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Sepsis, Cytokine Storms, and Immunopathology: The Divide between Neonates and Adults

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ABSTRACT

Sepsis can result from a variety of pathogens, originating from a range of sources. A vast range of presenting symptoms is included in the catch-all term of "bacteremia," making diagnosis and prognosis particularly troublesome. One underexplored factor contributing to disparate outcomes is the age of the patient. Neonatal sepsis in very-low-birth-weight infants can result in vastly different immunological outcomes unique from sepsis in adults. It is also becoming increasingly clear, both from preclinical experimental models and clinical observations, that the age and history of previous microbial exposures can significantly influence the course of infection from sepsis and cytokine storms to immunopathology. In this study, we will explore key differences between neonatal and adult sepsis, experimental models used to study sepsis, and how responses to the surrounding microbial universe shape development of the immune system and impact, positively or negatively, the course of disease. *ImmunoHorizons*, 2021, 5: 512–522.

INTRODUCTION

Sepsis resulting from bacterial bloodstream infections remains a serious clinical concern and is currently defined as a "life-threatening organ dysfunction caused by a dysregulated immune response that occurs as the result of an infection" (1). The dysregulated immune response is evident by the initial hyperinflammatory response, driven by proinflammatory cytokines and chemokines (2). In the United States, sepsis rates have been continuously increasing over the last 20 years; hospitalizations due to sepsis have increased from 1.2% of all hospitalizations in 2005 to 2.7% in 2014 and up to 5.8% in 2017 with ∼11 million sepsis-related deaths (3–5). Sepsis persists as a significant clinical burden, with the total cost of hospitalizations due to sepsis at $38 billion in 2017, up from $20 billion in 2011 (4, 6). Despite this increase in sepsis cases and costs, the mortality rate in the U.S. has dropped from 31.9% in 2005 to 17.1% in 2014 (3). This dramatic decrease in mortality from sepsis is likely a result of implementation of early goal-directed treatment, as broadly applicable drugs remain elusive (7). Increased readmission rates also likely contribute to increased total hospitalization numbers with reduced mortality but does highlight the potential of recurrent sepsis due to sepsis-induced immunosuppression (also referred to as immunoparalysis) (8). These trends may be, in part, due to expansion of definitions of sepsis and diagnosis criteria, as less severe cases are included in the

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Abbreviations used in this article: CLP, cecal ligation and puncture; EOS, early-onset sepsis; GBS, group B Streptococcus; LOS, late-onset sepsis; SPF, specific pathogen–free.

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classification of sepsis with improved recognition of disease (9). The cause of sepsis can vary widely between cases, in large part because the umbrella term "sepsis" includes a wide range of disease symptoms resulting from a variety of bacterial pathogens originating from different organs and tissues and affects patients spanning from very young to very old.

Sepsis occurring in adults is frequently attributed to the progression of an infection following surgical complications; however, translocating pathogens from tissue infections, such as appendicitis, pneumonia, or urinary tract infections can also serve as the nidus of sepsis. In a 2010 study of elective surgical cases in the U.S., 1.2% of patients developed sepsis after surgery (10). Similarly, surgery necessitated by trauma was found to be a major risk factor for disease among orthopedic patients, with 2% of trauma patients developing sepsis and 0.5% of non-trauma patients developing sepsis (11). The most common sepsis-associated pathogens in adults include Gram-negative species *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and Gram-positive species *Staphylococcus aureus* and *S. epidermidis* (12–14). Frequencies of Gram-negative versus Gram-positive bacteria as sepsis-causing agents vary between studies and locations, but rates are generally reported to be approximately equal. The strongest influence on the type of bacteria that causes sepsis is the site of initial infection, with Gram-negative infections being more likely with gut-originating sepsis (14). Infections in the lower respiratory tract can lead to sepsis and are among the most common nosocomial infections in intensive care units. The use of mechanical ventilation is considered a strong risk factor for development of infection in the lower respiratory tract that can lead to sepsis, and incidence rates of ventilator-associated pneumonia vary from 5 to 66% of ventilated patients, depending on the length of time mechanical ventilation is needed (15). The longer a patient requires the use of a ventilator, catheter, or IV, the higher their risk of developing infections in the related tissue (Table I).

Sepsis occurring in neonates, or infants younger than 3 months, is classified as neonatal sepsis. Risk factors for neonatal sepsis include low gestational weight or young gestational age, with very-low-birth-weight infants (<1500 g) being most at risk for infections. Neonatal sepsis includes both early-onset sepsis (EOS), or bacteremia, and late-onset sepsis (LOS). Historically, the age of onset for EOS was defined as less than 72 h old, although this criteria is not a universally defined and may include 7 d old or less (16). Although this delineation is based on age of sepsis onset after delivery, it is also based on route of pathogen entry, with the assumption that EOS results from maternal pathogen transmission in utero or during the birthing process (17). Group B *Streptococcus* (GBS) and *E. coli* are among the most common pathogens transmitted via this route (18). Screening for GBS at 35–37 wk gestational age has been

TABLE I. Summary of characteristics of neonatal sepsis and adult sepsis

	Neonatal EOS	Neonatal LOS	Adult
Route	Maternal pathogen transmission In utero During birth	Contamination of skin-resident bacteria during clinical procedures	Surgical complications
		Enteric pathogens translocating the intestinal lumen	Translocating pathogens from tissue infections (e.g., appendicitis, pneumonia, urinary tract infections)
Common causative pathogens	GBS	Skin-resident bacteria (such as *S. epidermis*)	Similar rates of Gram-negative versus Gram-positive bacteria
	E. coli	Gut-resident bacteria (such as *E.coli*)	Gram-negative species: *E. coli*, *K. pneumoniae*, *P. aeruginosa* Gram-positive species: *S. aureus* and *S. epidermidis*
Incidence	0.26 cases per 1000 live births in 2010 (21) Maternal GBS screening initiatives contribute to a decreased incidence from 1.8 cases per 1000 live births in the early 1990s (20)	0.31 per 1000 live births (22)	1.2% of patients develop sepsis after elective surgery (10) 2% of patients develop sepsis following trauma-associated surgical cases (11)
Risk factors	Low birth weight	Low birth weight	5–66% of ventilated patients develop ventilator-associated pneumonia (15) Surgery necessitated by trauma among orthopedic patients
	Failure to screen for maternal GBS colonization	Prolonged use of antibiotics	Prolonged need for mechanical ventilation, i.v., or catheter increases risk of infection in associated tissues
	Failure to administer intrapartum antimicrobial prophylaxis		

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recommended since 2002 for this reason, with those identified to be at risk receiving intrapartum antimicrobial prophylaxis (19). Screening initiatives over the past decade have significantly reduced EOS by GBS (20), resulting in incidence of GBS EOS decreasing from 1.8 cases per 1000 live births in the early 1990s to 0.26 cases per 1000 live births in 2010 (21). Despite the reduction in cases of EOS, LOS rates have been stable from 2006 to 2015 at 0.31 per 1000 live births (22). Acquisition of pathogens resulting in LOS occurs following birth and can range from contamination of skin-resident bacteria (such as *S. epidermis*) during clinical procedures to gut-resident bacteria (such as *E. coli*) translocating the intestinal lumen (23) (Table I).

Cytokine response to bacterial inflammation

Neonates and infants respond to pathogens very differently than adults, and neonatal sepsis is thus unique from adult sepsis. Classically, bacterial-induced inflammation includes the production of innate cytokine responses (e.g., IL-1β, IL-6, IL-12, and TNF-α). Characterization of the bloodstream response during sepsis at various ages revealed neonates clustered separately from adults, with older infants and children somewhere between, representing a spectrum of age-related responses and decreased TLR and iNOS signaling in neonates compared with other groups (24). Even within the scope of neonatal sepsis, a significant difference in cytokine responses has been noted between EOS and LOS, suggesting postnatal age may influence disease (25). Although both EOS and LOS patients had a significant increase in serum IL-6, LOS patients uniquely had an increase of anti-inflammatory cytokines IL-10 and IL-4 (26) (Fig. 1).

IL-6 is classically defined as an innate inflammatory cytokine in response to bacterial threats. Concentrations of circulating IL-6 are consistently elevated during many acute conditions, such as in trauma due to injury, burns, or surgery, as well as during sepsis (27). The correlation between higher IL-6 levels and increased disease severity and mortality suggests it may be an effective marker for sepsis, particularly early in the course of disease, for cases without a positive blood culture (28, 29). Outside of sepsis, IL-6 has been implicated as a source of immunopathology in neonates in the setting of influenza (30), which suggests its potential to target in neonatal sepsis (28). IL-6 has also been established as a biomarker in both fetal inflammatory response syndrome or intra-amniotic inflammation during pregnancy (31–33), further demonstrating the role of IL-6 in infection-associated inflammation in early life. This does not exclude adults from generating IL-6 during bacteremia. Interestingly, cytokine storms following IL-6 initiation cluster uniquely from TNF-α– and IL-12–initiated cytokine storms (34, 35). However, pervasive observations suggest IL-6 may perform immunosuppressive functions on dendritic cells and T cells (36–40), inhibiting further release of TNF-α and IL-1b, whereas increasing the circulation of anti-inflammatory mediators IL-1Rα, IL-10, and TGF-β (27). Indeed, the successful use of tocilizumab, an anti–IL-6 receptor mAb, in other inflammatory disorders supports the potential for its use in sepsis (41). In experimental sepsis models, injections of anti–IL-6 mAb improve colonic barrier function after disturbances associated with sepsis (42). Anti–IL-6 mAb therapies may also contribute to the reduced expression of the complement receptor C5aR with beneficial effects (43). The complement cascade is innately activated during bacterial infections, but the complement products, including C5a, may contribute to ongoing inflammation (44, 45). Interestingly, the complement pathway in neonates, particularly premature infants, may be perturbed when compared with adults (46, 47), although the role of complement in neonatal sepsis has yet to be fully explored (48).

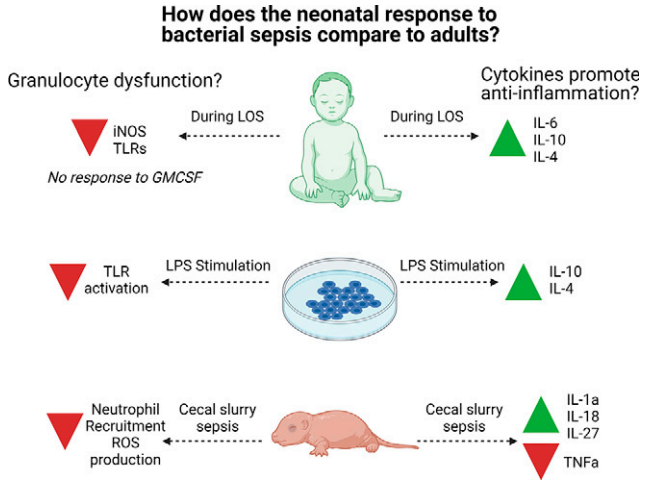

FIGURE 1. The neonatal response to bacterial sepsis differs compared with the adult response when looking at clinical data from LOS, in vitro data from human cells, and in vivo animal models.

Observations suggest cytokine differences may contribute to immune dysfunction and sepsis outcomes. The figure was created with BioRender.

In contrast to the one-time belief that the neonatal immune system was characterized by immaturity and more innate-like cells, adaptive immune system components, including B cells and T cells that are abundant in the fetus and neonate, can produce and secrete cytokines and express markers of resident memory phenotypes (49). The composition of WBCs is generally similar between neonates and adults, although neonates do have a noted increase in immature neutrophils (50–52). Several neonatal immune cell types may directly regulate aberrant inflammation and promote tolerance (53). Traditionally, the neonatal immune system is biased toward a Th2 immune response, including regulatory and anti-inflammatory responses, whereas adult immune responses favor Th1 effector phenotypes (54), and neonatal T cells exhibit higher production of IL-4, IFN-γ, and IL-2 following anti-CD3/CD28 activation compared with those of adults (55). Similarly, whereas both neonatal and adult PBMCs had increased IL-1β, IL-6, IL-8, CCL3, and TNF-α production following LPS stimulation, neonates showed a significant increase in IL-10, and only adults showed a significant increase in IL-12 (24), which is substantially reduced in neonates (56). During the course of neonatal sepsis, differences in cytokine release have been noted when comparing very

preterm infants (<32 wk gestational age) to preterm infants (32–36 wk gestational age), with a strong spike in the innate cytokines TNF-α, IFN-γ, and IL-6 and anti-inflammatory cytokines IL-4 and IL-10 in preterm infants, which were absent in very preterm infants (57) (Fig. 1). This observation is comparable with the differences observed between EOS and LOS, with the LOS signature resembling the more robust cytokine response in the preterm infants (26), again suggesting postnatal age is a determining factor of immune response.

Although most studies focus on the classic proinflammatory cytokines IL-1β, IL-6, and TNF-α, the initial innate response in neonates likely includes other cytokines. Preclinical data suggest the involvement of IL-1α over IL-1β in determining lethality following cecal slurry sepsis (58). Another member of the IL-1 superfamily, IL-18, was noted to be significantly elevated in uninfected neonates, particularly very-low-birth-weight infants (59), and contributed to pathology during cecal slurry sepsis (60). The potential role of IL-18 involves promoting the release of IFN-γ and TNF-α and activating IL-17A, which induces gut pathology and perpetuated bacteremia and induced mortality (60). Similarly, IL-27 was elevated in neonates, which may predispose them to severe sepsis, as IL-27 reduces the innate immune function of macrophages and promotes an anti-inflammatory T cell response (61).

In addition to WBCs, RBCs may also contribute to immune regulation in neonates. $CD71^+$ erythroid cells, a population of RBCs, can suppress inflammation resulting from bacterial threats such as *E. coli* (62). These cells may contribute to feto-maternal tolerance (63) and remain in circulation for the first few weeks following parturition (64). Despite the observed function of immunosuppression, the contribution of $CD71^+$ erythroid cells to susceptibility of sepsis remains to be clearly defined (64, 65). Understanding these cytokines and the cell populations they act on during neonatal sepsis will be key to identifying potential therapeutic points in the future.

The progression of sepsis

The primary therapeutics for sepsis consist of broad spectrum antibiotics; however, more precise treatments are needed, particularly in cases with a negative blood culture, in which the causative agent is unknown, and to avoid escalating microbial resistance to currently available antibiotics (66). Efforts to strategically administer antibiotics and contain the spread of antibiotic-resistant bacteria have resulted in 18% fewer deaths in 2019 compared with reports from 2013; yet, more than 35,000 people die each year due to antibiotic-resistant bacteria (17, 67). Additionally, increased exposure of antibiotics, particularly during early life, has its own drawbacks. Before GBS screening guidelines were implemented, 12% of newborns were exposed to antibiotics prior to birth and has since more than doubled to ∼30% (68). Although antibiotic usage in early life can reduce occurrences of EOS, prolonged use of antibiotics in early neonates can increase the risk of LOS, necrotizing enterocolitis, and long-lasting disruption of the infant's newly forming gut microbiome, in addition to potential development of antibiotic-resistant GBS (69, 70). The development of new therapeutic protocols to treat and potentially control the overwhelming inflammatory response and potentially ease pressure from increased antibiotic use and emergence of antibiotic-resistant bacteria has been a decades-long goal in the sepsis field.

Numerous trials have been conducted in adults, testing agents designed to temper sepsis hyperinflammation, although without much success, and the accumulating number of unsuccessful trials in neonates with sepsis has been equally disappointing (71, 72). Most therapeutics to treat neonatal sepsis have been those first vetted in adult sepsis, because of the protected status of neonates in clinical research. Drawing on the view that individualized immune responses need to be considered in adult sepsis cases (73), understanding the unique response in neonates may similarly advance the development of immunotherapy targeted to neonatal sepsis (29).

Recombinant human protein C became the first biologic therapeutic approved for severe sepsis and was met with controversy before it was removed from the market (74–76). In addition to failing replication in adult trials, it was unsuccessful in neonates, leading to increased hemorrhage and intracranial bleeding (77). Attempting to control overwhelming inflammation, many trials have used agents to directly block proinflammatory cytokines during the course of sepsis. Anti-TNF mAb therapy modestly decreased risk of mortality, but this decrease continues to be nonsignificant over decades of trials (78–81). Although it has not been directly tested in neonatal sepsis, use of anti-TNF mAb around parturition suggests it may increase neonatal neutropenia (82), which could be detrimental in overcoming disease.

Ig therapy has been widely tested to improve systemic opsonization and neutralization of bacteria and bacterial products, but it has had only modest success in sepsis clinical trials. As of 2016, Ig therapy has not been recommended by the Surviving Sepsis Guideline panel (83), because of a low certainty in the modest reduction of mortality (84–86). Despite these findings, there is still hope that the modest reduction in mortality suggests i.v. Ig therapy, specifically IgM, may have promise as a therapeutic option for adult sepsis. There is a noticeable difference, however, in the efficacy of Ig therapy in neonatal sepsis. Rather than displaying a modest reduction in mortality, clinical trials using Ig therapy in septic neonates had no effect on outcomes (87, 88). Ig therapy is thought to assist phagocytes in clearing the inflammatory bacterial products from circulation, but perhaps the variance in outcomes indicates phagocytes themselves are responding differently between neonates and adults.

CSFs, such as G-CSF and GM-CSF, have been tested as therapeutics to increase neutrophil and macrophage numbers to improve bacterial clearance during the course of sepsis. Similar to the aforementioned therapeutics, treatment with CSFs only led to modest improvement in recovery in clinical trials in adults (89), especially in those patients with sepsis-associated immunosuppression (90), although there is still caution around

their role in sepsis treatment (91). In stark contrast, no effect has been seen in clinical trials of neonates after G-CSF administration (92) or even infants with neutropenia (93). Although CSFs can delay apoptosis and improve function of adult neutrophils, in vitro studies found neonatal neutrophils may be more sensitive to apoptosis and were unresponsive to CSFs (94). Additionally, inhibition of necroptosis improved survival in an animal model of neonatal sepsis (95), and neonatal neutrophils and myeloid cells displayed reduced capacity to migrate and produce cytokines in response to bacterial stimuli (96, 97). Thus, increased sensitivity to apoptosis or necroptosis may explain why neonates are extremely susceptible to neutropenia (98).

The limited (at best) success of these trials has led to a new theme arising in the field of sepsis to understand long-term sepsis-induced alterations to the immune system and how these may contribute to recurring sepsis and recovery (99). Proinflammatory signals dominate the response at the onset of sepsis, but excessive production of both pro- and anti-inflammatory cytokines and chemokines partially overlap during the initial stage of sepsis (2), resulting in a considerable amount of indecision on what immunotherapy in sepsis would entail (100). Following acute hyperinflammation, the immune system seems to overcompensate and exhibits dramatic quantitative and qualitative alterations in multiple immune cell populations. In particular, there is significant lymphopenia, or reduction in number of circulating lymphocytes, occurring primarily via apoptotic death and, potentially, a consequence of the massive immune response. Lymphopenia can contribute to an immunosuppressed state, initial impairment in immunity to the sepsis-initiating infection, secondary nosocomial infections, and latent virus reactivation (101–108). Surviving patients recover from the sepsis-induced lymphopenia, but go on to display a prolonged state of immunoparalysis that extends the window of time in which they are highly susceptible to new infection, even from pathogens that are efficiently eliminated in healthy individuals (109–113). Consequently, there is considerable clinical interest in testing agents to help the immune system of sepsis survivors to functionally (as well as numerically) recover as quickly as possible. For example, cytokines that promote T cell proliferation (such as IL-7) or mAb-targeting proteins that stimulate or inhibit T cell function (such as anti-OX40 and PD-1/PD-L1) have shown promising results in preclinical models and clinical settings in adult sepsis patients (114–119). It is unclear whether these immunomodulatory approaches would also work in septic neonates, as no clinical studies have been performed to date. Although lymphopenia has been noted in neonates (120, 121), it remains unclear how it may contribute to sepsis outcomes, particularly as preterm infants may be lymphopenic compared with term infants (122). IL-7 may restore T cell function equivalently (or even better) in neonates, especially because IL-7 can stimulate circulating T cells to proliferate and promote T cell development in the thymus (123), which is potentially high in neonates. In contrast, mAb specific for OX40 or PD-1, for example, may only have limited activity in the neonate because the T cell compartment has had only a limited amount of time to develop. Much like the case for immune-based therapeutics in cancer patients, which show unique responses in individuals, it has been difficult to predict the best immunotherapy for individual sepsis patients or whether these approaches will restore the immune system to the presepsis state.

Animal models, what we have learned, and what still is left to learn

Preclinical experimental models of sepsis have been widely used to connect immune events from bacterial sensing to systemic inflammation involved in clinical outcomes. However, the clinical relevance of some of the most commonly used preclinical sepsis models has become a major discussion point in recent years, in part because of the variability in how some of the models are used from one laboratory to the next and because of limited adoption of the treatment methods used in the clinic. Cecal ligation and puncture (CLP) is a well-described animal experimental model in which the cecum is punctured, allowing release of fecal matter into the body and initiating a strong immune response and subsequent immune suppression. Although CLP has been used by many laboratories to mechanistically define sepsis-induced immunopathology, there is some disagreement about the extent to which CLP mimics the course of sepsis in humans or whether it is instead a model of surgical negligence marked by the formation of an intra-abdominal abscess (124). Because of the nature of the surgical procedure and dependency of CLP on gut microbiota to initiate the peritonitis, this model has been exclusively used in adult, albeit immunologically naive, mice. Prior to weaning, the limited microbiota and small size of the cecum makes CLP unviable in mouse pups, in which the polymicrobial cecal slurry model of sepsis or injection of cecal contents can be used as an alternative (125).

LPS endotoxemia has been used for many years to model the inflammation that occurs during systemic infection. Mice are considerably more resistant to bacterial endotoxins than humans (126), so infection with defined live bacteria has also been used to model sepsis to control the diversity and number of pathogens to which the animal is exposed. Both the LPS endotoxemia and live bacterial infection sepsis models have been adapted for use in neonatal and adult mice, revealing unique differences in the response and outcome. For example, neonatal mice are more susceptible to mortality following LPS injection because of an increased innate response that includes significantly increased TNF-α and IL-6 production (127). The exaggerated systemic inflammation following LPS injection in neonates also results in immunopathology to the CNS, potentially connecting the long-term clinical sepsis outcome of neurologic disorders to the systemic inflammation during neonatal sepsis (128). Intriguingly, transfer of adult T cells into the neonatal mice is sufficient to suppress this strong inflammatory response, suggesting the age of the immune system may affect the immune response (127). Similarly, adult *Rag*$^{-/-}$ mice are more sensitive to polymicrobial sepsis, and mortality rates are

similar to both neonatal wild-type and neonatal *Rag*$^{-/-}$ mice, providing further evidence of the potential role of components of the adaptive immune system in surviving sepsis (129). In contrast to the increased inflammation following LPS injection (127), neonates produce a subdued innate response following cecal slurry injection, which includes reduced IL-6 and TNF-α production. This response was increased during sepsis if neonates were pretreated with TLR agonists (such as LPS), which increased neutrophil recruitment (129). TLR agonist pretreatment also improved migration and cytokine secretion by neutrophils from term infants, although neutrophils from preterm infants only showed a modest improvement (97). Neutrophil dysfunction during cecal slurry-induced sepsis was noted in both neonatal and aged mice. Although neonates showed reduced neutrophil recruitment, reactive oxygen species production, and transcriptional changes during the course of sepsis, aged mice suffered from persistent inflammation, highlighting the potential differences in the immune response of the two age extremes (130) (Fig. 1). In work that implicated PD-1 on B cells rather than T cells, neutrophil recruitment and function during cecal slurry-induced sepsis was also improved in neonatal *Pd1*$^{-/-}$ pups (131). Therefore, it remains unclear whether the differences in the neonatal immune response results in reduced innate inflammation unable to control bacteremia or overwhelming innate inflammation that requires suppression. Potentially, these variances could result from the inherent difference between LPS injection and cecal slurry sepsis, as the former would only activate the TLR4 pathway, whereas the latter may activate a variety of innate bacterial-sensing pathways in addition to TLR4. Although no significant differences have been found in TLR4 expression on innate leukocytes between neonates and adults, TLR2 was slightly decreased in neonates and through neonatal sepsis (132, 133). Additionally, signaling downstream of TLRs and the resulting cytokine production are suppressed in neonates (134–136). Specifically, neonatal monocytes had reduced phagocytic ability, oxidative burst, and pathogen clearance compared with monocytes from adults, which was further reduced in preterm infants (133, 137).

One caveat of the LPS, cecal slurry, or CLP models of sepsis is the rapid binary response of the animal, with the potential for death or recovery to occur within hours, underscoring the importance of models that recapitulate the intermediate stage (138). Models that use clinical pathogens and relevant routes of entry may allow for the extended examination of how the immune system responds to bacterial threats (139). To specifically explore the gut-originating routes of neonatal sepsis, disruption of the microbiota by oral antibiotics worsened sepsis in neonatal mice gavaged with *K. pneumoniae* (140). Similarly, disruption of specific factors in maternal milk during breastfeeding worsened sepsis following enteric *E. coli* translocation (141). These models have important implications for understanding the role of diet and microbiota in early life in protection from gut-originating pathogens. Indeed, randomized administration of probiotics reduced LOS in a clinical trial, and an oral route of pathogen entry resulted in increased infectivity in an animal model of LOS (142, 143). These later observations suggest the intestinal microbiota may influence the infectivity of the bacteria, but clearly, the contributions of the microbiota in influencing the host and the bacteria must be considered.

Dysbiosis of the gut microbiota while the patient remains in the hospital environment can increase risk for dissemination of bacteria through the body through noninfectious acute injuries, antibiotic usage, or acute illnesses, such as influenza (144), which can harbor or encourage the growth of opportunistic pathogens. There is a wealth of information demonstrating how the composition of the gut microbiome can influence systemic immunity, so it is quite possible that the combination of immune system maturation and gut microbiome changes can together define the way the immune system responds during a septic event. Nearly all investigators use mice housed under specific pathogen–free (SPF) conditions, which helps to improve experimental reproducibility (among other things) (145). The immune system in SPF mice, however, is highly naive and more closely resembles the immune system of a human neonate (146). As a way to incorporate this important aspect of human biology into preclinical modeling, a number of investigators have recently developed models in which the murine immune system becomes matured (resembling what is seen in adult humans) after exposure to environmental microbes or a defined panel of pathogens (147). Similarly, cohousing SPF laboratory mice with mice purchased from local pet stores permits the transmission of various mouse pathogens and commensals to the laboratory mice, resulting in a more diverse gut microbiota (148) and subsequent maturation of the host immune system (146, 148). Interestingly, these "dirty" mice show increased cytokine responses and mortality after CLP, cecal slurry injection, or LPS challenge compared with SPF mice. Microbial exposure was associated with changes in TLR expression, particularly on phagocytes, suggesting the lack of complexity in the microbial environment of SPF-housed mice may not allow for rigorous modeling of the human response to bacterial threats. One potential benefit of having microbially experienced mice in the preclinical experimental sepsis toolbox may be the additional level in which new therapies may be tested prior to moving into clinical trials, as recent data highlighted the ability of "wildling" mice to better replicate the outcomes of immunotherapeutics tested clinically (149). The feasibility of using neonatal mice born from dirty parents in sepsis research remains to be determined, but it is tempting to speculate how such mice could advance the neonatal sepsis knowledge base.

CONCLUSIONS

Although antibiotics will remain the primary treatment for bacterial-induced sepsis for years to come, the rise of resistance in microbes coupled with the need to assess long-term care for patients that survive sepsis makes understanding the initiation and resolution of inflammation a priority. Additionally,

appreciation of how sepsis presents and the immune system's subsequent response at various stages of life will offer unique guidance and potential therapeutics specific to each age. As described above, disparities in how the immune system initiates, orchestrates, and resolves inflammation results in a variety of outcomes following sepsis. Although preclinical experimental models have defined these cytokine/chemokine cascades as potential biomarkers, future work must address which cascade points are beneficial to resolving infections and which are deleterious in driving excessive inflammation, appreciating that these points may be unique to type, dose, and route of pathogens that evoke sepsis, genetic background, and status of the immune system of septic hosts.

DISCLOSURES

K.A.K. is an inventor on United States nonprovisional patent application 15/880,658, Compositions and methods for modulation of dietary and microbial exposure. The other authors have no financial conflicts of interest.

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