



Current gaps in sepsis immunology: new opportunities for translational research

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Increasing evidence supports a central role of the immune system in sepsis, but the current view of how sepsis affects immunity, and vice versa, is still rudimentary. The European Group on Immunology of Sepsis has identified major gaps that should be addressed with high priority, such as understanding how immunological alterations predispose to sepsis, key aspects of the immunopathological events during sepsis, and the long-term consequences of sepsis on patient's immunity. We discuss major unmet topics in those three categories, including the role of key immune cells, the cause of lymphopenia, organ-specific immunology, the dynamics of sepsis-associated immunological alterations, the role of the microbiome, the standardisation of immunological tests, the development of better animal models, and the opportunities offered by immunotherapy. Addressing these gaps should help us to better understand sepsis physiopathology, offering translational opportunities to improve its prevention, diagnosis, and care.

Introduction

Sepsis and septic shock definitions were revised in 2016 to address key limitations of the previous iterations that included a simplistic illness model, which implied that sepsis transitions from a systemic inflammatory response to a compensatory anti-inflammatory response syndrome. Other limitations included a lack of explicit unifying clinical criteria^{1,2} and outdated understanding of sepsis pathophysiology.¹ Sepsis-3 definitions have shifted the focus away from a non-specific inflammation to sepsis as organ dysfunction caused by a dysregulated host response to infection with the sequential organ

failure assessment as the central diagnostic component.^{3,4} 1 year after the introduction of Sepsis-3, the WHO highlighted sepsis as a global health priority.⁵

Although Sepsis-3 is not free from controversy,^{6,7} the newly set focus on the host response and organ derangements has prompted new ways to approach sepsis immunobiology to identify unmet challenges in basic and clinical sepsis research.⁸ To address this deficit, the European Group on Immunology of Sepsis (EGIS) has identified key gaps in the current knowledge on sepsis immunology. The Sepsis-3 definitions refer to sepsis as dysregulated or dysfunctional host immune

Key messages

- The timeline of immunological events in sepsis must be elucidated, identifying the immunological changes induced by sepsis and those present before sepsis, which constitute risk factors for the disease
- Our knowledge on sepsis immunology is mostly based on what we learnt from studies in blood. We need to place more emphasis on understanding the immunological alterations occurring in organs, and their implications in the pathophysiology of sepsis
- We identify pending research challenges regarding key actors of the immune system, beyond T lymphocytes and antigen presenting cells (B cells, myeloid derived suppressor cells, neutrophils, neutrophil extracellular traps)
- A unified mechanistic framework is needed to understand the association between the immunity and pathological responses in sepsis.
- The microbiome plays a major but poorly understood role in shaping the immune response to infection, which could influence the risk of sepsis and the outcome after sepsis is established
- Sepsis survivors show important immunological alterations, which could play major pathogenic roles in the consequences of this disease in the long term
- Animal models that better mimic human immunopathology in sepsis are needed
- Reinterpretation and better standardisation of common immunological tests and emerging tests might offer new opportunities for improving detection and severity stratification of this disease
- Individualisation of treatment based on immunological profiling could help to improve the chances of immunotherapy to work
- Addressing the immunological gaps in the pre-sepsis phase, during sepsis, and in the post-sepsis phase will help to design better preventive, diagnostic, and treatment approaches to lower the morbidity and mortality associated with this disease

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and non-immune responses to infection without explicitly defining the nature and mechanisms of those dysregulations. In our view, this reflects the poor understanding of sepsis immunopathology. The lack of clear immune blueprints in sepsis generates many ambiguities and conceptual challenges. We review and discuss the major gaps in sepsis immunology at the pre-sepsis stage, during sepsis evolution, and at the post-sepsis period.

Gaps in the pre-sepsis period

An immunological profile predisposing to sepsis

Compelling evidence exists to support the connection between the basal immunological status and sepsis (table 1). As many as 24% of children with primary immunodeficiency diseases suffer from sepsis during their childhood.⁹ In a cohort study with 98 344 individuals, Warny and colleagues¹⁰ showed that, in the general population, lymphopenia (lymphocyte count $<1 \cdot 1 \times 10^9$ cells

per L) was associated with an increased risk of sepsis. Similarly, Furst and colleagues¹¹ documented that low concentrations of immunoglobulins for prolonged periods (IgG <100 mg/dL, IgM <20 mg/dL) increased the risk of life-threatening infectious episodes. Low concentrations of immunoglobulins increase mortality risk in patients with moderate severe sepsis.¹² In patients presenting to emergency departments with suspected acute infection, the presence of markers of early immunosuppression (neutrophil and monocyte programmed cell death protein 1 [PD-L1, also known as CD274] and programmed cell death protein-ligand 1 [PD-1, also known as CD279]; monocyte human leukocyte antigen-DR [HLA-DR]) is associated with subsequent sepsis.¹³

Factors inducing immunological susceptibility to sepsis

One of the major factors affecting the function of the immune system is genetic variability of the host. The impact of genetic variation on the immunopathogenesis

	Translational implications	Potential solutions
Pre-sepsis		
Identification of the host's factors impairing immunity	Identifying the factors impairing immunity may help to establish corrective measures	Promoting healthy ageing, proper control of chronic conditions (eg, diabetes, COPD, obesity, renal and cardiovascular insufficiency, HIV infection) and optimised nutrition could improve immune systems' ability to prevent, combat, and develop homeostatic responses to infection (thereby diminishing the risk of sepsis)
Defining immunological profiles associated with an increased risk of sepsis	Identifying profiles associated with an elevated risk of sepsis will allow implementation of specific preventive measures: correction of immunological deficiencies (ie, IgG, IgM), enhanced surveillance of potential infections, antimicrobial prophylaxis in susceptible individuals, and vaccination	Developing algorithms combining clinical information (ie, asplenia, treatment with immunosuppressors) with laboratory data (immunological phenotyping of whole blood, expression of immune related genes, functional responses to specific microbial antigens, etc) to detect those individuals predisposed to sepsis; developing international consortia to identify immunogenetic factors associated with sepsis by exome sequencing or genome-wide association studies
Sepsis		
Causes and evolution of lymphopenia	Precise understanding of the mechanisms underlying lymphopenia and their individualised correction will likely improve outcomes	The use of humanised mouse chimeras with transplanted human hematopoietic cells could help to study mechanisms leading to lymphocyte depletion following induction of sepsis; these models could help to evaluate the true impact of check-point blockade and IL-7 therapies aimed to preserve or restore T cells' function; developing longitudinal clinical studies to evaluate lymphocyte concentrations and function in blood before and after sepsis in individuals at risk could help to identify lymphopenia when it appears, and the impact of sepsis on lymphocytes' biology
The role of B lymphocytes in sepsis onset and progression	Understanding the implications of B-cell counts, their fluctuation and functionality (eg, mounting a fully functional antibody responses) will should provide vital diagnostic clues and serve as theragnostic component of personalised therapies with exogenous immunoglobulins	Implementing studies centred on the biology of B cells in patients with acute sepsis and in sepsis survivors
The role of neutrophils and NETs in sepsis	The balance between mature and immature forms of the neutrophils and the dynamics of NETs formation or clearance is relevant to the control of the pathogen and to the pathogenesis of organ failure following infection; profiling concentrations of mature or immature neutrophils and NETs could help to detect sepsis early and to predict its prognosis	Implementing new methods to quantify mature and immature forms of neutrophils and NETs (ie, analysers providing delta index, gene expression of neutrophil granule-related genes combined with histone quantification); evaluating functionality of immature neutrophils (bacterial phagocytosis and killing via the production of reactive oxygen species, chemotaxis, activation); improving functionality of immature neutrophils (ie, with G-CSF) might normalise innate immune responses; seeking drugs for preventing excessive release of NETs or promoting its clearance could avoid or improve endothelial and tissue damage
COPD=chronic obstructive pulmonary disease. NET=neutrophil extracellular traps. G-CSF=granulocyte colony-stimulating factor.		
Table 1: Major gaps in pre-sepsis and sepsis immunology, translational implications, and potential solutions		

of sepsis can influence predisposition to this disease and its prognosis, which should be reflected in study designs. Most available studies use the candidate gene approach dealing with, for example, cytokine genes that can affect the inflammatory response, genes encoding for pattern-recognition receptors (like Toll-like receptors), or genes that shape the response to infections.^{14,15} As such studies often suffer from poor reproducibility, an alternative could be conducting well planned and well powered data-driven genomic studies. Typically, these works are developed in consortia and lead to highly reproducible associations. Small-scale consortia aimed at studying the role of the immune system in sepsis employ approaches based on exome sequencing¹⁶ or genome-wide association studies.^{17,18} However, since exome sequencing studies miss regulatory regions of genes that are likely to be crucial in regulating inflammation in sepsis, broader genome-wide sequencing studies are necessary to cover this important aspect. In addition, large genome-wide association studies of sufficient power that insure validation in independent cohorts are needed to provide robust information on common genetic variants influencing susceptibility and outcome in patients with sepsis.

Aging, diabetes, chronic obstructive pulmonary disease, obesity, cardiovascular and renal disease, and the chronic use of immunosuppressors are factors that impair the immune system's ability to prevent and manage infections constituting risk factors for sepsis.^{19,20} These conditions simultaneously induce a lasting latent inflammation and metabolic dysfunction frequently accompanied by endothelial injury.^{21,22} These chronic alterations have a potential to modify the homeostatic transmigration of leukocytes and their ability to mount functional antimicrobial responses. They can also facilitate vascular leakage of cells and proteins (eg, immunoglobulins) during the response to infection.

The role of micronutrients such as zinc has been proposed to be essential in resistance against sepsis due to its modulatory effect on the inflammatory response, chemotaxis, phagocytosis, and oxidative stress.²³ Vitamin D has an integral role in the functioning of the innate immune system. Low baseline 25-hydroxyvitamin D concentrations in serum are associated with an increased long-term risk of subsequent community-acquired sepsis.²⁴ Moreover, malnutrition has a negative effect on immunity, potentially increasing sepsis frequency.²⁵

Patients with HIV infection or AIDS with decreased concentrations of CD4 T lymphocytes in the blood, are also at greater risk for sepsis.²⁵ Neutropenia is a common complication in patients with cancer given cytotoxic chemotherapy, and can result in sepsis, septic shock, and exacerbated mortality.²⁶ Furthermore, patients after splenectomy and those undergoing transplantation are at increased risk of sepsis.^{27,28} Patients with important bleeding have an acute loss of leukocytes. Some leukocyte classes rapidly replenish *de novo* (neutrophils) but others (lymphocytes) cannot be replaced easily, especially in older

patients. Surgery *per se* represents an exposure with a substantial impact on immune competence, inducing both a transient activation of the innate immune response, accompanied by a rapid decline of monocyte HLA-DR, which is sustained in patients developing infection.²⁹

Consequently, many adverse profiles influence the host immune responses to infection, which in turn increases risk of developing sepsis and thus encourages the design and implementation of novel, targeted preventive interventions to reduce or eliminate such risks. These interventions could involve the correction of nutritional and the immunological deficiencies (eg, immunoglobulin replacement),²⁴ enhanced surveillance of the status of infection and infection-induced organ failure, vaccination, or the implementation of antibiotic prophylaxis before facing situations of risk, such as surgery.^{30,31} A special cohort of surgical patients consists of those receiving immunosuppressive medication after, for example, solid organ transplantation. Due to the suppression of cell-based immunity, predominantly T-cell responses, these patients possess a substantial risk for developing infections with unusual pathogens (eg, candida or listeria), and reactivation of latent viruses (eg, cytomegalovirus).³² Therefore, for surgical procedures involving a likely exposure of sterile compartments to microorganisms (eg, colorectal resections or transrectal biopsy), a peri-operative, single-shot application of antimicrobial prophylaxis to prevent surgical-site infections and sepsis is the current standard of care, irrespective of the patient's immune competence.^{33,34} Ultimately, it is hard to maintain the balance between host protection and harm, introduced by the selection of antibiotic-resistant bacteria and disruption of the gastrointestinal microbiota.

Immunological gaps during sepsis

The cause, emergence, and evolution of lymphopenia

T lymphocytes are part of the adaptive immune system and are responsible for the generation of memory against invading pathogens. CD4 T-helper cells support the production of specific antibodies by B lymphocytes and promote the bactericidal activity of phagocytes that together clear the infection. CD8 T lymphocytes recognise and kill virus-infected cells and tumour cells. Although a massive loss of lymphocytes occurs at the onset of sepsis, it is its persistence in a substantial subgroup of patients that correlates with mortality.³⁵ Elucidating the mechanisms underlying lymphopenia and lymphocyte restoration is thus of paramount importance (table 1 and table 2). Most data argue for apoptosis as the cause of sepsis-associated lymphopenia. Lymphocyte apoptosis appears to be driven by intrinsic (eg, mitochondrial p53) or extrinsic (eg, FAS) apoptotic cues, depending on the context,^{36,37} but data remain unclear. Beyond apoptosis, an excessive extravasation and aberrant recruitment to sites of inflammation, together with a hampered egress to the periphery due to low serum concentrations of chemotactic factors such

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	Translational implications	Potential solutions
Sepsis		
The role of MDSC	Monitoring MDSCs' counts and their fluctuations will help define immunosuppressive phenotype and predict the risk of flairs-ups and secondary hospital infections	Standardisation and development of consensus flow cytometry protocols for whole blood phenotyping of granulocytic or neutrophilic and monocytic MDSCs
Common elicitors and mechanisms of immune cell malfunction	Identification and characterisation of the so-called master inducers of generalised dysfunction of immune cells and their mechanisms of action will yield a better understanding of sepsis immuno-pathogenesis; a unified framework for sepsis-induced immune dysfunction should facilitate the design of new targeted treatments targeting immunity in sepsis	Developing assays on primary cell cultures from patients' blood and tissues and animal models to evaluate the role of hypoxia, redox unbalance (including mitochondrial failure), and metabolic switch of immunocytes as potential ultimate triggers for most of immune cells' aberrancies
Inadequate knowledge of organ-specific immunology (the concept of compartmentalisation of responses)	Organ-specific immunological alterations promote injury of individual organs or systems and contribute to the overall pathogenesis; targeted correction of these alterations will prevent or treat failure of the affected organs or systems	Identification of immunological biomarkers related to specific organs (beyond the blood) will improve the understanding of compartmentalisation and its therapeutic consequences; works on animal models and autopsy tissues will help to evaluate organ specific immune responses in sepsis
The exact timeline and sequence of immune alterations	Understanding the temporal sequence of individual immunological alterations will facilitate development of precise, time-matched, individualised corrective therapies.	Developing comprehensive clinical studies profiling innate, adaptive, pro-inflammatory and anti-inflammatory responses along the course of sepsis, by using flow cytometry and functional, transcriptomic, and proteomic assays.
Post-sepsis		
Long-term impact of sepsis on the immune system: consequences on the risk of infections, neurological and cardiovascular complications, and cancer	Identification and temporal characteristics of typical and rare post-septic immune derangements (and subsequent complications) will enable creation of a SOP for sepsis survivors; correction of post-septic immune derangements using individualised SOP will prevent or reduce the detrimental long-term sequelae in sepsis survivors	Monitoring persistence of lymphopenia or low HLA-DR levels could help to identify those individuals at risk of secondary infections, and to reinforce surveillance and prevention of infection in these individuals; using high-dimensional flow cytometry and functional assays complemented by methods that focus on the transcriptome and epigenome could help to obtain a wide picture of the immune status in patients after sepsis
MDSC=myeloid derived suppressor cells. SOP=standard operation procedure.		
Table 2: Major gaps in sepsis and post-sepsis immunology, translational implications, and potential solutions		

as sphingosine 1-phosphate,³⁸ are potential mechanisms contributing to the decrease of circulating T cells. In fact, circulating lymphocytes might accumulate at large numbers at damaged endothelia and tissues; major cardiovascular injuries that afflict patients with sepsis lend credibility to such a hypothesis.³⁹ The effect of hormonal imbalances on T cells in sepsis is another potential mechanism of lymphocyte dysfunction. The identification of risk factors associated with septic lymphopenia and its causes is still pending.

Regarding the restoration of lymphocyte counts observed in sepsis survivors, data suggest that peripheral mechanisms play a more prominent role than thymic output, especially given the strong apoptosis and involution of the thymus observed in sepsis.^{40,41} Thus, the role of homeostatic proliferation of T-cell clones in the periphery, probably driven by IL-7 and IL-15 signalling, has been implied by animal studies⁴² but not yet verified in patients. Whether the promising check point blockade and IL-7 therapies⁴³ can improve preservation of T-cell clonal spectrum remains speculative. In turn, the extent to which the complete T-cell receptor clonal repertoire of naive and memory T cells remains functional after a sepsis episode is doubtful.⁴¹ Besides the numerical loss or clonal shift, multiple dysfunctions in T cells' metabolism and activation in sepsis have been reported.⁴⁴ Nonetheless, the extent to which these cell-intrinsic impairments

contribute to post-sepsis immunosuppression has now been challenged,^{45,46} implying a prominent role of T cells' extrinsic cues in the post-sepsis environment (eg, defective antigen presentation). Also, the impact of sepsis on non-conventional, but biologically potent T-cell subsets such as natural killer T cells or $\gamma\delta$ T cells deserves more attention. For example, a dysfunctional response of natural killer T cells and cytotoxic T cells following infection or trauma, causing an excessive release of pro-inflammatory cytokines while being unable to eradicate target cells,⁴⁷ has been put forward as a major cause of hemophagocytic lymphohistiocytosis—a life-threatening hyperinflammatory syndrome with high mortality in adults frequently misdiagnosed as sepsis.⁴⁸ Finally, we need to understand in more detail the processes influencing lymphocyte differentiation and egress in the bone marrow.

The role of B lymphocytes in sepsis

B cells differentiate to plasma cells, the cells responsible for production of antibodies (immunoglobulins) against the infecting pathogen. However, the role of B cells in sepsis extends beyond immunoglobulin secretion. For example, B cells modulate the innate immune responses, cytokine production, and function as antigen presenting cells.^{49,50} Sepsis is associated with an accelerated loss of B lymphocytes, secondary to either lack of T-cell support or accelerated apoptosis through the mitochondrial

and death receptor pathways. Unexpectedly, this occurs in the presence of unaltered B-cell specific survival factor concentrations such as B-cell-activating factor or a proliferation-inducing ligand.^{51,52} B-lymphocyte loss is differential, with a greater loss of activated memory B-lymphocyte subsets.⁵¹ B lymphocytes that survive this accelerated apoptosis have an exhausted phenotype featuring a decreased MHC class II expression and increased interleukin-10 production.⁵³ Finally, the profound T-lymphocyte abnormalities seen in sepsis also impair T-cell-dependent peripheral maturation of B lymphocytes contributing to changes in functionality of B lymphocytes.⁵⁴ The memory B-cell niches that exist to rapidly recall antibody responses to new infections⁵⁵ are unlikely to be spontaneously replenished to pre-sepsis levels and might contribute to the long-term infection risk seen in sepsis survivors.^{56,57}

The role of neutrophils and neutrophil extracellular traps in sepsis

Neutrophils are first-line defence cells of innate immunity responding to the infecting pathogen. Severe microbial infection leads to an enhanced generation of granulocytes and the release of immature and mature forms of neutrophils from the bone marrow into the peripheral blood.⁵⁸ In sepsis, the presence of excessive amounts of immature granulocytes in the blood is linked to clinical deterioration.⁵⁹ Immature neutrophils from patients with sepsis have substantially diminished functional capacity, including both phagocytosis and respiratory burst.⁶⁰ Immature neutrophils show an increased spontaneous production and release of neutrophil extracellular traps,⁶¹ which are composed of chromatin DNA, histones, and granular proteins. A prolonged presence of neutrophil extracellular traps in vasculature or tissues (by their overproduction or inadequate removal) can lead to endothelial injury and hypercoagulation.^{62,61} Understanding the impact of balanced responses between mature and immature neutrophils and the mechanisms of endothelial and tissue damage mediated by these cells and neutrophil extracellular traps will likely open new treatment options in sepsis.

The role of myeloid-derived suppressor cells

Immature myeloid cells migrating into the blood during emergency granulopoiesis in response to infection could become functionally active myeloid-derived suppressor cells. Myeloid-derived suppressor cells, whose immunosuppressing properties have been studied in depth in malignant disease,⁶³ remain poorly explored in sepsis. They constitute a heterogeneous population of immature myeloid cells equipped with potent immunosuppressing functions acting both on innate and adaptive immune responses. Two major subsets of these cells have been described: granulocytic or neutrophilic myeloid-derived suppressor cells, and monocytic myeloid-derived

suppressor cells. Although experimental models of sepsis have reported a deleterious role of myeloid-derived suppressor cells,⁶⁴ patient data are scarce. Uhel and colleagues⁶⁵ described an association between increased neutrophilic myeloid-derived suppressor cell counts and occurrence of nosocomial infections after sepsis. Additionally, these cells have been proposed to sustain long-term immunosuppression in patients with chronic critical illness.⁶⁶ The definition of human myeloid-derived suppressor cells has been lacking unanimous phenotypic characterisation.⁶⁷ The published results have been generated typically from Ficoll-enriched cell fractions, which constitutes a major limitation for clinical studies. A better standardisation and development of protocols for whole blood phenotyping of myeloid-derived suppressor cells is essential.

The quest for common mechanisms of immune cell malfunction

Sepsis is characterised by a dysfunctional host immune response comprising both pro-inflammatory and anti-inflammatory or immunosuppressing components that affect all types of immune cells and their compartments (figure 1). Current models assume that a derailment from immune homeostasis underlies sepsis-associated acute and long-term mortality. Nevertheless, the causes of the immune dysfunction remain conceptually hard to grasp. One difficulty is that most studies investigate a single type of immune cell at a time, which can preclude obtaining a wider view of how sepsis impacts immunity in its entirety. Owing to these deficits, an increased interest exists to generate a unified framework of sepsis-associated immune dysfunction. One crucial question is whether the causes of various immune malfunctions converge at the level of one or several common elicitors for all immune compartments. Can hypoxia, the derailment of reactive oxygen species homeostasis, and the resulting redox unbalance (including mitochondrial failure) be considered as the ultimate trigger for most of immune cell aberrancies?^{22,68} Another candidate is the metabolic switch of immunocytes: most immune cells undergo dramatic rewiring of their cellular energy metabolism upon infection or exposure to pathogens.⁵⁴

The spatial dimension: organ-specific immunology

Immunological sepsis research in humans has focused almost exclusively on the circulating blood cells. However, the existing evidence indicates that the immune response is highly compartmentalised. For instance, murine experiments showed that blood and spleen leukocytes are rendered hyporesponsive by endotoxaemia or caecal ligation and puncture-induced sepsis in the acute disease stage, which is indicative of tolerance in these haematopoietic compartments.^{69,70} However, the functionality of alveolar macrophages,^{69,70} liver Kupffer cells,⁷⁰ renal cortex cells,⁷¹ intestinal epithelial lymphocytes,⁷² skin's CD8 T-cells,⁷³ and microglial cells⁷⁴

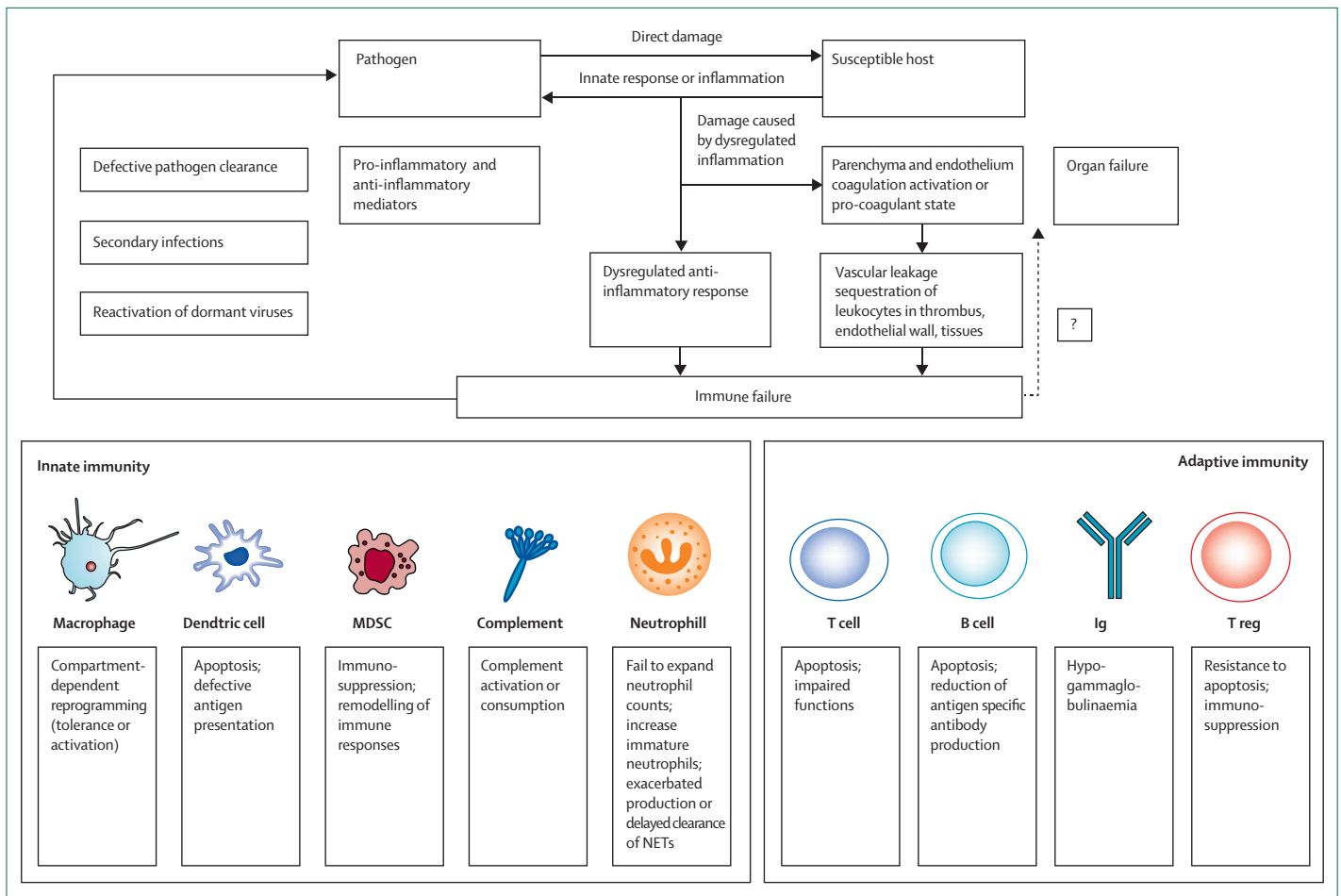


Figure 1: Key events in sepsis immunopathology

Pathogen associated molecular patterns and damage-associated molecular patterns are recognised by pattern recognition receptors (ie, Toll-like receptors), which initiate inflammation. What differentiates sepsis from uncomplicated infection is a dysregulated host response (ie, inflammation) that leads to various organ dysfunctions and systems activation (vascular endothelium, complement systems inducing a procoagulant state and injury in the parenchyma). This process normally occurs in a susceptible host with predisposing factors (ie, aging, chronic diseases, prior immunosuppression) who shows chronic endothelial injury, a loss of homeostasis (to contain inflammation), or ineffective antimicrobial host defence, which translates into high microbial burden and high innate immunity activation. At the same time, host mechanisms aimed at blocking this deleterious excess of inflammation (endotoxin tolerance, apoptosis, energetic failure, anti-inflammatory mediators release, epigenetic regulation, central and endocrine regulation) along with leukocyte loss by vascular leakage and sequestration in the tissues and thrombus, lead to profound immune failure in some patients with the effect on both the innate and the adaptive immunity. These events translate into the coexistence of a pro-inflammatory response with immunosuppression at the time of clinical diagnosis of sepsis. In turn, the immune failure might contribute to perpetuate organ failure, in an indirect manner (poor control of the pathogen, secondary infections, and reactivation of dormant viruses), but also in a direct way (endothelial injury mediated by leukocytes, cytokines, reactive oxygen species, and maintained activation of coagulation). ROS=reactive oxygen species. Ig=immunoglobulins. NETs=neutrophil extracellular traps; MDSCs=myeloid derived suppressor cell. T reg=T regulatory lymphocytes.

was shown to be unaffected or primed. Studies in healthy volunteers revealed that alveolar macrophages were primed after endotoxin administration,⁷⁵ a phenotype markedly different from the profound immunotolerant state of the blood monocytes in human endotoxaemia.⁷⁶ Furthermore, the initially suppressed ex vivo leukocytic cytokine production capacity was quickly restored after endotoxin administration in healthy volunteers and in mice,⁷⁶ whereas the in-vivo response to an endotoxin rechallenge remained impaired for a sustained period.⁷⁶ These findings suggest that compartments other than the blood participate in shaping immunosuppression. A study by Ferguson and colleagues⁷⁷ revealed that an endotoxin administration induced an opposite response

in monocytes versus adipose tissue in over 30 genes. Immunological exploration of the response specificity in other compartments (eg, the brain, endothelium) remains rudimentary. These considerations indicate a need to extend the research for immunological biomarkers in sepsis beyond the blood.⁷⁸ For example, several innovative nuclear imaging tracers that can quantitatively measure microglial activation in the brain in vivo have been developed.⁷⁹ Understanding organ-specific immune responses to sepsis should also help rationalise how the local control of the infectious focus (or the failure to do so) might affect the overall immunity and progression of sepsis and persistent inflammation, immunosuppression, and catabolism syndrome.

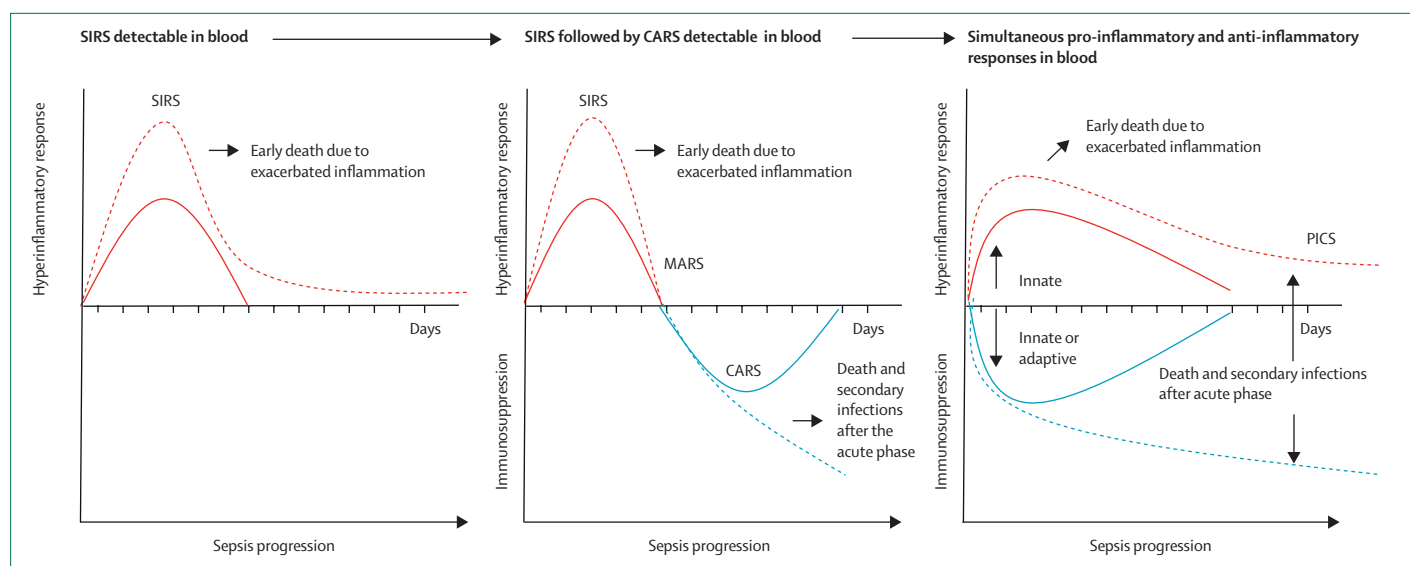


Figure 2: Historical evolution of the models explaining pro and anti-inflammatory responses during sepsis

Models were generated using data obtained from the blood during the sepsis episode. Lines represent prototypical hyperinflammatory (red) versus immune suppressive (blue) disease progression courses with return to immune homeostasis (solid) or pathological immune dysfunction (dashed). CARS=compensatory anti-inflammatory response syndrome. SIRS=systemic inflammatory response syndrome. PICS=persistent inflammation, immunosuppression, and catabolism syndrome. MARS=mixed antagonists response syndrome.

The temporal dimension: timeline and dynamics of immune alterations

The timeline of immunological alterations in sepsis is not well understood. Originally, a compensatory reaction was postulated to occur subsequently to the initial hyperinflammatory phase of sepsis (figure 2). However, suggestive evidence demonstrated that, in sepsis, anti-inflammatory reactions arise concomitantly with the pro-inflammatory response.⁷⁸ As early as 1995, three research groups independently reported that the increase of IL-10 correlated with the rise of tumour necrosis factor, IL-6, and IL-8 in patients;^{80–82} this finding was later reproduced by pre-clinical sepsis studies, which demonstrated an identical response pattern.^{83,84} Thus, septic plasma typically represents a mixed hyperinflammatory and immunosuppressing milieu^{85,86} that can subsequently modulate circulating leukocytes in various ways.^{87,88} In addition to IL-10, also plasma cortisol, norepinephrine, and other endocrine factors can contribute to the alteration of the immune status.⁸⁹ Because it is difficult to define the exact onset of sepsis, the alterations of the immune status observed in patients with sepsis feature large variations in the time-lag with respect to the initial infectious insult. For example, in patients after cardiac arrest resuscitation, the circulating immune cells displayed altered functions already at 3 h after admission.⁹⁰ This phenomenon occurred even earlier in patients undergoing arterial surgery, in which the alteration of the ex vivo cytokine production and HLA-DR expression on monocytes was evident already before the end of the surgery.^{89,91} The presence of damage associated molecular patterns and the alteration of the immune status of circulating cells occurs already at the

trauma scene, far before the patient admission to the emergency room.⁹² Finally, it is unclear whether some of the immunological alterations observed in sepsis (eg, depressed expression of HLA-DR on monocytes) precede its onset or are induced by sepsis (or if both options occur concurrently).

Immunological gaps post-sepsis

The role of post-sepsis persistent immune alterations in pathogenesis

Alterations of the patients' immune system after sepsis have been postulated as the molecular foundation of the epidemiologically proven increase in health-care costs and long-term mortality (table 2).^{56,93,94} Evidence favouring this concept is, however, sparse.^{95–97} It is necessary to direct efforts toward a holistic approach, incorporating data from routinely used methods, such as high-dimensional flow cytometry and functional assays, complemented by methods that focus on the transcriptome and epigenome. Identification of novel therapeutic targets of post-septic immune disorders requires an improved understanding of the underlying pathomechanisms. Therefore, animal models and the timing of post-septic and long-term analyses must be defined. A major issue is the reprogramming of immune cells due to epigenetic and metabolic changes that might determine a long-lasting dysfunction of immunity.^{98,99} Considering the rapid turnover of leukocytes during infection, changes in the bone marrow as the site of haematopoiesis are of special interest. Moreover, the impact of the microenvironment in the periphery on recruited immune cells deserves more attention.¹⁰⁰

	Additional gaps	Translational implications	Potential solutions
Immunological monitoring	Lack of standardisation of immunological tests for clinical applications	Developing a standardised profiling of the immunological alterations in sepsis will enable its use in a more individualised manner to predict, diagnose, and treat patients	Simple test based on flow cytometry to phenotype immunological cells using automated table-top cytometers (ie, for evaluating endotoxin tolerance in peripheral monocytes); using public repositories such as Gene Expression Omnibus or Arrays Express is helping to identify and validate gene expression signatures reflecting population heterogeneity; developing multicentric prospective studies with unified protocols is needed to evaluate the performance of each immunological test
Immunological biomarkers	Utility of existing biomarkers obtained from routine clinical analytics and the emergence of new potential immunological biomarkers	Re-interpretation of common analytics involving elements of the immune or inflammatory response or advancing the immuno-monitoring to new areas could contribute to the differential diagnosis of sepsis and to assess its prognosis	Circulating ferritin concentration enables an accurate detection of the macrophage-like activation syndrome in sepsis; sepsis frequently presents with lymphopenia, thus, the diagnosis of lymphopenia during ongoing infection might serve as a marker for the onset of sepsis; failure to expand neutrophil counts could be benchmarked as a predictor of mortality in septic shock; phenotyping of monocytes and T regulatory cells, characterisation of their metabolism (eg, mitochondrial respiration) and fate (eg, cell cycle analysis, death mode) could replace or improve the efficacy of the current biomarkers; testing immunological biomarkers (beyond procalcitonin) to improve antibiotic stewardship
Immunotherapy	Rationale and efficacy of personalised immunotherapy in sepsis	Developing verifiable strategies aimed at personalising the type, dosing, and timing of immunotherapies will likely enhance their efficiency and improve patient outcomes	Evaluating PD-1 and PD-L1 expression and lymphocytes counts to select potential patients for treatment with check-point inhibitors; evaluating expression of IL-7 in plasma or expression of its receptor to select potential patients for treatment with IL-7; assessing concentrations of endogenous immunoglobulins to select patients for treatment with intravenous immunoglobulins; assessing expression of HLA-DR in monocytes to identify patients for treatment with GM-CSF
Microbiome	The role of the microbiome in immunity and pathophysiology of sepsis	Future research should focus on elucidating how the microbiotic disturbances can predispose to, exacerbate, and perpetuate the immune response dysregulation in sepsis; modulation of microbiome harbours a great anti-sepsis potential regarding preventive strategies and individualised treatments	Next generation sequencing studies to evaluate the profiles of commensal and pathogenic bacteria in parallel to evaluation of immunological changes induced by sepsis at the blood and organ level; evaluation in animal models and clinical trials of pro-biotics or prebiotics and (partial) recolonisation of the gut with a faecal microbiota to decrease sepsis incidence, to improve sepsis outcome and late mortality, with a parallel evaluation of the immunological changes
Animal models	Clinically relevant animal models, their standardisation and reproducibility	Developing an adequate diversity of animal models that maximally recapitulate specific sepsis phenotypes will enable a better understanding of sepsis pathophysiology (its types, evolution, and response compartmentalisation) and a more reliable testing of immunomodulators for their potential advance to clinical trials	Identification of models that provide acceptable clinical relevance (following the evolving understanding of sepsis pathophysiology); identification of must-do must-not-do study-design elements that recapitulate clinical practice

PD-1=programmed cell death protein 1. PD-L1=programmed cell death-ligand 1. HLA-DR=human leukocyte antigen DR isotype. GM-CSF=granulocyte-macrophage colony-stimulating factor.

Table 3: Additional gaps in sepsis immunology, translational implications, and potential solutions

Additional gaps

Lack of standardisation of immunological test for clinical applications

We face an exciting new era in which the promise of immunological biomarkers to improve sepsis care is becoming a reality. Nonetheless, it is necessary to develop efforts to standardise the immunological tests (table 3). For example, a standardised protocol for flow-cytometric leukocyte biomarker measurement demonstrated that the combination of neutrophil CD24 and CD279 (PD-1) and expression of HLA-DR on monocytes accurately predicted the clinical deterioration to sepsis in patients with suspected infection.¹³ However, flow cytometry requires an advanced technical know-how that is not widespread across clinical laboratories. Given that persistence of low expression of HLA-DR predicts both sepsis mortality and the occurrence of nosocomial infections,^{101–103} new technologies such as the development of automated bench-top cytometers

could simplify testing.¹⁰⁴ Well standardised functionality tests to measure endotoxin tolerance from peripheral monocytes might serve as a surrogate readout of immunosuppression status.^{105,106} Standardisation problems also affect the transcriptomic tests. Quantification of the IL-7 receptor gene expression in the blood helps to identify patients with sepsis at risk of death.¹⁰⁷ The neutrophil transcriptome is a promising new source of biomarkers in sepsis with lipocalin-2 (also known as gelatinase-associated lipocalin) and matrix metalloproteinase 8 among the most promising ones.^{108,109} Transcriptomics could identify endotypes of sepsis with distinct clinical and biological features and differential treatment responses.^{110,111} Next generation mRNA profiling methods could facilitate standardisation of gene expression-based test.^{108,112} Finally, microfluidics could help to develop point-of-care tests to quantify immunological parameters in an easy and reproducible manner.¹¹³

Useful biomarkers for sepsis from simple routine clinical analytics

Another pending issue regarding immunological monitoring is the insufficient exploitation of simple analytical tests. For example, changes in the ferritin concentration enable an accurate detection of the macrophage-like activation syndrome and the presence of a hyper-inflammation in sepsis.¹¹⁴ In sepsis, at least 50% of patients show lymphopenia at diagnosis¹⁰⁸ and lymphopenia (especially persistent) identifies a subpopulation of patients at higher risk of death.^{35,115,116} Additionally, a lack of neutrophil count expansion can be associated with poorer outcomes in septic shock.¹¹⁷ The potential role of the neutrophil-to-lymphocyte ratio to predict mortality in sepsis has also been reported.¹¹⁸ An association has been shown between monocyte counts and mortality, the prevalence of bacteraemia, and organ dysfunction in patients with sepsis.¹¹⁹ Immature granulocytes count could help in ruling in¹²⁰ and ruling out¹²¹ sepsis. Monocyte complexity and neutrophil fluorescence intensity are promising cell population data to diagnose the presence of this disease.¹²²

New immunological features with potential as biomarkers in sepsis

A multitude of soluble mediators such as procalcitonin, C-reactive protein, and IL-6 (and other cytokines or chemokines) have been proposed as biomarkers in sepsis. So far, only procalcitonin has been officially sanctioned to aid antibiotic stewardship.¹²³ One of the main obstacles in clinical use of these markers is that they are relatively unspecific and neither comprehensively reflect the entire magnitude and extent of the response to infection nor can help to identify the entities involved in immune dysregulation. The identification and characterisation of new immune features (readouts) or detection of different T-cell populations and their functions hold potential as more valuable prediction and monitoring tools.^{124–126} Evaluating the potential translational applications of different types of monocytes or T regulatory cells (activated, exhausted, with markers of homing)¹²⁷ and monitoring cell cycle analysis, apoptosis, and mitochondrial functionality have been gaining momentum. Finally, use of immunological biomarkers could help to guide antibiotic treatment.

The rationale and potential efficacy of personalised immunotherapy in sepsis

Immunotherapy aims to improve sepsis outcomes by modulating (depressing or boosting) pathological immune responses to infection. After early animal studies evidenced the importance of pro-inflammatory mediators, immune cell dysfunction, and apoptosis for sepsis outcomes, immunotherapy emerged as a key approach. Spectacular successes of anti-inflammatory^{128,129} and immunostimulatory¹³⁰ therapies in various animals, including non-human primates, reinforced this notion.

However, the subsequent clinical sepsis trials based on the same premise failed. One important reason for that failure is the heterogeneity of sepsis and the lack of consensus on when or how the host response should be manipulated in patients. The unsuccessful clinical trials that tested various anti-inflammatory agents have shown that curbing excessive inflammation cannot improve outcome in all patients with sepsis and identification of sub-cohorts on the basis of the disease severity itself is not optimal.^{36,131} Conversely, the more functionally specific immune status-based criteria should be better suited to stratify patients for immunotherapy. Other investigators advocate the use of immunostimulatory approaches to restore defective immune functions, subsequently reducing susceptibility to secondary infection and late sepsis mortality.^{132–134} However, a reflexive preference of immunostimulation might, again, turn out to be too simplistic (and harmful) given that the presence of the immunosuppression status is currently judged on the basis of readouts from a single compartment (ie, the blood) and immunosuppression can be mixed or absent in other systems and organs. For example, a recent study calculated that secondary infections are responsible for only 10% of overall sepsis mortality in the intensive care unit, raising doubts about the potential benefit of immune stimulation in unselected sepsis populations.¹³³ Theragnostics could be the key to any effective host-directed treatment—ie, identification and enrolment of those patients who will most likely benefit from a given intervention. Such a therapy personalisation should be based on a continuous standardised monitoring of specific immune biomarkers in the blood and other compartments. Depending on the net balance of pro-inflammatory and anti-inflammatory responses, patients could be treated with either anti-inflammatory drugs (eg, IL-1 receptor antagonist) or immuno-stimulatory agents (eg, granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-7, or anti-PD-L1). The principle of a biomarker-guided immunotherapy has been shown by proof-of-principle studies using immunostimulatory cytokines such as interferon- γ , GM-CSF, and IL-7, or removing immunosuppressing mediators by selective extra-corporeal therapies.^{134–137} Finally, humanised mice might be a feasible alternative in preclinical immunotherapy modelling.¹³⁸

The influence of microbiome on the immune system

Preclinical studies show that microbiome-dependent metabolic pathways can drive distinct immune responses to invading pathogens.¹³⁹ The gut microbiome plays a protective role in sepsis by maintaining the gut barrier, regulating leukocyte function, and modulating innate and adaptive immunity.^{139–141} Clinical studies have underscored the extreme perturbations of the microbiome (termed dysbiosis) in patients with sepsis.^{139,142} Dysbiosis has been associated with poor outcome although the underlying mechanisms are not yet understood.^{139,143} The intestinal microbiome of a patient with sepsis is characterised by a

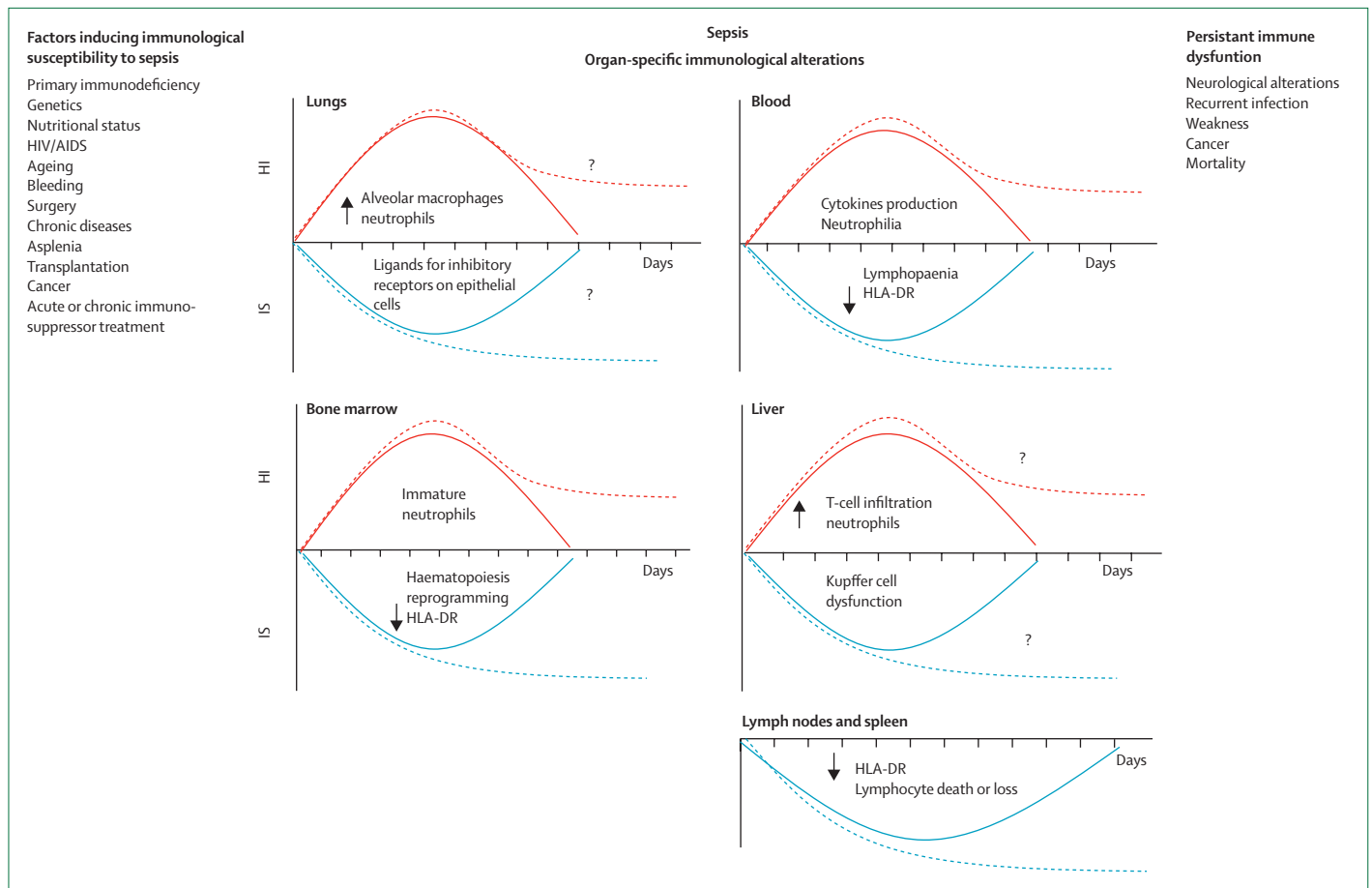


Figure 3: Conceptual framework proposed by European Group on Immunology of Sepsis for approaching sepsis immunopathology

The proposed model by our group emphasises the importance of the factors impairing immunity or predisposing to sepsis, the discrete organ-specific host immune response components, and finally the alterations of immunity after sepsis and their potential participation in sepsis consequences. For illustrative reasons different organs have been linked to specific immune responses that, in part, remain speculative. For example, although the loss of lymphocytes in spleen is well documented, the immune reactions taking place in liver or lung are less well characterised. Lines represent prototypical hyperinflammatory (red) versus immune suppressive (blue) disease progression courses with return to immune homeostasis (solid) or pathological immune dysfunction (dashed). HI=hyperinflammatory response. IS=immunosuppression.

loss of diversity, lower abundances of the key commensal genera, and overgrowth of opportunistic pathogens.^{139,142} How does the microbiome exert its protective effects in sepsis? Which components of the microbiome are doing the job? Which pathways are used by the metabolites that are excreted by all these microorganisms? What is the role of the other kingdoms such as the virome, mycobiome, and parasitome? In addition, other limitations include the large variation between and within individuals, the limited mechanistic knowledge, and the scarce number of trials that investigate microbes as a treatment for sepsis. The potential of microbiome-modulating, preventive, and treatment strategies in patients with sepsis is enormous. Examples include the use of probiotics and or prebiotics and (partial) recolonisation of the gut with a faecal microbiota transplantation.¹⁴⁴ These strategies can in theory be used to decrease sepsis incidence and to improve sepsis outcome and decrease late sepsis mortality.

The necessity of standardised animal models for sepsis

Although several disease models have already been following established standardisation blueprints, the sepsis modelling field has only recently attempted to fill this void¹⁴⁵ by releasing expert consensus guidelines for Minimal Quality Threshold in Pre-clinical Sepsis Studies (known as MQTiPSS).¹⁴⁶ This delay has been, in part, caused by an enduring reliance on the erroneous (homogenous) endotoxin or lipopolysaccharide model. Given the now recognised heterogeneity of sepsis, an adequate standardisation of (clinically relevant) animal models of sepsis will likely enhance their reproducibility and translational potential.¹⁴⁵ As good modelling practices overlap across fields, the standardisation should duplicate the successful guidelines from other diseases such as stroke¹⁴⁷ and malaria,¹⁴⁸ while developing strategies for (pre-clinical) sepsis. In the long-term, the standardisation of sepsis models should focus on the two main areas that are equally crucial for the bench-to-bedside translation:

Search strategy and selection criteria

A group of European experts met in Berlin, on May 23–24, 2018, to constitute the European Group on Immunology of Sepsis (EGIS). The panel was formed by nine immunologists, ten translational researchers, eight physicians of intensive care medicine, one of respiratory medicine, and one of infectious diseases. During an open discussion, the experts identified the key gaps in sepsis immunology, either in pre-clinical or clinical research. Following the meeting, each expert was commissioned to provide a short paragraph discussing each gap, its translational implications, and potential future ways to address them. Each member of the EGIS identified the most relevant bibliography through searches of PubMed for articles published in English, on the basis of their specific expertise using self-defined queries that matched their assigned thematic gap. Priority was given to the works published since 2000, which constituted 94% of the articles referenced in this Review. All the authors constructively reviewed the identified literature and approved the final citation list. A first version of the article was drafted by October, 2018, which underwent two additional internal revision rounds. Final draft was approved at a subsequent EGIS meeting in Valladolid, Spain, February 14–15, 2019.

identification of models that provide acceptable clinical relevance (following the evolving understanding of sepsis pathophysiology) and identification of must-do must-not-do study-design elements that recapitulate clinical practice. For example, inclusion of components such as advanced age, comorbidities (eg, diabetes, obesity), and testing beyond rodent models is especially crucial.¹⁴⁹ The standardisation needs to be balanced because excessive micromanagement creates an artificial, idiosyncratic environment that lowers multilaboratory reproducibility.¹⁵⁰

Conclusion

The way in which we study the immunological basis of sepsis must be approached anew and should consider the integration of multifactorial changes that occur at the molecular, cellular, organ, and systemic level. Addressing the gaps identified in this Review will help to better understand the immunological factors predisposing to sepsis, those participating in its pathogenesis, and, finally, those contributing to its complications in the long term (figure 3). Studying these gaps will consequently help to implement new immunology-based strategies to improve sepsis prevention, detection, and care.

Contributors

All authors contributed to the writing of the manuscript. Final integration and editing were done by JFB-M, IR, MFO, and MS-H. Tables and figures were drafted by RA, GM, JFB-M, IR, MFO, and SBF. All the authors critically reviewed the manuscript.

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