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Immunity

Review

The immunology of sepsis

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SUMMARY

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to an infection. This recently implemented definition does not capture the heterogeneity or the underlying pathophysiology of the syndrome, which is characterized by concurrent unbalanced hyperinflammation and immune suppression. Here, we review current knowledge of aberrant immune responses during sepsis and recent initiatives to stratify patients with sepsis into subgroups that are more alike from a clinical and/or pathobiological perspective, which could be key for identification of patients who are more likely to benefit from specific immune interventions.

INTRODUCTION

Sepsis has been described as the quintessential medical disorder of our time because it is not only a leading cause of morbidity and mortality in hospitalized patients but often also the direct result of the improvements in the medical care for patients with various disorders for which, until recently, no treatments were available (Deutschman and Tracey, 2014). Over the course of thousands of years, the meaning of the term sepsis has evolved. Historical definitions from the time of Hippocrates onward are depicted in Figure 1. Now, sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). Septic shock is a subset of sepsis with profound circulatory, cellular, and metabolic abnormalities associated with a greater risk of mortality than with sepsis alone (Shankar-Hari et al., 2016c). In the current sepsis definition, the terms *dysregulated* and *host response* are not explicitly defined but conceptualized as maladapted responses within the immune and non-immune systems that are in the causal pathway to organ dysfunction and death.

A recent Global Burden of Diseases report highlights that sepsis is common, with nearly 50 million cases globally per year (Rudd et al., 2020). Sepsis affects all ages. While the site of infection and causative pathogen in sepsis vary by geographic location and age, bacterial infections of the respiratory system and gastrointestinal systems are most common. Sepsis had an estimated mortality of 11 million in 2017, which equates to an age-standardized mortality of 148 per 100,000 population, representing nearly 20% of all deaths globally (Rudd et al., 2020). When patients with sepsis require admission to critical care units, one-in-three patients do not survive 30 days (Machado et al., 2017; Shankar-Hari et al., 2017b) and mortality varies by the age, comorbid status, number, and type of organ dysfunctions (Prescott et al., 2020; Rhee et al., 2017; Shankar-Hari et al., 2016b). Further, patients who survive sepsis have a longer-term risk of rehospitalization and death (Prescott et al., 2015b; Shankar-Hari et al., 2020). Nearly 50% of sepsis survivors are re-hospitalized at least once within a year, and one-in-six sepsis survivors do not survive the first year (Prescott and Angus, 2018; Prescott et al., 2016; Shankar-Hari et al., 2016a).

Despite more than three decades of research and more than 200 randomized controlled trials, we do not have a single treatment that consistently saves lives in sepsis patients (Marshall, 2014). Treatment of sepsis remains largely supportive with simple measures such as source control, timely antibiotics, resuscitation, and supportive care for organ dysfunction (Evans et al., 2021). In a hope for more successful clinical trials and better outcomes, the critical care community now considers the value of subgrouping sepsis patients either on measurable characteristics that inform treatment response (predictive enrichment) or outcome (prognostic enrichment) or to identify two or more homogeneous subgroups with common clinical and laboratory features (subphenotypes) or specific pathobiological abnormalities that could be targeted (endotypes) (Shankar-Hari and Rubenfeld, 2019; Stanski and Wong, 2020).

Here, we review current knowledge of immune dysregulations in sepsis. We describe mechanisms that contribute to (a return to) homeostasis during and after bacterial encounters, with subsequent attention for immune imbalance characterized by concurrent proinflammatory and immune suppressive aberrations. Our review focuses on the immune alterations in sepsis of bacterial origin. We note that sepsis could occur from viral, fungal, or parasitic infections and that non-immune alterations form part of the dysregulated host responses concept in sepsis. At present, sepsis remains an ill-defined syndrome, and we argue that successful implementation of additional technologies and bedside computational support may enable real-time immunological profiling of individual patients and inform patient selection for clinical trials of targeted therapies.

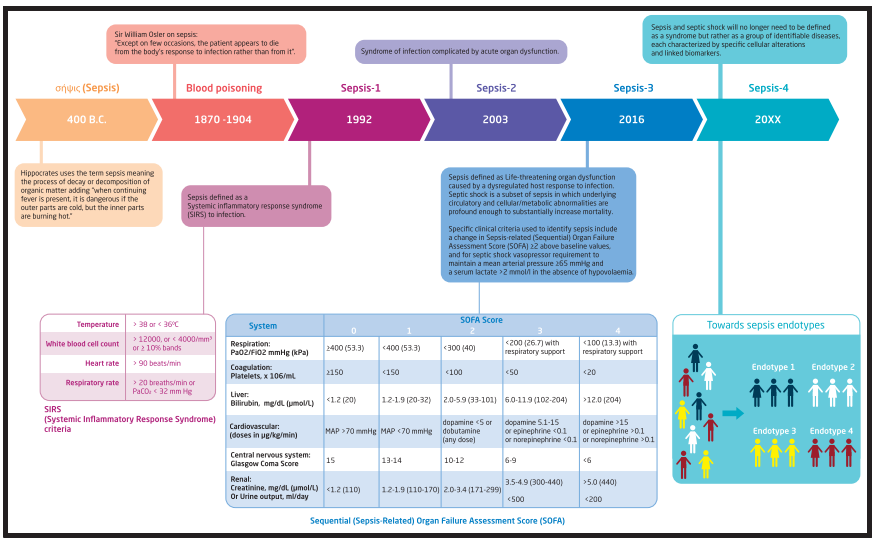

Figure 1. The evolving meaning of sepsis
Historical definitions used for sepsis depicted from the time of Hippocrates onward. SIRS: Systemic inflammatory response syndrome; SOFA: Sequential Organ Failure Assessment.

FROM HOMEOSTASIS TO IMBALANCE

The immune system is equipped with a range of cell membrane associated and intracellular pattern recognition receptors (PRRs) that can detect pathogens through their ability to recognize pathogen-associated molecular patterns (PAMPs), conserved motifs expressed by microbes (Kumar et al., 2013; van der Poll et al., 2017). Main PRR classes include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain and leucine-rich repeat containing gene family (NLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), C-type lectin receptors (CLRs), and DNA-sensing molecules (Kumar et al., 2013). Induction of innate immunity further occurs through inflammasomes—cytosolic multiprotein oligomers that, upon activation and assembly, promote a number of downstream events, including caspase-1 mediated cleavage of the proinflammatory cytokines interleukin-1β (IL-1β) and IL-18 (Lamkanfi and Dixit, 2014). A balanced immune reaction to a pathogen typically is localized and characterized by inflammatory, anti-inflammatory, and repair responses, with elimination of microorganisms and return to normal homeostasis (van der Poll et al., 2017). In such a protective immune response, different cell types become activated through an interaction between PAMPs and PRRs, triggering intracellular signal transduction pathways and activation of key transcription factors such as nuclear factor κB (NF-κB) and activator protein (AP)-1, which coordinate inflammatory responses. Soluble components of expected responses to infection include proinflammatory cytokines, chemokines, proteins released from activated neutrophils and platelets, complement products, and coagulation factors (Murphy et al., 2017). The vascular endothelium supports a protective immune response by increasing its expression of adhesion molecules and widening its gap junctions, enabling the adherence of immune cells and their migration to sites of infection. Moreover, the adaptive immune system is triggered by presenting antigen via dendritic and other cells to B and T lymphocytes, resulting in the production of pathogen-specific antibodies and memory for subsequent infection by the same pathogen (Murphy et al., 2017).

The trillions of commensal bacteria that colonize the gut also play an important role in homeostasis and host defense against invasion by pathogens. A healthy microbiota provides resistance against colonization and invasion by harmful microorganisms by utilizing both direct and indirect mechanisms (Kim et al., 2017; Pamer, 2016). The microbiota directly competes for nutrients, maintains epithelial barrier function, and produces antibacterial peptides. The microbiota can also regulate the production of antimicrobial proteins by host cells. An example is the regeneration of islet-derived protein IIIγ (REGIIIγ), which is produced by Paneth cells upon TLR-mediated stimulation of epithelial cells and dendritic cells by commensal-microbe-associated molecular patterns such as lipopolysaccharide (LPS) and flagellin (Kamada et al., 2013; Kim et al., 2017). Among the pleiotropic effects of short-chain fatty acids (SCFAs), which are the main metabolites produced by the microbiota, is their ability to drive monocyte-to-macrophage differentiation and inhibition of histone deacetylase 3. Through this inhibition, butyrate mediates macrophage metabolism and further induces the production of antimicrobial peptides to enhance antimicrobial activity in murine models of infection (Schulthess et al., 2019). Other gut bacteria interact with gut epithelial cells in order to enhance IgA production by B cells and induce T helper 17 (Th17) cell differentiation.

Disruption of the gut microbiome can lead to a transition from homeostasis to disease. Large observational patient studies have provided indirect evidence that gut microbiome disruption predisposes to sepsis (Adelman et al., 2020; Baggs et al., 2018; Prescott et al., 2015a). An altered gut microbiota can increase gut barrier permeability and translocation of pathobionts toward distant organs (Adelman et al., 2020; Dickson et al., 2016; Haak and Wiersinga, 2017). Disruption of the intestinal microbiota can additionally impact distant organs such as the bone marrow and the lung, leading to diminished effectiveness of host defense against infection. By using an *Escherichia (E.) coli* sepsis model in neonatal mice, Deshmukh and colleagues have shown that the microbiota regulates neutrophil homeostasis (Deshmukh et al., 2014). Commensal Gammaproteobacteria, Gram-negative bacteria, which express cell-surface LPS, activate IL-17A production by innate lymphoid cells via a TLR4-induced signaling cascade, which triggers an increase in plasma granulocyte colony-stimulating factor. This could subsequently stimulate neutrophil recruitment from the bone marrow into the bloodstream in order to combat invading pathogens such as *E. coli* (Deshmukh et al., 2014). Gut-derived SCFAs can influence the immunological environment in the lung (Sencio et al., 2020;

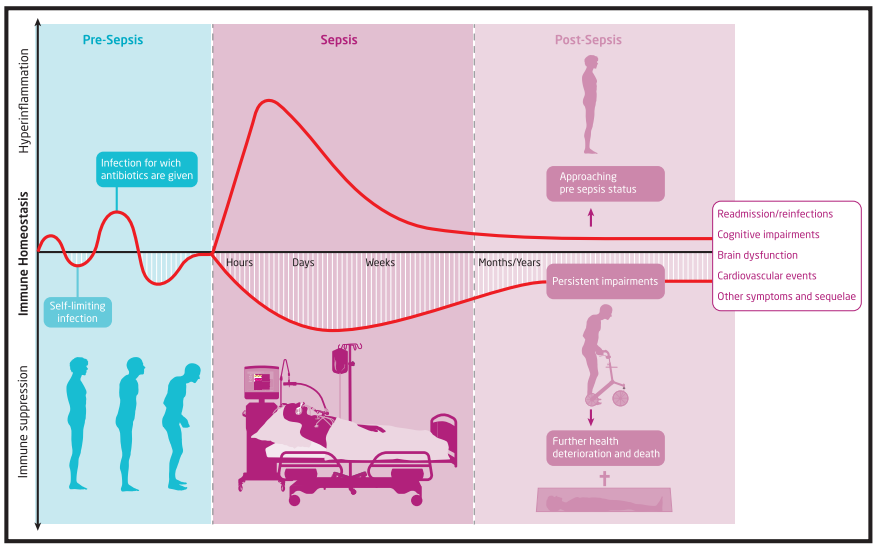

Figure 2. Immune trajectories and outcome pre-, during, and post sepsis

This conceptual model of the immune trajectories pre-, during, and post sepsis related to outcome shows that in sepsis, inflammatory and immunosuppressive responses occur concurrently. Traditionally, the early proinflammatory response to the invading pathogen or danger signals has been associated with the development of multi-organ failure and early mortality, while the anti-inflammatory response with reactivation of latent viral infections and delayed mortality. The host response seen in patients that do survive the early course of sepsis during hospitalization is characterized by persistent inflammation, immunosuppression, and catabolism syndrome (also called PICS). The extent of proinflammatory and immunosuppressive responses and their relative contribution to sepsis-associated immunopathology vary between individual patients. Patients that do recover from sepsis can return to pre-sepsis health status after several weeks to months but could also survive with persistent impairments such as cognitive impairments, brain dysfunction, cardiovascular events, and an increased change of hospital readmission in the weeks to months after discharge. These late sequelae are associated with reduced quality of life and increased healthcare utilization and can eventually lead to further health deterioration and death. A patient's individual clinical course depends on pathogen virulence, size and site of infection, comorbidities, availability of healthcare resources care, etc.

Trompette et al., 2014). Mice in which the gut microbiota is disrupted by antibiotics show increased bacterial dissemination, inflammation, and mortality during pulmonary infection with *Streptococcus (S.) pneumoniae*, *Klebsiella pneumoniae*, *Burkholderia pseudomallei*, or *Mycobacterium tuberculosis* when compared with controls (Clarke, 2014; Dumas et al., 2018; Lankelma et al., 2017a; Schuijt et al., 2016). Moreover, alveolar macrophages derived from gut microbiota-depleted mice show upregulation of metabolic pathways and altered cellular responses, leading to a diminished capacity to phagocytose *S. pneumoniae*, hence resulting in a less pronounced immunomodulatory response (Schuijt et al., 2016). Dysbiosis has been associated with poor outcome in patients with severe infections (Adelman et al., 2020; Haak and Wiersinga, 2017; Krezalek et al., 2016). The gut microbiome of the septic patient is characterized by a decrease in diversity, a lower relative abundance of *Firmicutes* and *Bacteroidetes* with decreased numbers of commensals such as *Faecalibacterium*, *Blautia*, and *Ruminococcus* spp., and an overgrowth of opportunistic pathogens such as *Enterobacter*, *Enterococcus*, and *Staphylococcus* (Haak and Wiersinga, 2017; Lankelma et al., 2017b; McDonald et al., 2016). Most recent data have not only shown the disruptive impact of the septic response, including the effects of treatment, on the composition of the bacterial microbiome, but also on the other kingdoms, such as viruses, fungi, and protozoa (Haak et al., 2021). The impact of these altered gut kingdoms on immune pathways and the host defense against invading pathogens remains undefined.

Sepsis is regarded as an unbalanced immune response, wherein pathogens have evaded protective defense mechanisms and continue to multiply, resulting in persistent stimulation of host cells and injury, and associated with a failure to return to homeostasis (van der Poll et al., 2017). In this unbalanced response, many of the immune mechanisms initially activated to provide protection have become detrimental, both related to excessive inflammation and immune suppression. Longitudinal analyses of immune reactions from early pathogen-host interactions to clinically manifested sepsis in humans are lacking, making a time-dependent reconstruction of sequential proinflammatory and immune-suppressive reactions during the pathophysiological path toward the "septic host response" speculative. Once in the hospital, the host response in patients with sepsis shows signs of concurrent hyperinflammation and immune suppression, involving partially different cell types and organ systems (van der Poll et al., 2017) (Figure 2). The mechanistic underpinnings of the concurrent hyperinflammation and immune suppression and the persistent longitudinal immune system changes in critically ill sepsis patients have yet to be clarified. Persistent immune stimulation in sepsis is not only caused by invading pathogens but also by the release of "damage-associated molecular patterns" (DAMPs, or alarmins), endogenous molecules liberated from injured cells. DAMPs activate PRRs that oftentimes also sense PAMPs, initiating a vicious cycle with sustained immune activation and dysfunction (Deutschman and Tracey, 2014; van der Poll et al., 2017). Patients who remain dependent on intensive care after primary therapeutic measures frequently develop a chronic critical illness termed "persistent inflammation, immunosuppression, and catabolism syndrome" or PICS, which involve many different cell types, organ systems, and pathophysiological mechanisms, with prolonged hyperinflammation, immune suppression, dysregulated myelopoiesis, and catabolism represented by muscle wasting and cachexia as main features (Darden et al., 2021; Gentile et al., 2012).

PROINFLAMMATORY MECHANISMS IN SEPSIS

The "dysregulated host response to an infection" captured in the present Sepsis 3.0 definition (Singer et al., 2016) relates to concurrent unbalanced hyperinflammation and immune suppression. Among the many different cell types and mediator networks implicated in sepsis-associated excessive inflammation, leukocytes (neutrophils, macrophages, natural killer cells), endothelial

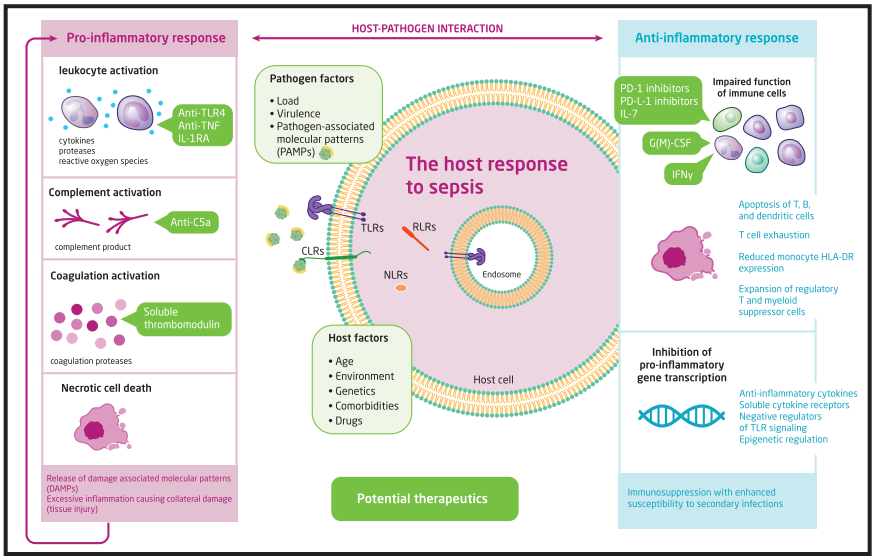

Figure 3. Sepsis immunopathogenesis and immunomodulatory interventions

The host response to sepsis starts with the recognition of an invading pathogen by the immune system. Cell-surface and intracellular pattern recognition receptors, such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), C-type lectin receptors (CLRs), and retinoic acid-inducible gene-like receptors (RLRs) recognize pathogen-associated molecular patterns (PAMPs) after which the inflammatory response is initiated. The scope of the individual immune response depends on pathogen (e.g., microbial load and virulence) and host factors (e.g., age, genetics, comorbidities, and medication). A dysregulated host response can contribute to organ failure and death. The proinflammatory response is characterized by the release of proinflammatory mediators (such as cytokines, proteases, and reactive oxygen species) by multiple cell types, activation of the complement system, the coagulation system, and the vascular endothelium. This can result in the release of alarmins or damage-associated molecular patterns (DAMPs) that can further exacerbate the proinflammatory response and contribute to tissue injury. The anti-inflammatory response is characterized by an impaired function of immune cells (e.g., apoptosis of T cells, B cells and dendritic cells, exhaustion of T cells, expansion of regulatory T cell and myeloid-derived suppressor cells, and reduced human leukocyte antigen-DR isotype (HLA-DR) expression by antigen-presenting cells) and a diminished capacity to produce proinflammatory cytokines, which is in part regulated by epigenetic mechanisms, the release of anti-inflammatory cytokines, and negative regulators of TLR signaling. The green boxes indicate a selection of interventions that specifically target different steps of the inflammatory response in sepsis, ranging from cytokine and complement blocking agents to immune stimulating drugs such as checkpoint inhibitors.

cells, cytokines, complement products, and the coagulation system are prominently featured (Figure 3). Early preclinical studies have introduced the term "cytokine storm" to indicate the strong systemic release of proinflammatory cytokines in experimental animals exposed to intravenous challenges of viable bacteria or their products; these animal models have demonstrated that elimination of proinflammatory cytokines such as tumor necrosis factor (TNF), IL-1β, IL-12, and IL-18 provides strong protection against organ damage and mortality (Wiersinga et al., 2014). While there is consensus now that acute systemic challenge models have little relevance for human sepsis, uncontrolled activity of proinflammatory cytokines still is considered to contribute to injury in sepsis.

Neutrophils can contribute to hyperinflammation in sepsis through the release of proteases and reactive oxygen species. Neutrophils can release neutrophil extracellular traps (NETs) composed of a network of chromatin fibers containing antimicrobial peptides and proteases including myeloperoxidase, elastase, and cathepsin G (Brinkmann et al., 2004). NETs contribute to antibacterial defense mechanisms by trapping and subsequently killing bacteria (Li et al., 2010), and inhibition of NET formation by DNase leads to an increased bacterial burden in blood and a decreased survival of mice with abdominal sepsis (Czaikoski et al., 2016). However, like many components of innate immunity, NETs act as double-edged swords in infection. Excessive NETosis during sepsis can be detrimental through various mechanisms, including induction of intravascular thrombosis and multiple organ failure (McDonald et al., 2017). Histones are abundant in NETs, and NETs can adhere and activate the endothelium during sepsis, resulting in vascular damage in a histone-dependent manner (Clark et al., 2007). NETs and histones can directly damage endothelial and epithelial cells (Saffarzadeh et al., 2012), and cell-free histones mediate lethality induced by high dose LPS or TNF, as well as cecal ligation and puncture (CLP)-induced sepsis in mouse models (Xu et al., 2009). Macrophages can also produce extracellular traps; their potential role in sepsis has not been studied in detail (Doster et al., 2018).

Close links exist between the coagulation and complement systems, which can be viewed upon as two evolutionary cascades originating from shared ancestral pathways. Complement activation results in the release of the anaphylatoxins C3a and C5a, which exert potent proinflammatory activities, including recruitment and activation of leukocytes, endothelial cells, and platelets (Merle et al., 2015). While complement activation is a vital component of protective innate immunity, uncontrolled activation can injure tissues and cause organ failure (Merle et al., 2015). Activation of the coagulation system can be considered part of the innate immune response to an invading pathogen and the term "immunothrombosis" was introduced to support this concept (Engelmann and Massberg, 2013). In agreement, elements of the coagulation system can trigger important innate defense mechanisms and inhibition of coagulation has been shown to impair antibacterial defense in several infection models (van der Poll and Herwald, 2014). In sepsis, activation of the coagulation system becomes unbalanced, resulting in a tendency toward thrombosis in the microvasculature (Levi and van der Poll, 2017). The most severe manifestation of sepsis-associated coagulopathy is disseminated intravascular coagulation (DIC), which apart from thrombosis, can be associated with bleeding due to consumption of clotting factors, anticoagulant proteins, and platelets (Levi and van der Poll, 2017). Tissue factor is the primary initiator of blood coagulation by forming a complex with clotting factor (F) VIIa, thereby inciting blood coagulation by activating FX and FIX (Grover and Mackman, 2018). Tissue factor is constitutively expressed by perivascular cells, such as fibroblasts, pericytes, and epithelial cells, which is important for hemostasis and

vascular integrity. Tissue factor is induced on endothelial cells, monocytes, and macrophages by microbial agents and a variety of inflammatory mediators, including cytokines and complement factors (Grover and Mackman, 2018; Levi and van der Poll, 2017). Under pathological conditions, high quantities of bioactive tissue factor are present in microvesicles derived from several cellular sources, which can bind to other cells, such as activated platelets, neutrophils, and endothelial cells, thereby amplifying coagulation. Early studies have documented the crucial role of tissue factor-FVIIa in models with relevance for sepsis: inhibition of this pathway in humans and non-human primates strongly diminishes coagulation activation after infusion of LPS or bacteria respectively (Levi and van der Poll, 2017). The prothrombotic state in sepsis is aggravated by concomitantly compromised activity of three main anticoagulant pathways, i.e., antithrombin, tissue factor pathway inhibitor (TFPI), and the protein C system (Levi and van der Poll, 2017). Herein, inflammation-driven disruption of the glycocalyx, a glycoprotein-polysaccharide layer covering the endothelium and essential for maintaining its physiological anticoagulant properties, plays a key role, together with decreased expression of thrombomodulin, an endothelial cell receptor that catalyzes the production of the natural anticoagulant activated protein C after forming a complex with thrombin. Platelets further contribute to coagulation and inflammation, both directly through cell-to-cell contact (e.g., complex formation with leukocytes) and indirectly through the release of proteases and other mediators (Kerris et al., 2020). Platelets increase endothelial cell adhesion and leukocyte extravasation at sites of inflammation and enhance activation of neutrophils (McDonald et al., 2012). Platelets and platelet-derived microparticles express phospholipids (including phosphatidylserine), which increase the activity of tissue factor, FVa and FXa, thereby facilitating coagulation (Kerris et al., 2020).

Bimodal interactions exist between coagulation and innate immunity. Neutrophil elastase and cathepsin G, together with externalized nucleosomes, stimulate coagulation and intravascular thrombus growth *in vivo* by mechanisms that involve enhanced tissue factor and FXII-dependent coagulation and local proteolysis of the coagulation inhibitor TFPI (Massberg et al., 2010). Platelets contribute to hyperinflammation and thrombosis in sepsis by mechanisms that partially involve neutrophils. Activated platelets can attract leukocytes to sites of infection, form complexes with neutrophils, reduce their threshold to release NETs, and enhance their ability to kill pathogens (Clark et al., 2007; Jenne et al., 2013). NETs in turn promote platelet adhesion, activation, and aggregation (Fuchs et al., 2011). In mouse models of sepsis platelet aggregation, thrombin activation and fibrin clot formation occur within NETs *in vivo*, which is crucial for the development of sepsis-induced intravascular coagulation (McDonald et al., 2017). Intravascular coagulation induced by NETs is dependent on a cooperative interaction between histone H4 in NETs, platelets, and the release of inorganic polyphosphate (McDonald et al., 2017). The extent of NET formation is predictive of the development of DIC and mortality in patients with sepsis, further pointing at a role for NETs in sepsis associated coagulopathy (Abrams et al., 2019).

Tissue factor and the clotting factors FVIIa, FXa, thrombin, and fibrin can induce proinflammatory signaling, particularly by activation of members of the G-protein-coupled protease-activated receptor family (Alberelli and De Candia, 2014). Complement factors can activate coagulation proteases and vice versa. For example, FIXa, FXa, FXIa, and thrombin can convert C3 and C5 into C3a and C5a, respectively, whereas C5a and the membrane attack complex (C5b-9) can stimulate expression of tissue factor on endothelial cells (Keragala et al., 2018). C5a can facilitate clotting further by disturbing the endothelial glycocalyx function. Recent studies have implicated inflammasomes and gasdermin D (GSDMD) in the interaction between inflammation and coagulation. Triggering of inflammasomes results in the activation of inflammatory caspases (Lamkanfi and Dixit, 2014). Among these, caspase-1 can be activated by the bacterial products flagellin and type III secretion system (T3SS) proteins, while caspase-11 can be triggered by cytoplasmatic LPS (Hagar et al., 2013; Kayagaki et al., 2013; Zhao et al., 2011). Both caspase-1 and caspase-11 cleave GSDMD, which can form pores in plasma membranes, thereby facilitating IL-1β release, IL-18 release, and pyroptosis (Evavold et al., 2018; Liu et al., 2016; Sborgi et al., 2016). Recent research has implicated GSDMD-mediated pyroptosis of monocytes and macrophages, mediated by caspase-1 or caspase-11, in activation of the coagulation system by permitting the release of tissue factor containing microvesicles from pyroptotic cells (Wu et al., 2019). Tissue-factor-mediated DIC elicited by intravenous injection of the *E. coli* T3SS inner rod protein EprJ is abolished in *Casp1*$^{-/-}$ and *Gsdmd*$^{-/-}$ mice; likewise, infection with viable *E. coli* and administration of rod proteins from other bacterial strains (*Burkholderia* BsaK and *Salmonella* PrgJ) trigger severe coagulation activation in wild-type but not *Gsdmd*$^{-/-}$ mice (Wu et al., 2019). Genetic ablation of the IL-1β or IL-18 receptor does not affect EprJ-induced coagulation, confirming the role of pyroptosis herein (Wu et al., 2019). Pyroptosis also was crucial for coagulation activation induced by LPS, as demonstrated by systemic coagulopathy in LPS-challenged wild-type mice primed with poly I:C, but not in *Casp11*$^{-/-}$ or *Gsdmd*$^{-/-}$ mice (Wu et al., 2019; Yang et al., 2019). Similar observations have been made after infection with *E. coli* or during polymicrobial sepsis induced by CLP (Yang et al., 2019). GSDMD enhanced tissue factor activity by inducing calcium release and subsequent phosphatidylserine exposure on leukocytes (Yang et al., 2019). Of note, some data suggest that GSDMD-mediated increased phosphatidylserine exposure is sufficient for tissue factor release, not requiring pyroptosis (Yang et al., 2019). Caspases other than caspase-1 and caspase-11 can also contribute to coagulation activation through GSDMD cleavage. Transmembrane protein 173 (TMEM173) is an endoplasmic protein that acts as an amplifier of inflammation (Ishikawa and Barber, 2008). Recently, myeloid cell TMEM173 (STING) has been shown to drive tissue factor release and blood coagulation via GSDMD activation in experimental sepsis in mice induced by CLP, *E. coli*, or *S. pneumoniae* (Zhang et al., 2020). Caspases involved in TMEM173-mediated GSDMD cleavage depend on the inciting pathogen: caspase-11 for *E. coli* and caspase-8 for *S. pneumoniae* (Zhang et al., 2020). An additional mechanism of coagulation activation in sepsis may be mediated by high mobility group box 1 (HMGB1). This nuclear protein acts as a DAMP once released into the extracellular environment and inhibition of HMGB1 exerts protective effects in preclinical sepsis models (Wang et al., 1999; Yang et al., 2004). Exogenous HMGB1 can form molecular complexes with LPS that can be transported into the lysosomes of

macrophages and endothelial cells via the receptor for advanced glycosylation end-products (RAGE), resulting in HMGB1-mediated destabilization of lysosomal membranes with subsequent release of LPS into the cytosol and activation of caspase 11-dependent pyroptosis and NLRP3 inflammasome activation and lethal coagulation activation (Deng et al., 2018). HMGB1-mediated GSDMD activation and pore formation is necessary to activate tissue factor through phosphatidylserine exposure, rather than via GSDMD-mediated pyroptosis (Deng et al., 2018). Together, these data place caspase-mediated GSDMD cleavage, resulting in tissue factor release, at the center stage of DIC and expose inflammasome activation as an important link between inflammation and coagulation.

IMMUNE SUPPRESSION IN SEPSIS

Immune suppression in patients with sepsis involves different cell types and characteristics and is related to enhanced apoptosis of immune cells, T cell exhaustion, reprogramming of cells through epigenetic changes, and reduced expression of activating cell surface molecules (Hotchkiss et al., 2013; van der Poll et al., 2017). Immune suppressive changes have been implicated in the increased susceptibility of sepsis patients to secondary infections, often caused by opportunistic pathogens, and viral reactivation (Ong et al., 2017; Otto et al., 2011). Apoptosis especially occurs in $CD4^+$ T cells, $CD8^+$ T cells, B cells, natural killer (NK) cells, and follicular dendritic cells, and both death receptor- and mitochondrial-mediated pathways are involved (Boomer et al., 2011; Chang et al., 2007; Hotchkiss et al., 1999; Shankar-Hari et al., 2017a). Sepsis-associated B cell depletion is related to enhanced apoptosis or deficient helper T cell support and occurs post-activation, affects the memory B cell subsets the most (Shankar-Hari et al., 2017a), and remaining B lymphocytes have an exhausted phenotype characterized by decreased major histocompatibility complex class II (MHC class II) expression and increased production of the anti-inflammatory cytokine IL-10 (Gustave et al., 2018). Interventions that inhibit or prevent apoptosis confer protection in several preclinical sepsis models, signifying the functional importance of enhanced apoptosis (Hotchkiss et al., 2013). Impaired autophagy, a process that removes redundant or dysfunctional cellular components, may also contribute to immunosuppression, i.e., mice with a reduced autophagy capacity in lymphocytes by cell-specific loss of *Atg5* or *Atg7* show an increased mortality together with immune dysfunction in abdominal sepsis, and Atg5 deficiency in T cells resulted in higher release of IL-10 by these cells during sepsis (Oami et al., 2017).

T cells from blood, as well as spleen and lung, from sepsis patients have a reduced capacity to produce cytokines (Boomer et al., 2011; Heidecke et al., 1999; Venet et al., 2004). $CD8^+$ T cells show attenuated cell proliferation, impaired cytotoxic function, and attenuated IL-2 and interferon (IFN)-γ production (Boomer et al., 2011; Danahy et al., 2016). The anti-inflammatory milieu in sepsis is further shaped by increased numbers of regulatory T (Treg) cells (Huang et al., 2010; Venet et al., 2004) and myeloid-derived suppressor cells (MDSCs), which represent a mixed population of predominantly immature myeloid cells that suppress effector immune cells, particularly T cells (Ost et al., 2016). MDSCs can impede immune functions through a number of mechanisms, including deprivation of L-arginine (essential for T cell functions), stimulation of Treg cell expansion and inhibition of macrophage and dendritic cell functions (Ost et al., 2016). Expansion of MDSCs is associated with an increased risk of secondary infections in critically ill patients with sepsis (Mathias et al., 2017; Uhel et al., 2017). Neutrophils show several immune compromised features in sepsis, including migration to a variety of chemoattractants, decreased intracellular myeloperoxidase and lactoferrin content, and reduced oxidative burst capacity (Demaret et al., 2015). Kinome profiling of neutrophils have uncovered impaired kinase activity in neutrophils from sepsis patients when compared with critically ill patients without infection, further suggesting an immune-suppressed neutrophil phenotype (Hoogendijk et al., 2019).

Recent studies have implicated checkpoint regulators in sepsis-induced immune suppression. Checkpoint regulators are membrane-bound proteins, which function as a second signal to direct the immune response (either inhibitory or stimulatory) to a specific antigen (Wakeley et al., 2020). A checkpoint regulator extensively studied in the field of sepsis is programmed cell death-1 (PD-1). Triggering of PD-1 on T cells results in release of immunosuppressive molecules and may culminate in apoptosis. Enhanced peripheral blood T cell expression of PD-1 was associated with attenuated T cell proliferative capacity, increased incidence of nosocomial infections, and increased mortality in patients with sepsis (Guignant et al., 2011). In addition, sepsis patients show enhanced expression of PD-1 on blood monocytes and granulocytes (Monaghan et al., 2012), and a postmortem study reports increased PD-1 expression on T cells and increased PD-L1 and PD-L2 expression on dendritic cells (Boomer et al., 2011). The expression of PD-1 on T cells and PD-L1 on antigen presenting cells show associations with lymphopenia, T cell apoptosis, and mortality in patients with sepsis (Boomer et al., 2011; Chang et al., 2014; Guignant et al., 2011). The functional relevance of these observational reports is supported by the finding of reduced apoptosis and enhanced IFN-γ production upon e*x vivo* treatment of $CD8^+$ T cells from sepsis patients with an anti-PD-1 antibody (Chang et al., 2014). Moreover, increased PD-L1 expression on neutrophils and monocytes correlates with an impaired phagocytic capacity of these cells, and *ex vivo* treatment with an anti-PD-1 antibody improved the phagocytic capacity of blood leukocytes harvested from sepsis patients (Patera et al., 2016). The pathobiological significance of the PD-1 pathway is further supported by reports of improved survival of septic mice with blocked or genetically eliminated PD-1 (Brahmamdam et al., 2010; Huang et al., 2009). These results have raised the hypothesis that PD-1 and/or PD-L1 pathway inhibitors may reverse sepsis-induced immune suppression and several phase I and phase II clinical trials with an anti-PD-L1 antibody have been done in sepsis patients; anti-PD-L1 treatment has been well tolerated (Hotchkiss et al., 2019a; Hotchkiss et al., 2019b; Watanabe et al., 2020) and induced an increase in absolute lymphocyte counts and monocyte human leukocyte antigen-DR isotype (HLA-DR) expression (Watanabe et al., 2020). Although abundant literature hints at possible roles of other checkpoint regulators in immune suppression, studies directly addressing their role in sepsis are scarce (Wakeley et al., 2020). Among these cytotoxic T lymphocyte antigen (CTLA)-4 is a negative

checkpoint regulator expressed on T cells that binds B cell activation antigens B7-1 (CD80) and B7-2 (CD86). $CD4^+$ T cells, $CD8^+$ T cells, and Treg cells show increased CTLA-4 expression after induction of abdominal sepsis in mice, and treatment with an anti-CTLA-4 antibody decreases sepsis-induced apoptosis in the spleen and improved survival (Inoue et al., 2011). Anti-CTLA-4 treatment also reduces mortality caused by fungal sepsis following bacterial sepsis from sub-lethal cecal ligation and puncture in mice, which is accompanied by increased IFN-γ production by splenocytes (Chang et al., 2013).

A key feature of immune suppression is a reprogramming of monocytes and macrophages, with an impaired capacity to produce proinflammatory cytokines upon *ex vivo* stimulation with bacterial agonists (also referred to as "LPS tolerance") and a reduced surface expression of MHC class II (Boomer et al., 2011; Döcke et al., 1997; Presneill et al., 2002). Blood leukocytes of septic patients have a diminished ability to release proinflammatory cytokines such as TNF, IL-1α, IL-6, and IL-12 following stimulation *ex vivo*, whereas their ability to release anti-inflammatory mediators such as IL-1 receptor antagonist and IL-10 is either unimpaired or enhanced (Adib-Conquy et al., 2006; Munoz et al., 1991; van Deuren et al., 1994). Reduced proinflammatory responses upon restimulation of blood leukocytes may relate to impairments in cellular NF-κB phosphorylation capacity, as indicated by intracellular flow cytometry analyses of *ex vivo* stimulated monocytes, $CD4^+$ T cells, $CD8^+$ T cells, B cells, and neutrophils from patients with sepsis (Hoogendijk et al., 2017). The anti-inflammatory phenotype observed in blood leukocytes in a LPS-tolerant state has also been demonstrated in organ-specific monocytes, including in the lungs in animal peritonitis and post-mortem septic patients (Boomer et al., 2011; Philippart et al., 2012; Reddy et al., 2001). Cross-tolerance refers to the finding that stimulation with one bacterial agonist can cause a reduced capacity to produce proinflammatory cytokines by multiple other agonists. For example, blood leukocytes obtained from human subjects injected with LPS *in vivo* displayed reduced *ex vivo* cytokine production in response to TLR2, TLR4, TLR5, and TLR7 ligands and to whole bacteria (de Vos et al., 2009). DAMPs also induce a cross-tolerization with LPS, indicating multiple mechanisms that can induce reprogramming of mononuclear cells in sepsis (Aneja et al., 2008; Austermann et al., 2014). Of note, mouse studies have suggested that some cell types do not show this "tolerant" phenotype and even become primed, including alveolar macrophages, Kupffer cells, microglial cells, and lymphocytes in the intestinal epithelium and skin (Rubio et al., 2019). A characteristic feature of sepsis is diminished expression of HLA-DR on blood monocytes, which is a well-established surrogate marker for sepsis-induced immunosuppression that correlates with impaired outcomes such as a higher incidence of nosocomial infections and increased mortality (Landelle et al., 2010; Leijte et al., 2020).

Epigenetic regulation of gene expression—in particular, through histone modifications and DNA methylation—contributes to the reprogramming of immune cells in sepsis (van der Poll et al., 2017). Gene transcription is regulated by organization of gene loci on chromatin into transcriptionally active or silent states (Vachharajani et al., 2014). Histones shape DNA in a chromatin structure, whereby particular histone modifications can wind or unwind chromatin to make it inaccessible (heterochromatin) or accessible (euchromatin) for transcription, respectively. Histone acetylation of lysines typically endorses transcription, while methylation can support either active euchromatin or silent heterochromatin formation, depending on the lysine that is methylated (Vachharajani et al., 2014). Among different histone modifications, methylation of histone 3 lysine-4 (H3K4) and histone 3 lysine-27 (H3K27) is highly correlated with activation and repression of transcription, respectively. In a pivotal study, downregulation of marks of open chromatin, such as histone 3 lysine-4 trimethylation (H3K4me3), has been shown to underlie LPS-induced tolerance in monocytes (Foster et al., 2007). LPS-tolerant macrophages display increased amounts of the repressive histone modification H3K9me2 at the promoter regions of the genes encoding IL-1β and TNF (Chan et al., 2005; El Gazzar et al., 2009). Similar results have been obtained in LPS-tolerant blood monocytes from septic patients (El Gazzar et al., 2009). Molecular mechanisms underlying LPS effects on epigenetic regulation of gene transcription include increased expression of the histone demethylase KDM6B (JMJD3) through NF-κB activation (De Santa et al., 2007) and accumulation of the histone deacetylase sirtuin-1 at the promoters of *TNF* and *IL1B,* resulting in inhibition of gene transcription (Liu et al., 2011). Single-cell transcriptomics of peripheral blood mononuclear cells and dendritic cells from sepsis patients have disclosed an expansion of a unique $CD14^+$ monocyte population, named MS1, which (relative to other $CD14^+$ monocytes) displayed an immunosuppressive phenotype, i.e., reduced MHC class II expression and a reduced capacity to activate NF-κB and produce TNF upon *ex vivo* stimulation with LPS (Reyes et al., 2020). This study indicates that single-cell analyses might be useful to gain insight into distinct cellular phenotypes (proinflammatory versus immune suppressive) in sepsis (Reyes et al., 2020).

Infection-induced epigenetic changes are long-lasting. Mice recovered from abdominal sepsis show a sustained depression of dendritic-cell-derived IL-12 that lasted for at least 6 weeks, caused by histone modifications affecting transcription of the genes encoding IL-12p35 and IL-12p40 (Wen et al., 2008). Moreover, pneumonia in mice causes long-lasting epigenetic changes that tolerized macrophages and decreased their capacity to phagocytose antigen-nonspecific, unrelated bacteria (Roquilly et al., 2020). Recent mouse studies documented that sepsis induces epigenetic alterations in cells in the bone marrow (Davis et al., 2019). Bone-marrow-derived macrophages obtained from mice recovered from abdominal sepsis show decreased expression of mixed-lineage leukemia 1 (*Mll1*), an epigenetic enzyme, and impairs H3K4me3 (activation mark) at NF-κB-binding sites on inflammatory gene promoters (Davis et al., 2019). Bone marrow transfer experiments have demonstrated that epigenetic modifications initiated in bone marrow progenitor stem cells following sepsis results in long-lasting impairment in peripheral macrophage function (Davis et al., 2019). As such, these results suggest that immune-suppressive changes persist in subsequent generations of leukocytes, which may have long-term consequences related to morbidity and mortality.

The central nervous system (CNS) participates in the regulation of the immune response (Chavan et al., 2017). The CNS can modify peripheral immune functions through the neuro-immune inflammatory reflex, which comprises sensory input from peripheral tissues via the afferent vagus nerve to the CNS and

stimulation of the efferent vagus nerve resulting in acetylcholine mediated inhibition of TNF production (Rosas-Ballina et al., 2011; Wang et al., 2003). The contribution of disturbed neuro-immune interactions in sepsis-induced immune suppression remains to be established.

Sepsis subphenotypes and endotypes

While the Sepsis 3.0 definition distinguishes sepsis (organ dysfunction associated with infection) from infection per se (Singer et al., 2016), it does not capture the heterogeneity and the underlying pathophysiology of this syndrome. This heterogeneity is considered a major factor in the failure of immune modulatory trials in patients with sepsis, and it has been proposed that stratification of patients in subgroups with shared features can improve effects of therapies targeting specific pathophysiological mechanisms, in particular if patient classification is based on characteristics of the host response (Marshall, 2014; Stanski and Wong, 2020). In this regard, precision medicine, referring to diagnostic and therapy strategies that take individual patient characteristics into account, is only in its infancy in the field of sepsis (Shankar-Hari and Rubenfeld, 2019; Stanski and Wong, 2020). Key concepts in precision medicine are prognostic and predictive enrichment of patient populations. Prognostic enrichment relates to selection of patients with a high likelihood of a relevant disease outcome—in sepsis, usually mortality. Predictive enrichment refers to selection of patients who are more likely to respond favorably to a specific therapy based on a biological mechanism. For sepsis, predictive enrichment is hampered by a relatively limited understanding of the dominant pathobiological mechanisms driving this complex syndrome. There is general agreement that in order to successfully implement precision medicine in sepsis, concurrent application of prognostic and predictive enrichment is warranted (Shankar-Hari and Rubenfeld, 2019; Stanski and Wong, 2020). The terminology used to indicate subgroups is not consistent; here, we use the term endotype to indicate a biological subtype defined by distinct pathophysiological mechanisms and the term subphenotype to indicate a group characterized by a set of features not necessarily linked by a common pathophysiological mechanism (Reddy et al., 2020; Seymour et al., 2017). Because endotypes and subphenotypes are derived from different patient characteristics, they cannot be easily interconnected.

In recent years, attempts have been made to subdivide adult sepsis patients into distinct groups, using clinical and/or host response data and unbiased computational analysis tools such as machine learning, latent class analysis, and K means clustering (DeMerle et al., 2021; Reddy et al., 2020). These efforts have included both prognostic and predictive enrichment strategies and were pioneered by Hector Wong and colleagues in pediatric sepsis (reviewed in Wong, 2021). In adult patients, several investigators have identified sepsis subgroups with different disease outcomes and (in some studies) biological mechanisms using readily available clinical and routine laboratory data (Bhavani et al., 2019; Seymour et al., 2019; Zador et al., 2019; Zhang et al., 2018). One study has identified four subphenotypes based on trajectory modeling of repeated temperature measurements; four temperature trajectory groups were identified: "hyperthermic, slow resolvers" (14.9%), "hyperthermic, fast resolvers" (23.2%), "normothermic" (32.8%), and "hypothermic" (29.1%), with different ages, comorbidity frequencies and mortality rates (highest in hypothermic subjects), and inflammatory marker concentrations (higher in hyperthermic, slow resolvers) (Bhavani et al., 2019). Another investigation finds four subphenotypes with distinct profiles regarding type of organ dysfunction (respiratory, neurological, multiple organs) and mortality rates, with relevance for response to intravenous fluids (better in the profile corresponding with multiple organ dysfunction and the highest mortality) (Zhang et al., 2018). Four clinical subphenotypes have been identified using data obtained at presentation to the emergency department: the α subphenotype (prevalence 33%; with less organ dysfunction and a mortality of 2%), the β subphenotype (prevalence 27%; with more chronic illness and renal dysfunction and a mortality of 5%), the γ subphenotype (prevalence 27%; with more inflammation and higher temperature and a mortality of 15%), and the δ subphenotype (prevalence 13%; with higher lactate and more hypotension and a mortality of 32%) (Seymour et al., 2019). In simulation models the proportion of clinical trials reporting benefit, harm or no effect changed by varying the subphenotype frequency in the study population (Seymour et al., 2019).

While many earlier studies have evaluated the prognostic value of individual host response biomarkers in patients with sepsis (Pierrakos et al., 2020), the use of enrichment strategies in sepsis trials based on host response measurements has been limited thus far. In this regard, the MONARCHS trial represents an early example: this trial tested the effects of an anti-TNF antibody in patients with sepsis, wherein the *a priori* population for efficacy analysis were patients with high baseline IL-6 amounts as defined by a positive rapid test result (Panacek et al., 2004). With this strategy the investigators sought to enrich the population with patients with more severe systemic inflammation, anticipating that anti-TNF would have the greatest effect in this subgroup. Several trials evaluating the effects of immune stimulants in sepsis sought to enrich the population with patients suffering from immune suppression, as reflected by low expression of HLA-DR on circulating monocytes and/or low lymphocyte counts (Döcke et al., 1997; Francois et al., 2018; Hotchkiss et al., 2019a). The SCARLET trial, which evaluated the effect of soluble thrombomodulin in patients with sepsis-associated coagulopathy, used the platelet count and international normalized ratio to enrich the population for patients more likely to benefit from this anticoagulant treatment (Vincent et al., 2019). Retrospective analyses of clinical trial data testing the efficacy of recombinant IL-1 receptor antagonist in sepsis has demonstrated that, while this treatment does not convey benefit in the overall population, it did reduce mortality in subgroups identified by high baseline (endogenous) IL-1 receptor antagonist concentrations (Meyer et al., 2018) or hepatobiliary dysfunction and DIC (Shakoory et al., 2016). Subsequent studies indicate that high plasma ferritin concentrations may identify sepsis patients with a "macrophage activation-like syndrome", and hyperferritinemia is now evaluated as a biomarker for hyperinflammation that might inform patient selection in clinical trials investigating anti-inflammatory strategies (Karakike and Giamarellos-Bourboulis, 2019).

Other studies used "omics" techniques to obtain insight into the host response and identify subgroups of patients. As an example of a prognostic enrichment analysis using combined proteomic-metabolomic data, relative impairments of fatty acid

transport and β-oxidation, gluconeogenesis, and the citric acid cycle were found to be associated with a higher risk of sepsis related death; changes in the metabolome were correlated with alterations in the proteome and a seven-metabolite panel could predict mortality at the time of presentation to the emergency department (Langley et al., 2013). A study that combined human genetics, metabolite and cytokine measurements in patients, and testing in a mouse model exposed the involvement of the methionine salvage pathway in the pathophysiology of sepsis (Wang et al., 2017). High plasma concentrations of the pathway's substrate methylthioadenosine were associated with mortality in patients with sepsis and correlated with proinflammatory cytokine concentrations, indicating that increased plasma methylthioadenosine marks patients with disproportionate inflammation. Combination of methylthioadenosine and other variables produced 80% accuracy in predicting death (Wang et al., 2017).

Most studies published thus far have used blood leukocyte transcriptome data to stratify sepsis patients based on their immune response. In a prospective cohort entailing 98 children with septic shock, Hector Wong and colleagues used unsupervised hierarchical clustering to identify three endotypes, named subclasses A, B, and C (Wong et al., 2009b). Subclass A showed reduced expression of genes associated with adaptive immunity and glucocorticoid receptor signaling and presented with more severe disease and higher mortality rates. Corticosteroid therapy, prescribed at the discretion of the treating physicians, was independently associated with mortality in children in subclass A, suggesting that this classification method possibly can inform therapeutic decisions. This pioneering genomics work in pediatric sepsis has been used to generate a classification and regression tree model for mortality risk based on protein biomarkers, not only in children (Wong et al., 2012) but also in adults (Wong et al., 2014). Several groups independently described sepsis endotypes based on whole-blood leukocyte gene expression profiles in adult patients with sepsis (DeMerle et al., 2021; Reddy et al., 2020). In a prospective cohort of 265 patients with sepsis caused by community acquired pneumonia (CAP), two distinct sepsis response signatures (SRS) have been identified, named SRS1 and SRS2 (Davenport et al., 2016), which were validated in an independent cohort of patients with CAP or fecal peritonitis (Burnham et al., 2017). SRS1 was associated with a higher mortality, and its gene expression profile indicated an immune suppressive phenotype, with LPS tolerance, HLA-II downregulation, and T cell exhaustion (Burnham et al., 2017; Davenport et al., 2016). The SRS1 and SRS2 endotypes could also be identified in a secondary analysis of a randomized clinical trial ("VANISH") investigating the effect of corticosteroid therapy in septic shock (Antcliffe et al., 2019). Of interest, using a simplified model entailing seven genes, corticosteroid therapy has been found to be associated with increased mortality in the SRS2 endotype and no treatment effect in SRS1 patients, suggesting that this classification method could have relevance for therapeutic decisions (Antcliffe et al., 2019). However, in this respect, confirmation is needed, also considering that while SRS1 shows similarities with the pediatric subclass A, both suggestive of immune suppression, corticosteroid therapy has been associated with increased mortality in subclass A but not in SRS1 (Antcliffe et al., 2019; Wong et al., 2015); moreover, a recent re-analysis of the aforementioned VANISH trial provided further evidence that corticosteroid exposure may be associated with increased mortality among adult septic shock endotype A patients (Wong et al., 2021). Other investigators have identified four sepsis endotypes, named MARS1 to MARS4, based on blood leukocyte genome-wide RNA expression data in patients with sepsis from different infection sources (Scicluna et al., 2017). MARS endotypes were validated in two independent cohorts, including the patient group used to derive SRS1 and SRS2 (Davenport et al., 2016; Scicluna et al., 2017), and were associated with distinct host response profiles and clinical outcomes (Scicluna et al., 2017). MARS1 was consistently related with a poor outcome, and its immune profile was indicative of blunted innate and adaptive immunity. MARS3 had a relatively low mortality risk, with an RNA expression pattern consistent with an upregulation of adaptive immunity and increased T cell function (Scicluna et al., 2017). Comparative analyses demonstrated considerable overlap between MARS3 and the earlier-described SRS2 endotype (Davenport et al., 2016; Scicluna et al., 2017). In yet another analysis, using whole-blood genome-wide RNA expression data from a collection of small studies, three subgroups have been identified, named "inflammopathic" (characterized by innate immune activation and a higher mortality), "adaptive" (adaptive immune activation; lower mortality), and "coagulopathic" (platelet degranulation and coagulation dysfunction; higher mortality and older) (Sweeney et al., 2018). Overlap was found with sepsis endotypes described earlier (Davenport et al., 2016; Wong et al., 2009a): the inflammopathic subgroup corresponded most closely to SRS1 and pediatric subclass B, while the adaptive subgroup corresponded to SRS2; a comparison with MARS endotypes was not done (Sweeney et al., 2018).

Collectively, these data indicate that blood leukocyte genomic data can be used to stratify sepsis patients in distinct groups with different immune profiles and clinical outcomes, and possibly with different responses to certain sepsis therapies. Much work still needs to be done, however. While similarities between subphenotypes and endotypes described by different groups of investigators are encouraging, clear differences remain. In this respect, an international effort to reach consensus on blood leukocyte genomic endotypes in sepsis would be of great help, with the field of oncology providing excellent examples (Bijlsma et al., 2017). Derivation of a relatively small set of genes reflective of (consensus) endotype identity would subsequently allow additional and independent studies to evaluate the relevance for sepsis pathophysiology and treatment responses on a much larger scale.

CONCLUDING REMARKS

Although our understanding of sepsis immunology has improved in recent decades, these insights have not translated into effective treatments. This makes a case to acquire a more robust and overarching understanding of the immune pathogenesis of sepsis. An increasing number of investigators seek to unravel the complexity of sepsis through high-dimensional data analysis, enabled by advances in "omics" immune-profiling technologies, allowing simultaneous analyses of multiple molecular layers such as RNA, proteins, lipids, and metabolites (Schuurman et al., 2021). In addition, it will be key to not only acquire a better

understanding of systemic immunity but also of local tissue-specific responses in sepsis (Cavaillon et al., 2020). In the future, treatment selection will likely be based on the immunological profile of a patient, possibly in combination with clinical phenotypes. This is exemplified by the ImmunoSep trial in which patients with sepsis are treated with the inflammation inhibitor IL-1 receptor antagonist or the immunostimulant IFN-γ depending on respectively elevated ferritin (indicating hyperinflammation) or decreased monocyte HLA-DR amounts (indicating immune suppression; https://www.immunosep.eu/) (ClinicalTrials.gov identifier: NCT04990232). Prognostic and particularly predictive enrichment strategies will decrease heterogeneity and thus aid in the design of future clinical trials to better target patient populations that may benefit from immunotherapy. For the next iteration of the sepsis definition, Sepsis-4, it has been envisioned that sepsis and septic shock are no longer defined as a syndrome but rather as a group of identifiable diseases, each characterized by specific cellular alterations and linked biomarkers (Abraham, 2016). A major challenge herein will be to not only distinguish pathobiological subgroups in the patient population now classified as "septic," but also to discover—through combined in-depth observational studies in patients and mechanistic studies in the laboratory—which pathophysiological and targetable pathway(s) drive(s) disease in an individual patient. Machine learning, the subfield of artificial intelligence (AI) that uses data and algorithms to emulate human learning, could aid in processing large amounts of data, detect meaningful patterns of information, and thereby aid in the diagnosis, prognostication, and treatment of sepsis (Vigilante et al., 2019).

More attention to prevention of long-term consequences of sepsis is warranted given the large proportion of sepsis survivors that experience impaired health status illustrated by cognitive impairments, brain dysfunction, cardiovascular events, and an increased rate of hospital readmissions in the weeks to months after discharge (Prescott and Angus, 2018; Prescott et al., 2020). These late consequences of sepsis lead to a reduced quality of life and often an inability to return to prior engagements (Prescott and Angus, 2018; Prescott et al., 2020). The underlying molecular mechanisms of these post-sepsis sequelae are still ill-defined. A recent study showed that, in humans, post-infection immune reprogramming can persist for up to six months after resolution of inflammation, providing a potential mechanism for the increased vulnerability to recurrent infection in post-sepsis patients (Roquilly et al., 2020). This in in line with several studies that suggested a trajectory of inflammation and increased immunosuppression in sepsis patients up to a year after ICU discharge as exemplified by persistently elevated systemic concentrations of high sensitivity C-reactive protein (CRP), IL-7, soluble PD-1, and soluble PD-L1 (Riché et al., 2018; Yende et al., 2019). Further insight into post-sepsis immunity could identify treatment targets to correct these immune derangements in order to improve long-term sepsis outcomes.

Almost three centuries after the first use of the term "sepsis" and more than 30 years after the first clinical definition of sepsis (Funk et al., 2009), sepsis remains an ill-defined syndrome that cannot be treated by specific therapeutics. Implementation of precision medicine for patients with sepsis, wherein immune therapy is guided by host response biomarkers reflecting targetable pathophysiological changes driving pathology in a time- and individual-dependent manner, will be the major challenge in the years ahead.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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