaling process,[155] or inhibition by bacterial products.[156] The levels of a wide variety of chemokines are increased during sepsis, including CXCL10 (IP-10), CCL5 (RANTES), CCL2 (monocyte chemoattractant protein 1), CCL3 (macrophage inflammatory protein 1α), and CXCL8.[157] The levels of other chemoattractive molecules also increase with sepsis, including complement proteins C3a and C5a, APPs, including cathelicidins and defensins, and components of invading bacteria themselves.[127,136] The importance of chemoattractive substances in the pathogenesis of severe sepsis is highlighted by studies showing that CXCL8 can be used as a stratifying factor for survival in children,[158] and C5a is implicated in sepsis-associated organ dysfunction in adults.[104] Chemokine investigations in infected neonates revealed that CXCL10 is a sensitive early marker of infection,[157] and low CCL5 levels may predict development of DIC.[149]

Damage-associated molecular patterns (or *alarmins*), such as intracellular proteins or mediators released by dying or damaged cells, may also active PRRs. For example, the damage-associated molecular pattern high-mobility group box 1 (HMGB-1) is involved in the progression of sepsis to septic shock in adults.[104,159] Macrophages or endothelial cells stimulated with LPS or TNF-α produce HMGB-1, which signals through TLR2, TLR4, and receptor for advanced glycation end products (RAGE).[160] HMGB-1 results in cytokine production, activation of coagulation, and PMN recruitment.[159,161] HMGB-1 mediates disruption of epithelial junctions within the gut via the induction of reactive nitrogen intermediates, leading to increased bacterial translocation.[162] The role of HMGB-1 and RAGE signaling in human neonates with sepsis has not been well characterized but has been shown to be involved in the pathophysiology of NEC in an experimental model.[163] Significantly lower soluble RAGE levels were found in human fetuses that mounted robust inflammatory responses and HMGB-1 levels correlated significantly with the levels of IL-6 and S100β calcium-binding protein in the fetal circulation.[164]

Other specific damage-associated molecular patterns, including heat shock proteins and uric acid, may also stimulate TLRs, regulate PMN function, and serve as immune adjuvants. Heat shock protein production in infected neonates has not been evaluated but polymorphisms in heat shock proteins increase the risk for acute renal failure in preterm neonates.[165] The levels of heat shock proteins are significantly elevated in infected adults and children.[166] Elevated heat shock protein 60 and heat shock protein 70 level measured within 24 hours of pediatric intensive care unit admission were associated with septic shock and there was a strong trend towards increased mortality.[167,168] Uric acid can increase cytokine production, PMN recruitment, and dendritic cell stimulation[169] and may also serve as an antioxidant.[170] The level of uric acid is reduced in the serum of neonates with sepsis as compared with control neonates.[171]

In addition to facilitating leukocyte attraction, proinflammatory stimuli result in production of vasoactive substances that decrease or increase vascular tone and alter vascular permeability (see Figure 152-4). These include platelet-activating factor, thromboxane, leukotrienes, NO, histamine, bradykinin,

and prostaglandins.[172,173] These substances are produced predominantly by host endothelium and mast cells. Activated PMNs produce phospholipase A_2 (PLA_2), the level of which is increased in the serum of neonates with sepsis[174] and leads to generation of vasoactive substances, including prostaglandins and leukotriene. Thromboxane produced by activated platelets and endothelin 1 produced by activated endothelium[175] are potent vasoconstrictors that participate in the development of pulmonary hypertension.[176-179] Overproduction of cytokines and vasoactive substances is associated with circulatory alterations and organ failure seen in severe sepsis and septic shock (Figure 152-5).[6,180-183]

THE ANTIINFLAMMATORY RESPONSE

If the pathogen is not contained locally and inflammatory homeostasis is not restored, SIRS may develop, and lead to MODS and death (see Figure 152-5).[184] The traditional paradigm for understanding the host response to sepsis consists of an intense proinflammatory response, or SIRS, temporally followed by a compensatory antiinflammatory response syndrome. This paradigm has been challenged by the failure of multiple antiinflammatory strategies to improve sepsis outcomes in adults.[185] New data in adults and children demonstrate simultaneous proinflammatory/antiinflammatory responses where the magnitude of either response may determine outcome.[186,187] Near simultaneous increases in antiinflammatory cytokine production (transforming growth factor β, IL-4, IL-10, IL-11, and IL-13) occur in neonates during infection, countering the actions of proinflammatory cytokines.[127,188,189] These mediators blunt the activation and recruitment of phagocytic cells, reduce fever, modify coagulation factor expression, and decrease production of reactive oxygen and nitrogen intermediates, NO, and other vasoactive mediators.[190-195]

Soluble cytokine and receptor antagonists produced during sepsis also modulate proinflammatory mediator action. Elevation of the levels of TNF receptor 2 (which regulates the concentration of TNF-α), soluble IL-6 receptor, soluble IL-2, and IL-1 receptor antagonist have been documented in neonatal sepsis with resolution after effective treatment.[189,196,197] The role of these regulatory cytokine inhibitors in the immune response to neonatal sepsis and septic shock has been incompletely characterized. Soluble RAGE competes with cell-bound RAGE for the binding of HMGB-1 and other RAGE ligands.[198] Soluble RAGE has antiinflammatory effects and its level is elevated in adults during sepsis.[199] Furthermore, soluble RAGE improved survival and reduced inflammation when given to infected adult rodents.[200] Serum soluble triggering receptor expressed on myeloid cells 1 may reduce inflammatory signaling for triggering receptor expressed on myeloid cells 1, and predict mortality in preterm neonates.[201]

MicroRNAs may regulate inflammation at the level of gene expression via several putative mechanisms.[202] Several pilot studies in rodents and humans have demonstrated regulatory functions for microRNA in neonates.[203-208] The impact of regulatory microRNAs and their effects on the host inflammatory response in neonates with sepsis are unclear.

Endogenous cortisol is induced by proinflammatory cytokines and attenuates the intensity of SIRS associated with severe sepsis and septic shock.[209] The use of cortisol in adults with sepsis has been controversial.[210,211] Cortisol production in newborn infants is significantly increased early in shock.[212] However, very preterm neonates may have relative adrenal insufficiency that may contribute to hemodynamic instability and hypotension. Cortisol replacement may be critical in these infants and deserves further study.[213] It is important to note, however, that in children with septic shock, adjunctive corticosteroid therapy is associated with repression of gene programs corresponding to the adaptive immune system.[214]

THE IMPACT OF GENETICS IN SEPSIS-ASSOCIATED INFLAMMATORY SIGNALING

A twin study which assessed the frequency of infections among monoygotic and dizygotic prematurely born twins concluded that 49.0% (p = .002) of the variance in susceptibility to late-onset sepsis was due to genetic factors alone.[215] The impact of genetics in the host response is also underscored by the increased risk for death from infection seen with African American race or male sex among low-birth-weight infants.[216] An ethnically unique single nucleotide polymorphism in the *TLR4* promoter region was significantly associated with gram-negative bacterial infections in preterm infants.[217] Several recent studies in newborn infants have demonstrated an association between small variations in DNA, specifically single nucleotide polymorphisms, and infection development and outcomes.[122,218-222]

Because TLRs play an essential role in recognition and response to pathogens, alterations in their expression, structure, signaling pathways, and function can have consequences for host defense. Polymorphisms or mutations in TLRs are associated with increased risk for infection in adults[223-226] and children[210,227,228] but are less well characterized in neonates. After confounders had been controlled for, the presence of a *TLR4* single nucleotide polymorphism was associated with a three-fold increase in the risk for gram-negative infections in VLBW infants.[222] Polymorphisms in the *TLR2*, *TLR5*, *IL10*, and *PLA2G2A* (which encodes PLA_2) genes were associated with the development of neonatal sepsis.[218]

Modifications in expression or function of costimulatory molecules necessary for TLR activation are also associated with an increased risk for infection. For example, the levels of LPS-binding protein (LBP; which binds intravascular LPS) and the LPS coreceptor CD14 are both increased during neonatal sepsis.[211,229,230] Furthermore, genetic variations in these proteins have been associated with increased risk for sepsis in adults.[231-233] Genetic polymorphisms in myeloid differentiation factor 2, a small protein involved in LPS signaling through TLR4, increase the risk for organ dysfunction and sepsis in adults[234] but the significance in neonates is unknown. Polymorphisms in post-TLR activation intracellular signaling molecules, including myeloid differentiation factor 88,[235] IL-1 receptor–associated kinase 4,[236] and NF-κB essential modulator,[237] are associated with invasive bacterial infection in older populations. Additional genetic polymorphisms in intracellular second-messenger inflammatory signaling systems with impact on neonatal sepsis risk and progression are likely to be uncovered with the implementation of biobanking and mining of stored samples.

Mutations have been identified in NLRs that are involved in the pathogenesis of Crohn's disease (*NOD2*)[238] and neonatal-onset multisystem inflammatory disease (*NLRP3*).[239] RLR mutations have been identified but have unknown clinical significance.[240] No mutations in specific domains of NLRs have been found in neonates with sepsis or NEC.[220,231-233,241-252] The importance of NLRs in *Listeria monocytogenes* infections in neonates is unknown.

INFLAMMATORY RESPONSE PROTEINS

COMPLEMENT

Complement is an important component of early innate immunity that facilitates killing of bacteria through opsonization and direct microbicidal activity. Complement components also possess chemotactic or anaphylactic activity that increases leukocyte aggregation and local vascular permeability. Furthermore, complement reciprocally activates a number of other important processes, such as coagulation, proinflammatory

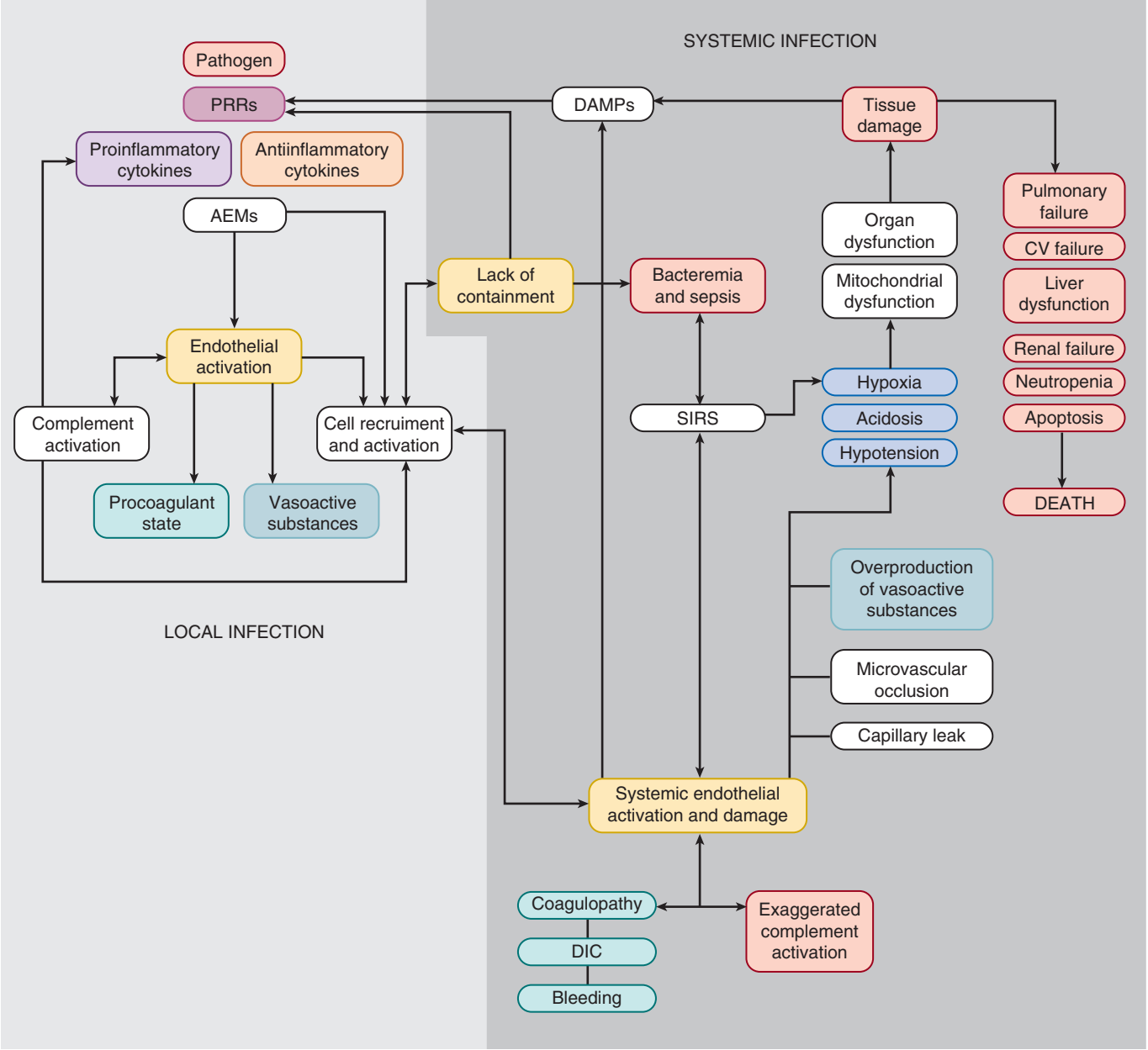

Figure 152-5 Pathophysiology of neonatal sepsis and septic shock. *AEMs,* Antimicrobial effector mechanisms; *CV,* cardiovascular; *DAMPs,* damage-associated molecular patterns; *DIC,* disseminated intravascular coagulation; *PRRs,* pattern recognition receptors; *SIRS,* systemic inflammatory response syndrome. (From Wynn JL, Wong HR: Pathophysiology and treatment of septic shock in neonates. *Clin Perinatol* 37(2):439–479, 2010.)

cytokine production, and leukocyte activation.[104] Contrary to its name, the *alternative* pathway is the primary mechanism of amplification of complement activation after C3 convertase assembly (which cleaves C3 to C3a and C3b). Dysregulation of complement activation may contribute to adverse effects in individuals with severe sepsis or septic shock. Neonates, particularly the very premature, exhibit decreased basal levels of complement proteins and function for both the alternative pathway and the classical pathway.[253,254] Moreover, as compared with adults, neonates exhibit gestational age–related degrees of depressed complement-mediated opsonic capabilities.[255] As such, complement-mediated opsonization is poor in premature neonates and limited in term neonates.[255,256]

Complement-mediated activation of leukocytes during sepsis occurs via up-regulated cell surface receptors (complement receptor 1 [CD35] and CR3).[257,258] C3b and C5a facilitate opsonization (primarily C3b), redistribute blood flow, and increase inflammation, platelet aggregation, and release of reactive oxygen intermediates (primarily C5a).[259,260] C5a-mediated local leukocyte activation also results in increased cytokine production with subsequent up-regulation of adhesion molecules on vascular endothelium and increased cell recruitment to the site of infection.[261] Data in adults link elevated C5a levels with multiple facets of sepsis-associated disease, such as DIC, cardiac dysfunction, increased proinflammatory cytokine levels, SIRS, apoptosis of adrenal medullary cells leading to adrenal

insufficiency, and PMN dysfunction.[104] Septic shock in adult humans was associated with extensive complement activation, C-reactive protein–dependent loss of C5a receptor on neutrophils, and the appearance of circulating C5a receptor in serum, which correlated with a poor outcome.[262] Deficiencies in C5a receptor found in term neonates as compared with adults may limit the ability to respond to C5a and therefore increase the likelihood of infection.[240] The expression of C5a receptor on preterm PMNs is unknown. The extent to which C5a or other complement proteins play a role in the development of disease in septic neonates remains to be determined.

Complement regulatory proteins modify the effects of complement and prevent potential damage due to overactivation. In particular, CD59 blocks C9 polymerization and target lysis, CD55 destabilizes CD35 and C3 and C5 convertases, and CD35 accelerates the deactivation of C3b.[263] Dysregulation of complement activation can lead to a vicious activation cycle that results in excessive cellular stimulation, cytokine production, endothelial cell activation, and local tissue damage promulgating SIRS and septic shock (see Figure 152-5).[264]

ACUTE-PHASE REACTANTS

In addition to the initial inflammatory response including complement activation, molecular detection of PAMPs promotes IL-1β and IL-6 production, which in turn increases the production of multiple other innate proteins that possess valuable immune function and serve to reduce pathogen load.[265] Acute-phase reactant proteins, produced predominantly in the liver, include C-reactive protein (opsonin), serum amyloid A (cellular recruitment), lactoferrin (reduces the level of available iron/antimicrobial peptide lactoferricin), procalcitonin (unknown function), haptoglobin, fibronectin (opsonic function), pentraxin 3 (binds C1q and activates the classical complement pathway), MBL, and LPS-binding protein.[127,211,229,265-270] Acute-phase reactant proteins have been studied in neonates with sepsis primarily to assess them for diagnostic utility rather than immunologic function. In particular, elevated plasma concentrations of C-reactive protein and LPS-binding protein are often associated with early-onset sepsis.[229,271] The levels of IL-10 and C-reactive protein were significantly higher in preterm infants who did not survive sepsis, pneumonia, or NEC.[272] A lack of sustained increase in the production of C-reactive protein and serum amyloid A during sepsis has also been associated with a fulminant course.[273]

PASSIVE IMMUNOGLOBULIN

The fetus receives antibodies from the mother via active placental transfer, with a significant increase beginning around 20 weeks' gestation. As a result of a shorter period of gestation, preterm neonates have lower IgG subclass levels as compared with term neonates, particularly IgG1 and IgG2 subclasses.[274] Preterm neonates (24 to 32 weeks' gestation) with low IgG levels (serum total IgG levels below 400 mg/dL at birth) were at increased risk for development of late-onset sepsis but not death compared to those with levels above 400 mg/dL. However, IgG titers and opsonic activity to CoNS were not predictive of late-onset CoNS sepsis.[275] Reliance on other means of innate immune defense likely provides the premature neonate with alternative microbial control mechanisms. Despite the presence of maternally derived immunoglobulin and acute-phase reactant proteins, neonates exhibit impaired opsonizing activity compared with adults, which likely increases the risk for progression of infection.[276] Complement plays a critical role in immunoglobulin-mediated opsonization and effector cell phagocytosis.[277] Although immunoglobulin has many putative beneficial immunologic functions, most of these have not been demonstrated or examined in preterm infants.[278] The dependence on complement for effective immunoglobulin-based opsonization and pathogen clearance may help explain the lack of efficacy of intravenous immunoglobulin to prevent sepsis or death from sepsis in neonates.[279-285]

ANTIMICROBIAL PROTEINS AND PEPTIDES

APPs are the most phylogenetically ancient means of innate immune defense against microbial invasion. Present in nearly every organism, including bacteria, plants, insects, nonmammalian vertebrates, and mammals, these small, often cationic peptides are capable of killing microbes of multiple types, including viruses, bacteria, parasites, and fungi, largely by disruption of the pathogen membrane.[286] Constitutive expression of APPs occurs in humans on barrier areas with consistent microbial exposure such as skin and mucosa. After microbial stimulation, both release of preformed APPs and inducible expression are thought to contribute to early host defense.[287] Importantly, there is no evidence for the development of microbial resistance to APPs that target fundamental components of the microbial cell wall. Some APPs can bind and neutralize microbial components such as endotoxin, precluding engagement with TLRs and other PRRs, and diminish inflammation. Many APPs can potentially reduce the intensity of the inflammatory response associated with the presence of bacterial toxins.[288-290] Because endotoxemia is an important contributor to neonatal MODS and death with sepsis and NEC,[84] LPS-binding/blocking strategies, including use of synthetic APPs, may have a significant positive impact on outcomes.[288,291]

Bactericidal/permeability-increasing protein (BPI) is a 55-kDa protein present in the respiratory tract, PMN primary granules, and plasma. BPI exerts selective cytotoxic, antiendotoxic, and opsonic activity against gram-negative bacteria.[288] Plasma BPI concentrations were higher in critically ill children with sepsis syndrome or organ system failure than in critically ill children without sepsis syndrome or organ system failure, and BPI levels positively correlated with the pediatric risk for death score.[265] PMNs from term neonates are deficient in BPI, potentially contributing to the increased risk for infection.[292] Whereas term neonates demonstrate up-regulation of plasma BPI during infection, premature neonates showed a decreased ability to mobilize BPI on stimulation,[293] which may contribute to their risk for infection with gram-negative bacteria. Polymorphisms in BPI increase the risk for gram-negative sepsis in children, but the impact of these polymorphisms in neonates is unknown.[294] Compared with PMNs from adults, PMNs from term neonates produce similar quantities of defensins but reduced quantities of BPI and elastase.[292,295,296] Recombinant BPI ($rBPI_{21}$) treatment was associated with improved functional outcome, reduced amputation, but no difference in mortality in a multicenter study of children with severe systemic meningococcal disease.[297]

Lactoferrin is the major whey protein in mammalian milk (in particularly high concentrations in colostrum) and is important in innate immune host defenses. Lactoferrin is present in tears and saliva and has antimicrobial activity both via binding iron and by direct membrane disruption activity via a portion of its amino-terminal lactoferricin.[298] Lactoferrin is also an alarmin (e.g., HMGB-1 or IL-33), capable of activating leukocytes, binding endotoxin, and modifying the host response by acting as a transcription factor that regulates mRNA decay.[299,300] Bovine lactoferrin has been shown to reduce the incidence of bacterial and fungal sepsis[301,302] and NEC in preterm infants.[303]

Lysozyme is present in tears, tracheal aspirates, skin, and PMN primary and secondary granules and contributes to degradation of peptidoglycan in bacterial cell walls. Secretory PLA_2 can destroy gram-positive bacteria through hydrolysis of their membrane lipids.[174] PMN elastase is a serine protease released by activated PMNs with microbicidal function and is believed to play a role in the inflammatory damage seen with PMN recruitment, particularly in the lung.[116,136] Cathelicidin and the

defensins are other APPs that possess antimicrobial properties.[304] Cathelicidin is present in the amniotic fluid, vernix, skin, saliva, respiratory tract, and leukocytes. α-Defensins are cysteine-rich 4-kDa peptides found in amniotic fluid, vernix, spleen, cornea, thymus, Paneth cells, and leukocytes. β-Defensins are found in the skin, GI tract, urinary system, reproductive organs (placenta, uterus, testes, kidney), respiratory tract, breast milk, mammary gland, and thymus.

In addition to microbicidal action, APPs have a wide range of immunomodulatory effects on multiple cell types from both the innate immune system and the adaptive immune system.[287,305,306] These immunomodulatory effects include altered cytokine and chemokine production, improved cellular chemotaxis and recruitment, improved cell function (maturation, activation, phagocytosis, reactive oxygen intermediate production), enhancement of wound healing (neovascularization, mitogenesis), and decreased apoptosis.

The cytosolic granules of PMN are rich in APPs, including α-defensins, lactoferrin, lysozyme, cathelicidin, soluble PLA_2, and BPI. Gestational age–related decreases in the umbilical cord blood concentration of several APPs (cathelicidin, BPI, calprotectin, soluble PLA_2, α-defensins) in comparison with maternal serum levels have been drescibed.[307] Plasma APP deficiencies may contribute to the increased risk for infection associated with prematurity, and their absence may increase the risk for endotoxemia. Compared with term neonates, preterm neonates showed lower human β-defensin 2 levels in umbilical cord blood.[308] Up-regulation of APPs (defensins) occurs in blood of infected adults[309] and children (defensins, lactoferrin).[310] The effect of sepsis on the production of plasma APPs in neonates has not been investigated in detail.

COAGULATION

The development of a procoagulant state in the surrounding microvasculature allows the trapping of invading pathogens and prevents further dissemination (see Figure 152-5). In general, the intrinsic pathway amplifies coagulation after initiation by the extrinsic pathway.[311] Reduced levels of vitamin K–dependent factors (factors II, VII, IX, and X), reduced thrombin generation, reduced consumption of platelets with formation of microthrombi, and reduced levels of counterregulatory elements (inhibitors) increase the risk for bleeding in infants and children.[312] During sepsis, a microvascular procoagulant state develops via stimulation of phagocytes, platelets, and endothelium, resulting in expression of tissue factor.[313,314] Tissue factor–mediated activation of the coagulation cascade results in activation of thrombin-antithrombin complex, plasminogen activator inhibitor type 1, and plasmin-α_2-antiplasmin complex,[315] as well as inactivation of protein S and depletion of the anticoagulant proteins antithrombin III and protein C.[316-318] Decreased activated protein C levels were associated with increased risk for death from sepsis in preterm neonates.[319] A randomized controlled trial of activated protein C revealed no change in mortality among pediatric patients with sepsis, but term infants younger than 60 days old experienced increased bleeding.[320]

The coagulation cascade is intimately tied to inflammation and complement activation.[104] Cytokine production increases expression of endothelial tissue plasminogen activator inhibitor type 1. Plasminogen activator inhibitor type 1 inhibits fibrinolysis by inhibiting the conversion of plasminogen to plasmin, which in turn is important for the breakdown of fibrin. Deposition of fibrin in small vessels leads to inadequate tissue perfusion and organ failure.[321] Increased plasminogen activator inhibitor type 1 levels are associated with increased IL-6, nitrite, and nitrate levels (metabolites of NO production), the development of organ failure, and increased mortality.[321] Sepsis is associated with thrombocytopenia in neonates,[322] which is attributed to reduced megakaryopoiesis in the setting of consumption with clot formation.[323] Decreased platelet function in preterm neonates with sepsis further increases the risk for bleeding.[324] In extremely low-birth-weight infants, platelets are hyporeactive for the first few days after birth, complicating the ability of the immune system to contain a microbiologic threat and increasing the risk for hemorrhage.[325] Clotting can lead to propagation of inflammation via thrombin-induced production of platelet-activating factor. PMNs activated by platelet-activating factor or platelet TLR4 may then contribute to further endothelial injury and dysfunction, leading to the development of a vicious clotting-inflammation-clotting cycle. Activated platelets may be consumed in clot formation and/or may also be removed from the circulation by the liver,[326] potentially resulting in thrombocytopenia, particularly during gram-negative and fungal infections.[196,322,327]

Systemic activation of coagulation is associated with consumption of clotting factors and increased risk for bleeding, prolonged proinflammatory responses, and DIC.[128,149,328] This finding is consistent with the elevated serum levels of IL-6[52] and high frequency of DIC seen with disseminated herpes simplex virus infection.[329] In adult mice, protease-activated receptor 1 plays a major role in orchestrating the interplay between coagulation and inflammation.[330] Protease-activated receptor 1 may modify the endothelial response during neonatal sepsis and thus is a target for therapeutic intervention.

ROLE OF VASCULAR ENDOTHELIUM

Recent studies have shown the critical importance of vascular endothelial activation in the early recognition and containment of microbial invasion. In transgenic mice, it was shown that pulmonary endothelial cells sense blood-borne bacteria and their products,[156] whereas alveolar macrophages patrol the air spaces.[331] These data illustrate the role of endothelium and help to explain in part the occurrence of acute respiratory distress syndrome (ARDS) and persistent pulmonary hypertension of the newborn associated with severe sepsis in the absence of a primary pulmonary infectious focus. Expression of TLRs allows endothelium to become activated in the presence of microbial components, leading to production of cytokines, chemokines, and adhesion molecules (e.g., vascular cell adhesion molecule, intercellular cell adhesion molecule, L-selectin, P-selectin, and E-selectin). These substances are all necessary to attract immune cells (primarily PMNs) to the site of infection and to facilitate pathogen containment.[150-153,156] Vasoactive substances released from activated leukocytes, platelets, and endothelial cells include platelet-activating factor, thromboxanes, leukotrienes, NO, histamine, bradykinin, and prostaglandins.[172,173] The balance of NO and endothelin 1, a vasoconstrictor, may be disrupted with endothelial damage, favoring the constrictive effects of endothelin 1 and leading to ischemia and injury.[175] This phenomenon may explain in part why NO inhibitors increased mortality in adults with septic shock.[301] Stimulated endothelium can be a double-edged sword, however, because excessive activation can lead to systemic overproduction of cytokines and vasoactive substances (including NO). Endothelial cell apoptosis, detachment from the lamina, and alterations in vascular tone combine to promote capillary leak, leading to hypovolemia, shock, and organ failure[156,302,303] (see Figure 152-5). Release of myeloperoxidase from PMNs may also injure surrounding endothelium.[332] Activated or damaged endothelium establishes a prothrombotic environment that can result in local microvascular occlusion[314] or progress to DIC.[333]

The glucocorticoid receptor is the target for cortisol, the primary endogenous glucocorticoid in humans, produced in the zona fasciculata of the adrenal glands. Endothelial glucocorticoid

receptor is a critical negative regulator of inducible NO synthase expression and NF-κB activation,[334] demonstrating a protective role of the endothelium during sepsis. Studies have revealed a potential role of plasma angiopoietin during pediatric septic shock.[335] The level of angiopoietin 1, which protects against vascular leak, was reduced, whereas the level of angiopoietin 2, which promotes vascular permeability, was elevated, highlighting a novel potential therapeutic opportunity to reduce end-organ injury. The roles for endothelial glucocorticoid receptor and angiopoietin 1 in neonatal sepsis are unknown.

The role of endothelium activation during sepsis and septic shock in neonates, particularly in premature neonates, has been less well investigated. Toxins from GBS have been shown to damage pulmonary endothelium[336] and likely participate in pulmonary complications associated with GBS pneumonia such as ARDS and the development of persistent pulmonary hypertension of the newborn.[337] The levels of the adhesion molecules E-selectin and P-selectin, expressed and secreted by activated endothelium, are increased in the serum of neonates with sepsis[136] and likely reflect significant endothelial activation. Endothelial TLR4 activation impaired intestinal perfusion in an experimental model of NEC, via endothelial NO synthase–nitrite–NO signaling.[338]

INNATE IMMUNE CELLULAR CONTRIBUTIONS

The PMN is the primary effector of innate immune cellular defense. Endothelial cells produce activating cytokines and chemokine gradients that recruit circulating PMNs to the site of infection. Expression of cell adhesion molecules by PMNs and endothelium allows cells to roll and extravasate into surrounding tissues. Activated PMNs phagocytose and kill pathogens via oxygen-dependent and oxygen-independent mechanisms. IL-1β is produced by activated PMNs largely via an NLRP3-ASC-caspase 1–dependent* mechanism that amplifies the recruitment of additional PMNs from the bone marrow to the site of infection.[339]

Activated PMNs may release reactive nitrogen species, reactive oxygen species, and proteolytic enzymes via activation of membrane-associated NADPH oxidase. These reactive intermediates and enzymes can lead to destruction of nonphagocytosed bacteria but can also cause local tissue destruction, including neonatal endothelial and lung injury, as well as surfactant inactivation,[116,340] and thus play a role in progression from sepsis to MODS.

Neonatal PMNs exhibit quantitative and qualitative deficits as compared with adult PMNs.[295,341] Respiratory burst activity is suppressed in PMNs during neonatal sepsis and may contribute to poor microbicidal activity.[342-344] Compared with adult PMNs, neonatal PMNs exhibit delayed apoptosis,[345,346] as well as sustained capacity for activation (CD11b up-regulation) and cytotoxic function (reactive oxygen intermediate production) that contributes to tissue damage.[347] Reduced apoptosis with prolonged survival of PMNs may result in improved bacterial clearance but may also paradoxically increase the risk for sustained PMN-mediated tissue damage. Increased serum PMN elastase, urokinase plasminogen activator, and urokinase plasminogen activator receptor levels are found at the time of presentation in infected neonates.[136]

With PMN death, DNA (chromatin), histones, and APPs are expelled into the environment and serve to trap bacteria (neutrophil extracellular traps [NETs]).[348] The formation of NETs can occur after activation of platelet TLR4[349] and may lead to excessive local inflammation and tissue damage.[350] High early levels of circulating free PMN-derived DNA produced by NETs are associated with MODS and death.[351] NETs contain destructive proteases capable of killing bacteria even after the PMN has died.[352] Formation of NETs is reduced in PMNs from preterm neonates and nearly absent in term neonates[353] but may occur with sustained cellular stimulation.[354] NET formation may result in collateral damage to surrounding tissues when the target microbe is too large to be effectively phagocytosed (e.g., fungal hyphae).[355] The contribution of NET production to detrimental outcomes in infected neonates is unknown but excessive NET formation with collateral tissue injury may contribute to the poor outcomes seen in preterm neonates with fungal infections.[356]

Rapid depletion of bone marrow PMN reserves during infection, particularly in neonates,[357] can lead to neutropenia, with consequent impaired antimicrobial defenses and significantly increased risk for death.[358] In a multivariate analysis, neutropenia and metabolic acidosis were associated with fatal neonatal sepsis.[359] Neutropenia is particularly common in gram-negative sepsis in neonates.[360] Release of immature PMN forms (bands), which exhibit greater dysfunction than mature PMNs,[361] may further predispose to adverse outcomes. Murine neonates with experimental sepsis exhibit delayed emergency myelopoiesis (a process by which the host repopulates peripheral myeloid cells lost early during sepsis), that is independent of TRIF and myeloid differentiation factor 88.[362] Interventions aimed at addressing reduced PMN numbers in neonates have included provision of mature PMNs[363] and prophylaxis or treatment with colony-stimulating factors (granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor). Despite strong biologic plausibility, these interventions have been unsuccessful at reducing the neonatal infectious burden.[364-366] In a metaanalysis, treatment with colony-stimulating factor therapy (granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor) in a subgroup ($n = 97$) of neutropenic neonates (absolute neutrophil count less than 1700/μL) with culture-positive sepsis (largely gram-negative and GBS) significantly reduced the risk for death (relative risk, 0.34; 95% CI, 0.12 to 0.92).[365] Therefore, stimulation of granulopoiesis may be beneficial under these specific circumstances, although further studies focused on this subpopulation and outcomes are needed.

Irreversible aggregation and accumulation of newborn PMNs in the vascular space after stimulation leads to decreased diapedesis, rapid depletion of bone marrow reserves, vascular crowding,[367] and increased likelihood of microvascular occlusion.[368] Neonatal PMN deformation compared with adult PMN deformation is reduced at the baseline, which increases the risk for occlusion.[367] Furthermore, low blood pressure/flow states seen during septic shock further exacerbate existing microvascular ischemia.[295] In combination, these deficiencies increase the propensity for systemic spread of infection, and set the stage for microvascular occlusion.

OTHER INNATE CELLULAR CONTRIBUTIONS

Many other cells besides PMNs are involved in the development of an immune response to infection. Monocytes, macrophages, and dendritic cells amplify cellular recruitment through production of inflammatory mediators, activation of endothelium, phagocytosis and killing of pathogens, and antigen presentation to T and B cells of the adaptive immune system. The primary functions of monocytes are the synthesis of crucial inflammatory proteins[369] and antigen presentation to naïve $CD4^+$ T cells.[370]

The patterns of cytokine production can promote the differentiation of naïve $CD4^+$ T cells into distinct subtypes of T cells that serve important roles in the clearance of pathogens. For example, T-helper 1 (T_H1) cells are produced from naïve $CD4^+$ T cells after exposure to IFN-γ and IL-12, and support cell-mediated immunity against intracellular pathogens through production of IFN-γ, TNF-α, and lymphotoxin. T-helper 2(T_H2) cells

*ASC, Apoptosis-associated speck-like protein containing a carboxy-terminal CARD.

arise in the presence of IL-2 and IL-4, produce IL-4, IL-5, and IL-13, down-regulate T_H1 responses, and support humoral immunity, as well as defense against extracellular parasites. A third subset of T_H cells, T-helper 17 cells, are generated in the presence of transforming growth factor β, IL-6, IL-21, and IL-23. These cells produce IL-17 and IL-22, which are important for defense against extracellular bacteria and fungi. Neonatal mononuclear cells exhibit a bias away from T_H1 cell–polarizing activity because of increased IL-6 and low TNF-α production.[371] This may be beneficial because of mobilization of antiinfective proteins/peptides that serve to protect the newborn during microbial colonization[266] and development of immune tolerance.[341] The adverse consequence is a reduced ability to respond to infection with microorganisms; particularly intracellular pathogens such as *Listeria* sp.[372] and mycobacteria.[373] Preterm infants (<30 weeks) may have greater attenuation of TNF-α and IL-6 secretion compared with term infants and adults.[277]

There is decreased monocytic recruitment to areas of inflammation during sepsis because of decreased chemotactic ability.[374] Although the levels of peripheral monocytes decrease early during sepsis (between 60 nd 120 hours), secondary to extravasation and differentiation into macrophages, sepsis-related elevation of macrophage colony-stimulating factor[375] results in a late increase in the number of peripheral monocytes (>120 hours).[376] In addition to altered cytokine production and suboptimal recruitment, monocyte phagocytic function is reduced during sepsis.[377] Antigen presentation to naïve $CD4^+$ T cells is an important immune function performed by monocytes. The decreased antigen-presenting function in monocytes from newborn infants is in part due to decreased MHC class 2 molecule expression[378] and decreased expression of costimulatory molecules, including CD86 and CD40.[379]

Monocytes leave the bloodstream, enter the tissues, and differentiate into macrophages and dendritic cells. Monocytes and macrophages are closely related to PMNs (common myeloid progenitor) and can kill pathogens by similar means. Circulating monocytes differentiate into macrophages after exposure to maturing cytokines, and exit the bloodstream into tissues. Important substances produced by stimulated monocytes/macrophages include complement components, cytokines (both proinflammatory and antiinflammatory), coagulation factors, and extracellular matrix proteins.[369] Located just below epithelial borders, macrophages encounter pathogens immediately after entry. Macrophages are avidly phagocytic and generate APPs to reduce bacterial burden, such as lactoferrin, defensins, transferrin, and lysozyme. In addition, macrophages play an important role in the amplification of the immune response through the production of cytokines and chemokines, as well as in antigen presentation to naïve $CD4^+$ T cells. Macrophages are poorly responsive to several TLR agonists.[380]

Dendritic cells are antigen-presenting cells that function as a liaison between the innate immune system and the adaptive immune system through induction of antigen-specific T cell–mediated immunity. Dendritic cells from newborn infants exhibit a reduced antigen-presenting function when compared with adult cells[379] and require increased stimulation for activation.[381] Evaluations of neonatal dendritic cell function suggest a tendency towards poor up-regulation of costimulatory molecules (CD80/CD86) and activation markers (CD83), poor stimulation of T-cell proliferation, and a tendency towards the induction of immune tolerance.[382] Although preterm and term infants and adults have similar numbers of "plasmacytoid" dendritic cells in their blood, the capacity to produce IFN-α on TLR9 challenge was significantly decreased in preterm neonates and may increase susceptibility to viral infections.[383] Dendritic cells in umbilical cord blood can effectively induce cytotoxic lymphocyte responses.[384] Depletion of dendritic cells has been reported in adult animals[385] and adult patients[386] with sepsis; their role in the immune response to neonatal sepsis is not well characterized.

Eosinophils phagocytose antigen-antibody complexes and release cytokines, chemokines, cytotoxic molecules, APPs, and other substances (prostaglandins, thromboxanes, leukotrienes) when stimulated.[387] Eosinophilia is commonly observed in neonates with sepsis due to *Candida* sp.[388] and bacteria,[389] and is seen in infants with NEC.[387] In infants of less than 26 weeks' gestation, eosinophilia (absolute eosinophil count more than $1000/mm^3$) may predict bacterial sepsis.[389] Eosinophilia in premature infants is not associated with production of IgE.[390] Studies have demonstrated an integral role for eosinophils in adult intestinal integrity and revealed a novel innate bactericidal nonphagocytic function via extracellular catapulting of mitochondrial DNA nets with associated bound toxic proteins.[391] The precise role of eosinophils in the neonatal immune response to sepsis and in maintenance of intestinal integrity has yet to be determined.

Mast cells play a role in the response to pathogen invasion as a part of the innate cellular immune system via production of histamines (which promote vasodilation and up-regulation of P-selectin), cytokines, PMN recruitment, bacterial phagocytosis, and antigen presentation.[392,393] Mast cell involvement was demonstrated in infants with erythema toxicum, where mast cell recruitment, degranulation, and expression of APPs occurs.[394] Adult rodents deficient in mast cells exhibit impaired PMN influx,[395] impaired clearance of enteric organisms, and decreased sepsis survival.[396] Mast cell production of histamine likely contributes to the vasodilation associated with sepsis and septic shock. Like eosinophils and PMNs, mast cells are capable of killing bacteria via generation of extracellular traps in adults.[397] This means of immune protection has not been investigated in neonates. Mast cells may also alter adaptive immune function by patterning the T_H2 immunophenotype seen in the neonate and therefore contribute to the increased risk for infection. Immature dendritic cells exposed to histamine during maturation (with LPS) exhibit altered T-cell polarizing activity with predominance towards the T_H2 phenotype via increased production of IL-10 and decreased production of IL-2.[398] Furthermore, mast cells from neonates were shown to secrete significantly more histamine after stimulation as compared with adults,[399] which may contribute to the development of shock.[400]

The role of natural killer (NK) cells in neonatal bacterial sepsis is incompletely defined. NK cell numbers increase with increasing gestational age,[401] Furthermore, a reduced percentage of NK cells present at birth may be a risk factor for late-onset sepsis in preterm infants.[402] It is noteworthy that the numbers of circulating NK cells are not significantly different in neonates with or without infection[370,403]; however, the numbers of circulating NK cells are decreased in newborn infants with shock.[404] The mechanisms used by NK cells to destroy bacteria include secretion of APPs (defensins), direct contact and lysis, antibody-dependent cellular cytotoxicity, and IFN-γ production.[405] In neonates with bacterial sepsis, NK cells are activated, as evidenced by up-regulation of CD69.[2,406] Despite activation, NK cell cytotoxicity is deficient in infants with sepsis and recurrent infections.[370,405] Although neonatal macrophages exhibit impaired baseline activation in response to IFN-γ,[341] NK cell–mediated production of IFN-γ can enhance their phagocytic capability. Further studies are necessary to more clearly define the role of NK cells in neonatal bacterial sepsis.

$CD71^+Ter119^+$ (erythroid) cells may contribute to the increased susceptibility of the neonate to infection by reducing the inflammatory response associated with bacterial colonization of the gut. For example, ex vivo TNF-α production by stimulated adult effector cells was reduced in the presence of murine neonatal splenic $CD71^+$ erythroid cells via an arginase 2–dependnent mechanism.[407] The $CD71^+$ erythroid population represents a large portion of murine fetal liver, neonatal spleen/bone marrow, and adult bone marrow.[408-410] Furthermore, the murine neonatal spleen contains large numbers of colony-forming progenitor cells for 2 to 3 weeks after birth.[411] Of note and in stark contrast

to the lymphoid and reticuloendothelial system roles of the spleen in the healthy adult, the spleen is normally a major site of erythropoiesis during fetal and neonatal life, to support rapid fetal and postnatal growth in the setting of significantly reduced erythroid reservoirs as compared with the adult reservoirs.[410,412,413] A lack of effect on neonatal murine survival to polymicrobial sepsis after adoptive transfer or diminution of $CD71^+$ erythroid splenocytes suggests that the impact of these cells on neonatal infection risk and progression may be limited.[414]

CONTRIBUTIONS OF THE ADAPTIVE IMMUNE SYSTEM

The contribution of the adaptive immune system in the neonatal host response to sepsis is uncertain. The 5- to 7-day interval required for development of an adaptive immune response—namely, the selection and amplification of specific clones of lymphocytes (B cells and T cells) that results in immunologic memory—argues against a central role for adaptive immunity in the protective response to early neonatal *bacterial* sepsis. As a result, the neonate is thought to largely depend on innate immunity for protection from infection during the first days of life. In adults, absence or dysfunction of the adaptive immune system has a profound impact on survival in preclinical models.[415] B cells (and in particular B-cell cytokine production) and not T cells were shown to be important in the early host response to experimental sepsis.[416] Studies using neonatal mice lacking an adaptive immune system showed no difference in polymicrobial sepsis survival as compared with survival of wild-type mice with an intact adaptive immune system.[3] Furthermore, there are many quantitative and qualitative differences in lymphocytes from neonates compared with lymphocytes from adults,[417] each with a respective proposed clinical impact.[87] As these findings illustrate, the contribution of adaptive immunity for protection and response against sepsis, and in particular which components are protective, is unclear in the most immature and requires further investigation.

Peripheral blood examination has yielded inconsistent changes in the percentage, number, and type of circulating lymphocytes during sepsis.[403,418-423] Moreover, changes related to the timing of sepsis onset (early-onset or late-onset sepsis) and prematurity have been incompletely characterized. T regulatory cells are abundant and potent at birth, facilitating inhibition of T_H1 cell immunity,[424] and perhaps mediating a state of immunologic tolerance.[425] Although the numbers of splenic T regulatory cells are increased in murine neonates and adults with sepsis, depletion of T regulatory cells had no effect on survival of murine adults.[2,426] Alterations in the number or function of T regulatory cells in human neonatal sepsis have not been reported.

Examination of peripheral blood to identify markers of sepsis has yielded a number of lymphocyte cell-surface molecules whose levels increase during sepsis. Activation of neonatal T cells is evidenced by increased CD45RO expression (present on T cells after antigenic stimulation) at the time of sepsis diagnosis,[419,427,428] and with congenital infection,[429] although changes in number may take several days to occur after stimulation.[430] Other markers of lymphocyte activation may be found at different time points during the course of infection. For example, expression of the activation marker CD69 is increased on T cells ($CD4^+$) early in the infectious process, whereas CD25 and CD45RO expression persists for several days.[406] Increased expression of $CD4^+$ T-cell carcinoembryonic antigen–related cell adhesion molecule 1 (CD66a) in preterm infants with late-onset sepsis may contribute to sepsis-associated immune suppression.[431] HLA-DR expression is increased on multiple cell types during neonatal sepsis.[406] In contrast to adults, a large portion of neonatal T cells produce CXCL8, which activates PMN and γδ T cells.[432] These data show that neonatal T cells are activated and are capable of playing a role in the host response to bacterial sepsis in vivo.

Neonatal lymphocyte function is skewed towards T_H2 responses, setting the stage for immune tolerance (T_H2) rather than immune priming for infection (T_H1).[417] Newborn infants must overcome that immune modulation in order to mount effective responses to specific infectious challenges and respond to vaccination. Examples of the impact of this immunopolarization include decreased IFN-γ production by $CD4^+$ and $CD8^+$ T cells as compared with production in children and adults.[433,434] The likely significance of decreased IFN-γ production is a reduction in activation of other immune cells, such as macrophages.

Reports of lymphocyte function in infected newborns are very limited. Expansion of lymphocytes after antigenic stimulation is important for development of sustained immunity. Decreased lymphocyte proliferative responses have been shown during the first 8 weeks of life in VLBW neonates,[421] and may predispose the premature neonate to development of late-onset sepsis. For example, T-lymphocyte function was depressed in infected newborn infants, and especially in those with multiorgan failure, versus healthy term or growing preterm infants.[435] Similarly, production of lymphocyte-associated cytokines after stimulation of umbilical cord blood peripheral blood mononuclear cells with GBS was significantly deficient in preterm and term infants compared with adults.[277] Cytomegalovirus infection in utero leads to the expansion and differentiation of mature cytomegalovirus-specific $CD8^+$ T cells, which have characteristics similar to adult $CD8^+$ T cells.[436] These cells showed potent perforin-dependent cytolytic activity and produce antiviral cytokines, highlighting the potential for adult-like immunocompetence of neonatal T cells under specific circumstances.

An important location for effective lymphocytic function during systemic bacterial infection is the spleen. The marginal zone of the spleen facilitates the clearance of bacteria, particularly encapsulated organisms, from the bloodstream. These functions are accomplished via the interaction of multiple leukocytes, including macrophages, dendritic cells, B cells, and T cells, within follicles of the spleen. The neonatal splenic marginal zone is immature, owing to a lack of antecedent antigen exposure and is virtually devoid of $CD21^+$ B cells.[412] As a result of this functional asplenia, there is decreased clearance of pathogens from the blood and potential for a more fulminant course with bacteremia.[437,438]

B cells are critically important in the adult host response to sepsis. Data suggest antibody-independent and antibody-dependent roles for B cells in the outcome of sepsis.[416] Studies deciphering the role of B cells in neonatal sepsis are very limited, and thus the role B cells play in the neonatal host response is unclear. After GBS meningitis, the level of IgM was increased, suggesting B cells from neonates can respond to pathogenic challenge.[439] Premature neonates with perinatal infection or nosocomial infection may show signs of humoral immunoparalysis, manifested by decreased IgM/IgG production ex vivo as compared with production in their healthy age-matched counterparts.[440] Sepsis in early life did not reduce serum antibody titers in preterm infants after heptavalent pneumococcal conjugate vaccine exposure but was associated with a reduced opsonization titer to a single serotype, suggesting the capacity to respond to vaccination or other immune challenge may be altered.[441]

In the setting of reduced classic adaptive immune function seen in early life as compared with the function in adults, innate lymphoid populations (which lack B cell receptor and T cell receptor) may play a significant role in protecting the neonate from infectious challenge.[442-448] Examples of innate lymphoid cells include γδ T cells, intestinal lymphoid cells, invariant NK T cells, mucosa-associated invariant T cells, and B1 cells.

Mechanistic investigations that fully explore the role of these newly discovered populations in the neonatal host response to sepsis are likely to uncover novel therapeutic opportunities.

IMPACT OF SYSTEMS BIOLOGY APPROACHES

Systems biology and the use of "omic" approaches have the potential to produce significant insights into the pathogenesis of sepsis. Genomic and proteomic approaches have yielded important data associated with septic shock in older populations.[449-457] The use of these modern techniques in the study of neonatal inflammation and response to pathogen challenge has only just begun.[136,198,458,459] The ability to profile genome-wide expression has significantly enhanced our understanding of the complexity of the host immune response to sepsis in children.[5,449,450,453,460] For example, genome-wide expression profiling revealed zinc homeostasis as an important feature of pediatric sepsis.[450,453,461] Prophylactic zinc supplementation reduced bacterial load and mortality in a murine model of peritoneal sepsis.[462] However, oral zinc supplementation did not alter mortality in neonates with probable sepsis.[463]

In a study of pediatric patients who met the criteria for septic shock,[6] a unique whole-blood transcriptomic response was found in neonates as compared with infants, toddlers, and school-age children. Neonates manifested the largest number of uniquely regulated genes, representing both innate and adaptive immune system pathways, and showed a predominance of down-regulated transcripts representing the adaptive immune system.[5] The number of up-regulated genes increased in proportion with developmental age. Investigation of the murine circulating leukocyte transcriptome revealed significant differences in the host immune response to sepsis across the age spectrum (neonate, young adult, elderly), *despite similar increases in mortality* among the neonates and elderly mice as compared with young adult mice.[1] These data underscore the impact of developmental age on the host immune response and suggest that therapeutics, which may have efficacy in older populations, may be ineffective in or possibly detrimental to neonates.

Because the transition to extrauterine life is associated with dramatic changes in physiology, the whole-blood transcriptome is likely to be quite different between both uninfected infants and in the host response to sepsis by timing after birth. Indeed, an unsupervised analysis of the whole-blood genome-wide transcriptome on prospectively collected peripheral blood samples from infants evaluated for sepsis revealed the major node of separation between groups (infected or uninfected) was the timing of evaluation relative to birth (early, lass than 3 days or late, more than 3 days).[464] Principal component analyses revealed significant differences between patients with early or late sepsis despite the presence of similar key immunologic pathway aberrations in both groups. This study highlights both the uninfected state and the host responses to sepsis are significantly affected by timing relative to birth.

A study of VLBW infants with blood culture–proven late-onset sepsis (59% CoNS) identified a 554-gene signature associated with sepsis, with increased expression of the TNF-α network, including matrix metalloproteinase 8 and CD177 among the most commonly up-regulated genes.[465] Elevated matrix metalloproteinase 8 mRNA expression and activity in septic shock correlated with decreased survival and increased organ failure in pediatric patients. Matrix metalloproteinase 8 is a direct activator of NF-κB.[466] Inhibition (genetic or pharmacologic) of matrix metalloproteinase 8 leads to improved survival and a blunted inflammatory profile in a murine model of sepsis. Most recently, a 52-gene network was uncovered and validated that accurately identified infected infants, who exhibited increased expression of genes for innate immune and metabolic pathways with decreased levels of adaptive immune transcripts.[467] Using a proteomics approach, Ng and colleagues[468] identified pro-apolipoprotein CII and a desarginine variant of serum amyloid A as promising biomarkers for late-onset sepsis and NEC in preterm infants.[468] It is very likely that implementation of unbiased "omic" approaches will reveal critical age-appropriate pathways and opportunities for therapeutic interventions aimed at improving neonatal sepsis outcomes.

SEPSIS-ASSOCIATED ORGAN FAILURE

Sepsis that leads to shock and organ failure carries the worst prognosis. SIRS contributes to the development of organ failure in neonates (see Figure 152-5).[52,148,181,469] Persistent decreases in capillary perfusion are associated with MODS and death in adults.[470] Lethargy, shock, and birth weight less than 1500 g were independent predictors of sepsis-related death.[471] In neonates, impairment of the cardiovascular system, manifested by poor perfusion or hypotension, is invariably associated with septic shock. Sustained poor organ perfusion in neonatal sepsis and septic shock due to cardiovascular dysfunction is associated with MODS affecting the kidney,[472,473] liver,[474] gut,[475] and central nervous system[476] (see Figure 152-5). The mechanism of organ failure may be decreased oxygen utilization associated with mitochondrial dysfunction rather than poor oxygen delivery to tissue.[477,478] On the basis of available evidence, it has been speculated that the prolonged SIRS associated with severe sepsis and shock leads to organ failure via a cessation of energy-consuming processes.[479,480] Development of severe NEC is also associated with severe sepsis, shock, MODS, and death.[84,481] The need for intubation or initiation of vasoactive medications, and hypoglycemia, thrombocytopenia, increased prothrombin time, or excessive bleeding as presenting laboratory signs of sepsis are risk factors for sepsis-related death.[359,475,482] Independent predictors of in-hospital late-onset sepsis death during the birth hospitalization were the presence of congenital anomalies (OR, 4.12; 95% CI, 1.60 to 10.60), neuromuscular comorbidities (OR, 3.34; 95% CI, 1.66 to 6.73), and secondary pulmonary hypertension with/without cor pulmonale (OR, 23.48; 95% CI, 5.96 to 92.49),[60] underscoring the impact of organ-level comorbidities that increase neonatal sepsis mortality.

CARDIOVASCULAR SYSTEM

The most common organ dysfunction associated with sepsis is cardiovascular dysfunction. Cardiovascular dysfunction associated with sepsis may lead to shock that is a composite of hypovolemic, cardiogenic, and distributive shock. Distributive shock is related to endothelial NO production that leads to excessive vasodilation. Cardiogenic shock may be related to mitochondrial death (induced by reactive nitrogen and reactive oxygen intermediates) with subsequent myocardial dysfunction. Abnormalities in peripheral vasoregulation and myocardial dysfunction may play a larger role in hemodynamic derangements in pediatric patients, especially infants and neonates.

In children, a non-hyperdynamic state with reduced cardiac output and increased systemic vascular resistance is most commonly observed in the setting of sepsis.[483-487] The hemodynamic presentation in neonates is much more variable[484] and is complicated by an unclear association between a normal blood pressure and adequate systemic blood flow.[488,489] Microcirculatory flow is impaired in term neonates even with mild to moderate severity of infection.[490] Preterm neonates with sepsis have relatively high left and right cardiac outputs and low systemic vascular resistances. However, a decrease in right or left ventricular output of more than 50% has been associated with increased mortality in neonatal sepsis.[491] An elevated left ventricular output

but normal ejection fraction in preterm neonates with septic shock suggests that septic shock in preterm neonates is predominantly due to vasoregulatory failure. Neonatal sepsis may or may not be associated with left ventricular diastolic dysfunction; however, cardiac injury as manifested by elevated levels of cardiac troponin T may complicate the clinical picture.[492,493] Abnormal peripheral vasoregulation with or without myocardial dysfunction is the primary mechanism for the hypotension accompanying septic shock in the neonate.[494] Therefore, infected neonates may present with hypotension and adequate perfusion (warm shock) or inadequate perfusion (cold shock). Myocardial dysfunction can lead to ventricular wall stretch that in turn elevates B-type natriuretic peptide levels. B-type natriuretic peptide levels are elevated in children with septic shock,[495] and increased levels have utility as prognostic indicators of death.[496] Plasma NO level is elevated in neonates with sepsis and shock compared wit those with shock alone.[182] Elevated serum lactate level (>3 mmol/L) distinguished nonsurvivors from survivors in a pediatric study that included neonates.[497]

IMMUNE SYSTEM

After severe sepsis or septic shock, there is an increased risk for subsequent infection and death in children and adults. This phenomenon is termed *immunoparalysis* and is associated with reduced MHC class 2 expression and TNF-α production by mononuclear cells after endotoxin stimulation. In addition to altered monocytic responses, there is significant loss of lymphoid $CD4^+$ T and B cells via caspase-dependent apoptotic pathways.[415,498] Whether by clonal selection, apoptosis, or elevated endogenous glucocorticoid levels,[499-501] lymphocyte loss may lead to a state of immune compromise after the acute phase of sepsis.[412,499,501-505] New data suggests that IL-7 may play an important role in promoting T-cell activation and prevention of apoptosis.[506] The importance of immunoparalysis has been convincingly demonstrated in infected adults[507-510] and children.[511] However, the clinical impact in the preterm neonate in whom adaptive immune function is less well developed is uncertain.[512,513]

In examinations of peripheral blood and postmortem spleens from infected adults, there is significant loss of B and $CD4^+$ T lymphocytes and dendritic cells,[386,498] resulting in decreased antigen presentation, antibody production, and macrophage activation.[514] Circulating peripheral absolute lymphocyte counts can drop significantly in adults with sepsis but this phenomena is also seen in critically ill adults who are not infected.[477] Sustained lymphopenia significantly increases the risk for secondary infection, MODS, and death in children.[505] Extensive loss of lymphocytes (both B and T lymphocytes) has been described in postmortem specimens from the thymus and spleen in infected preterm and term infants.[412,499,501-504] The number and the size of the follicles in the spleen decreased significantly and the total number of cells decreased by more than three times; similar changes were found in lymph nodes.[478] However, these histopathologic splenic findings are in contradiction to earlier reports where no differences were described in infected and uninfected infants.[412] Splenomegaly may occur in infants with late-onset sepsis and may be due to splenic congestion in the absence of hyperplasia of white pulp.[502]

The mechanisms responsible for immune alterations after sepsis are beginning to emerge. The intensity of the inflammatory response may be modified by neural-based mechanisms.[515] T cell–secreted acetylcholine acts on macrophages to reduce production of TNF, IL-1, IL-18, HMGB-1, and other cytokines.[516] The role of vagal tone in the neonatal host response to sepsis is unclear.

Discovery and characterization of the impact of epigenetic-mediated immune system functional alterations after sepsis is an area of intense research. DNA methylation and posttranslational modification of histone proteins (methylation, acetylation, phosphorylation, ubiquitination, SUMOylation) may occur after sepsis.[126,517] These DNA alterations may modify transcription factor access of gene-specific promoter regions, ultimately leading to short-term and long-term changes in gene expression and immune function. The DNA methylation pattern in the promoter region of the *CALCA* gene varied in different types of bacterial sepsis in preterm infants, suggesting its potential use as an epigenetic biomarker.[518]

Trained immunity, the term coined to describe an *adaptive* innate immune response, may also be a positive or negative consequence of sepsis in early life.[519] Mechanisms that underlie trained immunity are beginning to emerge, and include DNA methylation and modification of energy utilization pathways.[520,521] Nonspecific vaccine benefits and resistance to subsequent pathogen challenge after innate immune priming or previous infection are likely manifestations of trained immunity in neonates.[3,513,522] The cell types, extent, and duration of trained immunity-based modifications in neonates with sepsis have not been studied. Myeloid suppressor cells manifest immunosuppressive activity with sepsis[523] and were recently described in neonates. Myeloid suppressor cells are present at high frequency at birth and decline in number with postnatal age. They inhibit T-cell proliferative responses and IFN-γ production.[524] Reactivation of viral infection that may contribute to morbidity and mortality has been demonstrated in infected adults.[525] The impact of this phenomenon in neonates is unknown.

PULMONARY SYSTEM

Acute hypoxic respiratory failure, ARDS, and acute lung injury are common pulmonary complications associated with severe sepsis. Destruction of the alveolar capillary membrane leads to refractory hypoxemia. After direct or indirect insults to the lung, alveolar macrophages produce chemokines that mitigate PMN influx to lung parenchyma. Activated PMNs release reactive oxygen and reactive nitrogen intermediates that damage endothelial and epithelial barriers, leading to leakage of protein-rich edema fluid into the air spaces. Other pulmonary complications with severe sepsis may include secondary surfactant deficiency,[526] primary or secondary pneumonia,[527] and reactive pulmonary hypertension.[528,529] Infants with sepsis and persistent pulmonary hypertension of the newborn may require inhaled NO in addition to optimized ventilation strategies such as high-frequency oscillatory ventilation.[530] If oxygenation or tissue perfusion remains severely compromised despite optimal medical management, extracorporeal membrane oxygenation should be considered in neonates weighing more than 2 kg without contraindications.[531,532]

CENTRAL NERVOUS SYSTEM

The detrimental neurodevelopmental long-term impact of sepsis has been demonstrated in multiple studies and has been reviewed in detail.[533-537] Central nervous system injury is predominantly white-matter injury (loss of pre-oligodendrocytes), manifested by focal cystic periventricular leukomalacia, diffuse necrosis, or a combination of these entities.[538,539] Central nervous system injury is, in part, mediated by inflammation with or without direct pathogen invasion.[141,540,541] The impact of sepsis on central nervous system injury is intensified with lower gestational ages, highlighting the detrimental effects of sepsis on the developing brain.[539] The importance of evaluating the preterm infant for disseminated infection that may include meningitis cannot be overemphasized. A complete evaluation, including cultures of blood, urine and cerebrospinal fluid, is uncommon,[356] although one third of the cases of culture-positive meningitis in VLBW infants are associated with negative concurrent blood cultures.[542] Clinically apparent seizures may occur in 25% of VLBW preterm infants with meningitis.[542] Low-voltage background pattern, sleep-wake cycling, and seizure activity on

the amplitude-integrated electroencephalogram may be helpful to predict neurologic outcome in infants with sepsis or meningitis.[543] Significantly lower resistance, vasodilatation, and higher blood flow were noted in all the cerebral arteries of infants with sepsis. Increase in cerebral blood flow velocity was correlated with elevated IL-6 concentrations.[544] Alterations in blood flow in preterm infants, in addition to factors associated with sepsis, such as respiratory distress, hypercarbia, hypotension, and patent ductus arteriosus, contribute to the risk for intracerebral hemorrhage.

OTHER ORGAN SYSTEM CONTRIBUTIONS

Endocrine abnormalities may include altered thyroid function[545] and adrenal insufficiency associated with refractory hypotension.[546] Inadequate adrenocortical responses are associated with increased mortality.[547,548] Cortisol production in the neonate is significantly increased early in septic shock.[212] However, very preterm neonates can have relative adrenal insufficiency that may contribute to hemodynamic instability and hypotension. Hydrocortisone has not been evaluated in large prospective randomized clinical trials for the treatment of septic shock in the neonate but it has been shown to increase blood pressure, decrease heart rate, and decrease vasoactive medication requirements in preterm and term neonates in addition to its cytokine-suppressing effects.[549-551] If hydrocortisone treatment is considered, the obtaining of a pretreatment serum cortisol level is prudent in order to differentiate contributing causes of hypotension.

Sepsis was the most common cause (78%) of acute kidney injury in term neonates and was associated with high mortality (37%) (n = 49).[552] The frequency of acute renal failure (defined as a blood urea nitrogen level greater than 20mg/dL) in infected neonates was 26% and oliguria occurred in 15% of acute kidney failure cases.[553] Acute kidney injury in preterm neonates is associated with high mortality.[554] Hepatic injury and dysfunction are frequent associations with severe sepsis. The mechanisms include reduced hepatic perfusion associated with septic shock and mitochondrial energy failure. Reductions in coagulation and complement factors, acute-phase reactant proteins, and increases in the levels of transaminases and bilirubin are commonly seen, especially in association with decreased perfusion states. Energy expenditure and oxygen consumption are increased during sepsis,[555] and decreased mitochondrial oxidative function precipitated by hypoxia and the presence of reactive oxygen intermediates may lead to impaired growth, caloric deficiency, and energy failure.[556,557]

MULTIORGAN FAILURE/DYSFUNCTION

Sepsis that leads to MODS carries a dismal prognosis. Inadequate cardiac output and microcirculatory failure, which may be combined with formation of microthrombi and DIC, can lead to poor perfusion to the kidney,[472,473] liver,[474] gut,[475] and central nervous system.[52,148,181,469,476] Recent studies suggest that the mechanism of organ failure in sepsis may relate to decreased oxygen utilization associated with mitochondrial dysfunction rather than poor oxygen delivery to tissues.[479,480] Mitochondrial dysfunction can initiate activation of cell death pathways, including apoptosis, pyroptosis, necrosis, and NETosis (i.e. cell death mediated by NETs). Damage-associated molecular patterns (including nucleosomes and microparticles) created by activation of these cell death programs further amplify the host inflammatory response.

Free radicals play an important role in the inflammatory process of sepsis.[558] In a sepsis model in neonatal piglets, edaravone, a novel free radical scavenger, increased mean arterial pressure and cardiac output, lowered heart rate, reduced hydroperoxide, nitrite, and nitrate levels, delayed the TNF-α surge, prevented HMGB-1 level elevation, and was associated with longer survival times.[559] Increased plasma nitrite and nitrate concentrations are associated with the development of multiple organ failure in pediatric patients with sepsis[560,561] but have not been investigated in neonates.

FUTURE CONSIDERATIONS

The incidence of neonatal sepsis remains high and outcomes remain poor despite considerable technologic advances in the field of neonatology. Much remains to be learned about the impact of developmental age on the host response to sepsis and what facets are critically important. Important considerations for future investigations include the development and implementation of a generally accepted definition for neonatal sepsis, the use of homogeneous systems (only neonatal components) for human ex vivo studies, and transgenic approaches in preclinical models, alongside observational studies in humans to ensure meaningful findings.

Complete reference list is available at www.ExpertConsult.com.

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