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Reframing Sepsis Immunobiology for Translation

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Abstract

Sepsis is a common and deadly condition. The current framing of dysregulated host immune responses within the sepsis immunobiology model into pro-inflammatory and immunosuppressive responses for testing novel treatments, have not resulted in successful immunomodulatory therapies. Thus, the recent focus has been to parse observable heterogeneity into subtypes of sepsis to enable personalized immunomodulation. In this perspective we highlight that many fundamental immunological concepts such as resistance, disease tolerance, resilience, resolution, and repair are not incorporated into the current sepsis immunobiology model. The focus for addressing heterogeneity in sepsis should broaden beyond subtyping, onto identifying deterministic molecular networks or dominant mechanisms. We explicitly reframe the dysregulated host immune responses in sepsis as pathologic disruption and/or alteration in homeostasis of the immune-driven resistance, tolerance and resolution mechanisms occurring concurrently. Our reframing highlights novel treatment opportunities and could enable successful immunomodulation in the future.

Keywords: Sepsis, immunobiology, precision medicine, molecular mechanisms, subtyping, immunomodulation
Search strategy and selection criteria

References for this review were identified by individual contributors through searches of PubMed for articles published from June, 1992 (to coincide with the first publication of Sepsis Consensus definitions), to November, 2023, by use of the terms “Sepsis”, “immunobiology”, “phenotype”, “endotype”, “resistance”, “disease tolerance”, “resolution”, “repair”, “Immunomodulation”, “immune mediated inflammatory diseases”, “and “precision medicine”. Relevant articles were also identified through searches in the authors' personal files and previous state of the art reviews. Articles resulting from these searches and relevant references were reviewed by each contributor for their sections without any language restrictions.

Key messages

- The conventional sepsis immunobiology – modulation paradigm consisting of hyperinflammation and immunosuppression has failed to identify any immunomodulation treatment that improves outcomes for patients with sepsis in primary reports of randomised controlled trials, illustrating the need for reconsidering this paradigm.

- Resistance refers to effector mechanisms that reduce pathogen burden once the infection is established through detection, neutralization, killing or expulsion of microorganisms and production of inflammatory mediators (also referred to as inflammatory cost to the host).

- Disease tolerance refers to an evolutionary conserved defence strategy that limits the severity of infectious diseases, without directly affecting pathogen burden.

- Resilience refers to the capacity of the immune system to rapidly restore to the regulated state it was in prior to the infectious threat, whilst limiting inflammatory cost to the host. Clinical equivalents of the inflammatory costs to the host are the adverse outcomes in patients with sepsis.

- Resolution is conceptualised as a tightly regulated and active biological process that restores tissue homeostasis following inflammation.

- We reframe dysregulated host immune responses in sepsis as pathologic disruption and/or alteration in homeostasis of the immune-driven resistance, disease tolerance and resolution mechanisms occurring concurrently.

- Studies based on above reframing could eventually lead to classified sepsis subtypes or sepsis immune states that are complex treatable traits defined as measurable characteristics that (a) have clinical
consequences, (b) have multiple interacting molecular mechanisms and (c) are modifiable with repurposed drugs or yet to be discovered treatments.
Introduction

Sepsis is common and deadly, with global estimates of ~49 million incident cases per annum\(^1\) and ~11 million deaths per annum\(^1\). Sepsis is a medical diagnosis, informed by clinical history, physiological and laboratory data. In the current consensus definitions (referred to as Sepsis-3), sepsis is defined as a dysregulated host response to infection resulting in life threatening organ dysfunction and septic shock as a subset of sepsis with profound circulatory, cellular and metabolic abnormalities, associated with a greater risk of death than sepsis\(^2-4\).

To enable bedside diagnosis and management, the Sepsis-3 definitions and criteria have necessary compromises likely are instrumental to the observed heterogeneity in the dysregulated host responses. Indeed, in the Sepsis-3 definitions, infection can be suspected or microbiologically confirmed; with many critically ill patients with suspected infection in retrospect are classified as suffering from a non-infectious condition\(^5\). Although sepsis commonly arises from either bacterial or viral infections (a recent example being COVID-19), fungal, protozoal or parasitic infections, or combinations of pathogens (bacterial co-infections with influenza\(^6\) and malaria\(^7\)) can result in sepsis. The site of infection differs between patients and affects immune responses. Organ dysfunction is quantified with physiological derangements (e.g., hypotension), as well as treatment variables (e.g., mechanical ventilation). The illness severity is linked to host responses and varies between sepsis cohorts. We neither have a definition, nor widely accepted diagnostic test(s) for these dysregulated immune responses despite a plethora of biomarkers. As such, the clinical definition has minimal relationship to the current sepsis immunobiology framework\(^8\).

This context emphasises the need to explicitly define the dysregulated immune response in patients with sepsis. Defining dysregulated immune responses may identify previously unrecognised sepsis immunobiology, enable us to undertake more sophisticated immunological assessments, and highlight novel treatment opportunities. In this article, we attempt to define dysregulated immune responses by discussing how fundamental immunological concepts (such as immune resistance, disease tolerance, resilience and resolution) relate to sepsis immunobiology. Thus, after outlining the current sepsis immunobiology – modulation paradigm as a contributory reason to unsuccessful immunomodulation trials and as another rationale to reframe sepsis immunobiology, we summarise key lessons for success with immunomodulation in immune-mediated inflammatory diseases (IMIDs), generate a working definition for dysregulated immune responses in sepsis and end by
proposing a research road-map for reframing sepsis immunobiology. We acknowledge that this
case study will require global stakeholder engagement and further research to enable change.

**Conventional sepsis immunobiology - modulation paradigm**

Sepsis immunobiology has been reviewed recently\(^8\)-\(^{11}\), including in this issue by Cajander and
colleagues\(^{12}\). Dysregulated immune responses in sepsis are characterized by concurrent
hyperinflammation and immunosuppression, two normally opposing responses involving distinct cell
types and organ systems. Hyperinflammation is caused by the uncontrolled activity of pro-
inflammatory effector mechanisms, involving activated leukocytes and endothelial cells with
concomitant dysregulated production of oxygen and/or nitrogen radicals, cytokines and activation of
the complement and coagulation systems. While the activation of these mechanisms is part of innate
immune responses to infection (i.e. inflammatory and protective trade-off responses\(^{13}\)), their
uncontrolled activity can cause collateral damage and plays a key role in the pathogenesis of
sepsis\(^8\). These unbalanced responses also contribute to the development of immune suppression,
which involves different cell types\(^9,10,14\) and is associated with a higher risk of new infections including
reactivation of latent viruses. Sepsis induced immunosuppression results from widespread lymphocyte
programmed cell death\(^{15}\), an impaired functional state in T cells (exhaustion), relative increases in the
number of regulatory T cells, increases in myeloid-derived suppressor cells and lower surface levels
of human leukocyte antigen (HLA)-DR on monocytes, indicative of reduced antigen presentation
capacity\(^8\). These maladaptive responses are typically present to variable degrees in patients with sepsis,
and change over the natural history of sepsis between patients, which contributes to observed
immunological heterogeneity.

More than 200 randomized controlled trials have tested the hypothesis that modulating these dysregulated
immune responses could improve outcomes from all-cause sepsis. There are numerous reasons why none
of the trials has resulted in new immunomodulatory treatments for all-cause sepsis\(^{16}-^{19}\). It is possible that
eligibility criteria in clinical trials have not enrolled the sepsis subtype who may benefit from the
immunomodulator tested or that the immunomodulator was not administered in the right dose, or at the
right time to achieve optimal immunomodulatory effect. While we can identify and possibly correct
single biological derangements, it remains speculative whether blocking one or more elements of
maladaptive responses (such as excess cytokines like IL-6), or stimulating impaired host responses
(such as increasing lymphocyte counts and improving lymphocyte function) could improve outcomes from sepsis. Moreover, our understanding of how the host immune responses in sepsis change over time is limited due to lack of high-quality cohort studies with longitudinal multi-domain immunological data. Although not a focus of our review, how the non-immune component of the dysregulated host responses in sepsis interacts with the immune responses is incompletely understood. These form additional key reasons to reframe the sepsis immunobiology model into its component parts of the immune response to pathogens.

**Lessons from immune-mediated inflammatory diseases (IMIDs) for sepsis immunobiology**

IMIDs are clinically diverse conditions characterized by chronic inflammation, underlying immunologic dysregulation and end-organ damage. IMIDs include inflammatory arthropathies, e.g., rheumatoid arthritis (RA), spondyloarthopathies, connective tissue disorders (e.g., systemic lupus erythematosus), cutaneous inflammatory conditions, inflammatory bowel disease (IBD) and autoimmune neurological diseases. Historically, the cornerstone of treatment was broad immunosuppression, agnostic to pathogenesis, including glucocorticoids and/or agents such as methotrexate, azathioprine, cyclophosphamide and gold salts. Such therapeutics were only partially effective and dose limited by serious toxicities.

Recently, increased understanding of the pathogenesis established the pivotal role of inflammatory cytokines, particularly tumour necrosis factor (TNF), in disease etiology.\(^{20,21}\) TNF inhibition in RA comprised the first success, that was extended to include other IMIDs shortly thereafter. A broad range of cytokine inhibitors targeting for example the IL-6 receptor, IL-1, IL-4, IL-13, IL-17A/F, IL-12/23 and IL-23 are now in clinical practice\(^{21}\). Cell targeting agents such as abatacept (targeting the CD28/CTLA4 pathway) and B cell depleting biologics (anti-CD20) are efficacious in several IMIDs\(^{21}\). This ‘biologic’ revolution led to higher rates of response and remission with significantly reduced toxicity. Moreover, co-morbidities involving cardiovascular, bone and psychologic function were positively impacted, reflecting the benefits of modulating systemic inflammation. More recently oral Janus kinase inhibitors (e.g., baricitinib) have been approved that recapitulates the high levels of efficacy achieved with biologics.

This revolution in treatment is driving a transition from organ-affected classification to molecular-based classifications.\(^{20,21}\) The therapeutic efficacy of a single cytokine inhibitor, suggests the
existence of dominant signature cytokines in discrete diseases. A key example concerns IL-23p19 inhibition. Benefits accrue in psoriasis, psoriatic arthritis and IBD upon administration of IL-23p19 inhibitors, but not in RA or axial spondylarthritis, suggesting that these diseases have discrete etiopathogenetic features that can be parsed by cytokine therapeutics.\textsuperscript{20,21} In contrast, IL-17A inhibitors are effective in axial spondyloarthritis, psoriasis, psoriatic arthritis but not in RA or IBD.\textsuperscript{20,21} These complex inter-relationships of cytokine pathways in IMIDs could enable precision medicine-based approach, which may be applicable to sepsis given the similarities in cytokine profiles and success of similar interventions in COVID-19\textsuperscript{22}.

A further key development in IMID therapeutics was the recognition that strict control of inflammation enabled either more frequent remission or maintenance of low-disease activity state and prevented progressive target organ damage. Moreover, earlier intervention leads to substantially improved outcomes suggesting that the timing of interventions is critical to restore homeostasis. These concepts are useful when reframing sepsis immunobiology. Based on the IMID experience, detailed consideration should be given not simply to levels of individual cytokines but rather to the identification of networks of cytokines, defined as profiles, that are correlated with disease kinetics, current immune state, relevant co-morbidities and therapeutics and thereby probable trajectories of immunologically mediated tissue damage. This will be complex. Even in IMIDs in which dominant cytokine hierarchies have been identified, there are as yet no biomarkers, that positively or negatively predict treatment response. The availability of multiplex technologies, supportive software and artificial intelligence bioinformatic methodologies should bring new opportunities. For example, a network formed by plasminogen activator inhibitor type 1, IL-6, IL-8, monocyte-chemoattractant protein-1 and IL-10 persisted for over the first four days of acute sepsis; IL-6 had the maximum value as the treatment target cytokine, further supported by evidence in severe COVID-19 and mendelian randomization evidence.\textsuperscript{25}

**Incorporating additional key concepts when reframing sepsis immunobiology models**

*First,* humans can protect themselves from or recover from (survive) microbial threats using three distinct strategies: avoidance, resistance and disease tolerance. In sepsis, avoidance strategy has been bypassed and the human host has an established infection. Thus, in-terms of reframing sepsis immunobiology, humans depend on resistance, disease tolerance and related immunological
concepts of resilience and resolution to recover. Therefore, immune responses in sepsis include two

distinct (often opposing) immunological and metabolic programs of immune effector mechanisms

aimed at pathogen elimination (i.e., resistance) versus those aimed at limiting tissue damage,

promote repair/resolution (i.e., disease tolerance), leading ultimately to restoring immune system

homeostasis. Restoring homeostasis also depends on resilience, which is a trade-off between

resistance and disease tolerance mechanisms. Recent data suggest that identifying and targeting

mechanisms of immune resilience may be useful in infectious diseases. In the context of sepsis

(and infectious threat) the term immune resilience refers to the capacity of the immune system to

rapidly restore to the regulated state it was in prior to the infectious threat, whilst limiting the

inflammatory cost to the host. Clinical equivalent of the inflammatory cost to the host are the

adverse outcomes in patients with sepsis.

Resistance strategies protect the human host following sensing of microbial threat by reducing (or

eliminating) invading microbes through neutralisation or killing. Resistance strategies are functions of

the innate and the adaptive immune systems. Resistance strategies are anabolic and carry

substantial inflammatory cost to the host, as elimination of pathogens is accompanied by collateral

tissue damage and harm to normal tissue function. Inflammation has been conceptualised ‘as a

response to deviations from homeostasis that cannot be reversed by homeostatic mechanisms

alone’. In the context of inflammation, homeostasis refers to active maintenance of certain quantitative

characteristics of the system, known as regulated variables, within a desired range (set point), which is

altered during inflammation. Thus, resistance mechanisms in sepsis can be reframed as an altered

immune homeostasis caused by infection, resulting in inflammation of observable magnitude and

requires active intervention to restore baseline homeostasis of the immune system.

Disease tolerance refers to an evolutionary conserved defence strategy that limits the severity of

infectious diseases, without directly affecting pathogen burden. Disease tolerance reduces the host

susceptibility to metabolic dysfunction and tissue damage caused directly by pathogens or indirectly

by immune responses to pathogens. The establishment of disease tolerance to infection may also

involve mechanisms that pertain to host-microbiota interactions, such as those involving

microbiota-derived metabolites (e.g., butyrate). The microbiome of critically ill patients with sepsis is

disrupted, resulting in selection of microorganisms that can cause harm under certain circumstances.

This harm occurs via further dysregulation of host defence mechanisms and reduced production of
beneficial metabolites such as some short chain fatty acids. This link between disease tolerance and microbiome is poorly understood.

The successful therapeutic targeting of tissue damage control mechanisms in murine models also helps to establish disease tolerance as a mechanism of interest in sepsis. The best evidence comes from studies of haemopexin, a plasma protein that neutralizes the pathogenic effects of labile haem or soluble ferritin. Labile haem is a prototypical iron-based damage associated molecular pattern, generated as a by-product of haemolysis, which dysregulates host energy metabolism and regulated cell death, compromising disease tolerance to sepsis. These pathogenic effects of labile haem may explain why targeting different regulatory components of haem metabolism exerts protective effects against sepsis and other infectious diseases associated with haemolysis. Recently, in murine models of sepsis, therapeutic effects via disease tolerance mechanisms were reported with anthracyclines (e.g., daunorubicin, doxorubicin) through activation of DNA damage responses and autophagy pathways and tetracyclines (e.g., doxycycline) via the mitoribosomal inhibition of protein synthesis, perturbation of the electron transport chain, increased fatty acid oxidation and glucocorticoid sensitivity.

Conceptually, most immunomodulation trials in sepsis to-date have directly targeted selected components of immune resistance mechanisms. However, there are multiple causal pathways between infection ➔ immune resistance mechanisms ➔ outcomes. Thus, it could be argued that the effector pathways we have targeted in immunomodulation trials thus far may not be true proximate determinants of outcomes or altering them may not completely take away the excess risk from sepsis or that some patients could have suffered harm that offset any benefit in the trial population. A simple example is that most microorganisms can be recognized by a handful of pattern recognition receptors, which in turn can induce multiple effector responses. Thus, blocking single pathways may not improve sepsis outcomes, as was observed with TLR4 antagonist therapy. This is also supported by observations that molecular subtypes respond differently to treatments (e.g., hydrocortisone was associated with increased mortality in a subset of septic shock patients).

Second, the immune responses of the human host to microbial threat and microorganisms themselves have co-evolved. Thus, the acquired subversion mechanisms in microorganisms could target either human innate immune system detection or avoid inflammatory responses. However, to survive infections, the immune system must invoke responses appropriate to the scale of microbial
threat. The microbial threat could be scaled from low to high. Soluble pathogen associated molecular patterns (PAMPs) have least threat. The scale of microbial threat is higher when dead microorganisms are sensed and increases further when viable microorganisms are detected. The microbial load may be an additional factor. The scale of threat is highest when viable microorganisms that express genes encoding virulence factors which actively disrupt or alter host tissue homeostasis (so called vita-PAMPs) are sensed.\textsuperscript{44,45} The current microbiological assessments in sepsis are limited to whether a pathogen was identified and what class of pathogen it was. Whilst we acknowledge that immune responses differ by pathogen class (such as bacterial vs. viral\textsuperscript{46}), studying differences in immune responses to different scales of microbial threats could explain observable heterogeneity in sepsis\textsuperscript{45} and perhaps identify novel targets.

Third, every organ has a distinctive set of immune sensors and effectors, as organs consist of organ specific cells, resident immune cells and immune cells recruited during inflammation. In humans, each organ also has a unique resident immune cell composition,\textsuperscript{47} and proteomic signature.\textsuperscript{48,49} These organ specific cells and immune cells display numerous abnormalities in sepsis.\textsuperscript{50-52} In animal models of infection, there are organ specific differences in immune responses\textsuperscript{53}, which may also occur in patients with sepsis.\textsuperscript{,} Currently, this possibility that the immune responses within organs may be different from what is observed in blood and the risk of adverse consequences from immunotherapy are not explicitly considered during sepsis trials.\textsuperscript{54,55} For example, when immunostimulants are administered for blood level diagnosis of immunosuppression and lungs are not in a similar immunosuppressed state, then the lung injury could theoretically worsen. To explicitly test this hypothesis, we need to identify biomarkers that provide information on organ specific immune states to compare with blood immune state. This concept is supported by observations in acute respiratory distress syndrome\textsuperscript{56} and in COVID-19\textsuperscript{57}. We acknowledge that it is neither feasible to sample all vital organs to assess organ-specific immune states nor possible in every patient with sepsis. However, there are accessible spaces such as respiratory, urinary and gastrointestinal tracts, accepting that samples from these organs may get us closer to an organ-specific immune state, not necessarily the true tissue immune state (Figure-1). Alternatively, we could search for blood biomarkers that are reflective of immune dysregulations in specific organs. We highlight compartmentalisation of immune dysregulations as a concept that may have treatment implications, and therefore should be explored in future studies.
Fourth, the problem in sepsis immunobiology may not be the initial resistance mechanisms, but their failure to turn off following elimination of microbial threat. The resolution of inflammation is an active process associated with the expression of anti-inflammatory and reparative genes such as IL-10 and transforming growth factor-β, the removal of inflammatory cells and the restoration of tissue resident macrophages and dendritic cells.

Neutrophils provide a cardinal example of the complex interplay between the processes that support activation of an innate immune response and those that enable its resolution. Neutrophils are the most abundant circulating leukocytes, the first line of defence against infection as they phagocytose bacteria and tissue debris, release antimicrobial compounds and reactive oxygen intermediates and extrude their DNA as neutrophil extracellular traps. They are crucial to the early response to danger, but harmful when that response persists and activated neutrophils play a pivotal pathologic role in sepsis. Neutrophils are constitutively apoptotic cells, circulating for only hours following their release from the bone marrow before apoptotic death. Each day, some $10^{11}$ neutrophils are released from the bone marrow. Their apoptotic death and uptake by resident phagocytes activates counter-inflammatory and reparative responses. The processes of activation and resolution through apoptosis in the neutrophil are intimately intertwined. Caspase-1, initially identified as a key effector of apoptosis, also activates IL-1β through a multi-protein complex called the inflammasome and so initiates the host inflammatory response following pattern recognition receptor engagement. Caspase-8, the enzyme responsible for initiating apoptosis in response to extracellular signals, exerts anti-apoptotic activity following its tyrosine phosphorylation, a post-translational modification apparent in neutrophils from patients who have sustained trauma or sepsis that results in neutrophil-mediated apoptosis of epithelial cells.

The biologic processes that underlie the resolution of inflammation in sepsis are poorly understood. Understanding resolution mechanisms of relevance in sepsis could enable new treatment opportunities. Drugs such as acetylsalicylic acid (through enhanced production of lipoxins) or corticosteroids have pro-resolution activities. The cellular signalling pathways that sustain inflammation are complex and provide additional potential targets, including IL-1β, heat shock protein 90 and the NAD-generating enzyme Nampt, to accelerate the resolution of inflammation.

Fifth, trained immunity refers to the durable increased responsiveness of innate immune cells to secondary stimulation following prior exposure to microbial challenges and endogenous stimuli (such
as oxidized low-density lipoproteins, uric acid, aldosterone, catecholamines and S-100 proteins). Trained immunity is acquired via extensive metabolic and epigenetic reprogramming of innate immune cells induced by the primary microbial threat, either pathogens or components thereof (e.g., BCG vaccine, viral infections, β-glucan) and is expected to last for a few weeks or months after a primary challenge. Induction of trained immunity requires involvement of several metabolic pathways including glycolysis, oxidative phosphorylation, glutaminolysis, cholesterol metabolism, fatty acid oxidation and methionine and glutathione metabolism. These changes provide metabolites needed to induce and sustain the epigenetic and functional changes that characterize trained immunity.

Important epigenetic histone modifications involved in trained immunity are H3K4me3, which marks active promoters; H3K4me1, which marks distal enhancers; and H3K27 acetylation, which marks both active enhancer and promoter regions.

Understanding the role of trained immunity in the sepsis immune response is relevant, given that many sepsis events occur in the context of deteriorating health in the year preceding sepsis. Markers of trained immunity are acquired during sepsis in animal models and sepsis survivors often suffer from infection-related rehospitalisation in the months following primary sepsis admission. Sixth, the current subtyping of patients with sepsis (Figure-2) differs across investigations by study design, input data, the type of analyses and the terms used to describe the subtypes (e.g., subphenotypes, treatable traits, endotypes). Sepsis molecular subtypes are derived using data from cohort studies, input data being blood leukocyte gene expression data and the type of analyses being unsupervised clustering generating up to four molecular subtypes of sepsis (such as MARS (molecular diagnosis and risk stratification of sepsis) endotypes 1-4, sepsis response signature (SRS) subphenotype 1 and 2 or recently SRS-3 and others (“inflammopathic”, “adaptive” and “coagulopathic”). Sepsis clinical subtypes are derived using data from cohort studies and completed randomized controlled clinical trials, with input data including routine clinical data plus biomarker data (such as physiological variables, leukocyte counts and protein biomarkers in some of the analyses) and the type of analyses being unsupervised clustering. Up to 6 sepsis subtypes have been reported (Clusters 1-4; α, β, γ and δ clusters, Classes 1-6; subphenotype-1V and 2V from VANISH Trial and Subphenotype-1L to subphenotype-3L from LeoPARDS Trial). The key conceptual argument from Figure-2 is the need to test for mechanistic overlap between subtypes to generate one or more overlap subtypes or common subtypes across different studies.
Conceptual definition of dysregulated immune responses and research roadmap

We reframe the dysregulated host immune responses in sepsis as pathologic disruption and/or altered homeostasis of the immune-driven resistance, disease tolerance and resolution mechanisms occurring concurrently. This reframing provides additional opportunity for refining sepsis treatments (Figure-3).

Our conceptual definition of dysregulated immune responses also provides a tangible opportunity to highlight a research roadmap (Figure-4 and Table-1), that will need refinement as the field progresses. This roadmap could be grouped into two broad areas: (a) re-evaluation of currently available datasets to refine our proposed reframing of dysregulated immune responses; (b) considering how the future translational research enterprise could use systems biology approaches to determine dominant modifiable mechanisms, sepsis subtypes, incorporate scale of microbial threat with sepsis diagnostics and come to a broad agreement on minimum standards of rigour or framework for sepsis subtyping.

Revaluation of currently available and published datasets with a focus on exploring resistance, tolerance and resolution pathways and factors causing variations between patients with sepsis is an essential next step. This revaluation could be iterative in terms of input data, model testing and subsequent validation. Such analytic models can be applied across data formats, such as clinical data or biological data, to perform either integrative or explanatory (prediction) modelling.

Broadly, integrative models could be complete or partial. In complete-data integrative models, data are measured on the same individuals in the dataset, with the goal to build the relationships between different variables to explain findings at individual level. In partial-data integrative models, data are measured on different individuals often in different datasets, with the goal to build the relationships between different variables to predict at cohort level. Whilst such analyses require collection and storage of varied samples from large numbers of patients at different stages of clinical disease (from pre-sepsis to late resolution) and expensive necessitating collaborative working of laboratories with different areas of expertise, we note that such studies are already feasible given the wealth of publicly available datasets.
Published literature highlights that sepsis (susceptibility and clinical features) is associated with changes at genome, transcriptional, translational and post translational biological levels highlighted in Figure-4. Specifically, genetic associations and variants that underlie susceptibility to sepsis reported in pneumonia, COVID-19 and other subgroups have the potential to reveal molecular mechanisms underlying sepsis, through functional genomics. An example is the identification of multiple expression quantitative trait loci (eQTL) and protein quantitative trait loci (pQTL) significantly associated with life-threatening COVID-19 and pathogen specific host responses. There is limited information on eQTL and the relationship between eQTL and pQTL in all-cause sepsis. Whilst numerous epigenetic modifications are associated with sepsis, there has not been large scale studies to explore the impact of such changes on the resistance, disease tolerance and resolution components of the dysregulated immune responses. For example, presence of acquired epigenetic changes from environmental exposures could explain exaggerated innate immune responses seen in some patients with sepsis and could highlight new immune therapeutic interventions aimed at stimulating or repressing trained immunity. There is much important information available and more to be discovered with high-throughput assays of RNA expression, epigenetics, proteomics, metabolomics and other omics technologies, including at single cell resolution, on the different elements of the dysregulated immune responses in sepsis. Integration across these modalities is limited - for example, mRNA abundance may have little correlation with the concentration of the corresponding proteins. A further limitation is the difficulty in identifying causal relationships in highly multidimensional observational data. However, human genetics can also offer an approach to cut through this complexity and reveal causal mechanisms. There is also a need to address the limited information on the interrelationships between and variations within the transcriptional, translational and post translational levels in sepsis. Thus, a key element of the future roadmap is to perform large scale cohort studies, alongside approaches to enable causal inferences when evaluating multiple biological levels of data incorporating systems biology principles.

There is limited longitudinal biological information in deep immunophenotyping studies, with almost all information coming from the admission time point in patients with sepsis. The admission time point biological sampling provides a snapshot of immune effector responses, manifesting as immunological heterogeneity, as the time of transition from infection to sepsis is unknown. In future studies, analytically, this could be addressed with having controls with timed insult (e.g., major
elective surgery) and by using methods that model longitudinal information when the actual
measurement time is treated as uncertain (e.g., pseudotime analyses). Insight into the kinetics and
interrelationships of distinct immune dysregulations likely is key for not only risk assessment and
timely recognition of sepsis, but particularly for identifying central targets for therapeutics that can
prevent or reverse sepsis by restoring immune homeostasis.

The clinical utility of -omic profiling will be enhanced by availability of such data in advance of acute
illness, for example either with broad population level implementation of whole genome sequencing
or targeted assessments in high-risk patients or sepsis survivors. This will also enable us to explore
hypotheses such as the protective versus adverse autoimmunity inducing roles of anti-self antibodies
generated during infections. There is also the opportunity for point-of-care testing for specific
gene sets, for example based on multiplex RT-PCR, that could facilitate patient stratification based
on underlying immune state and/or biomarkers for specific treatable traits.

Our roadmap for research is ambitious. Our concepts may be refined when information on targetable
mechanisms within immune resistance, disease tolerance and resolution that are specific for sepsis
are identified using systems immunology principles. We currently lack the information needed to design
the diagnostic tests that can identify the mechanistic sepsis subtypes we suggest. The longitudinal
studies we propose will enable us to understand immunological trajectories, immune state transitions
and the validity of different treatments at different timepoints. At present it is a challenge to have
such detailed immunological information in near-real time for patient management. With global
stakeholder engagement, it should be feasible to translate our roadmap to clinical reality within the
next decade.

Conclusions

We reframe the dysregulated host immune responses in sepsis as pathologic disruption and/or
altered homeostasis of the immune-driven resistance, disease tolerance and resolution mechanisms
occurring concurrently. Sepsis subtypes are complex traits determined by the summation of patient’s
baseline health, inherited host features, environmental influences, and dysregulated immune
responses. To enable successful immunomodulation in patients with sepsis, modifiable
immunological traits or deterministic biological networks or molecular features need to be identified.
Legends

Table legend

Table-1: Examples of knowledge gaps, measurements, and analytic approaches

Table aims to provide tangible examples for the roadmap presented in the manuscript, based on reframed immunobiology illustrated in Figure-3 and Figure-4. Please see these additional references\textsuperscript{103-109} for concepts included in the table. We are not presenting an exhaustive list of possibilities. We envisage that our conceptual reframing will trigger discovery orientated new lines of research in sepsis immunobiology, which could eventually lead to improved outcomes in patients with sepsis, and in sepsis survivors.

Figure legends

Figure-1: Overview of the sepsis immunobiology and compartmentalisation of immune responses

Health is characterized by constant (re)circulation of the major cellular and humoral components of immune system via the bloodstream and lymphatic systems, providing surveillance of danger signals. Inflammation triggers include danger signals pathogen associated molecular structures (PAMPs) from pathogens, damage associated molecular patterns (DAMPs) from stress and tissue damage and homeostasis-altering molecular processes (HAMPs) from disruptions of cellular homeostasis. Sensors for these signals include pattern recognition receptors (PRRs) as well as stress sensors expressed on leukocytes and non-leukocyte cells such as epithelial cells and endothelial cells. When danger signals are sensed inflammation signals (IS), effector signals, homeostasis signals and inflammation pathways are generated. Organ dysfunction in sepsis results from altered tissue homeostasis (ATH) with minimal tissue damage. In the context of immune responses, every organ has organ specific cells (e.g., neurons, cardiomyocytes, hepatocytes, specialized epithelial cells in kidneys, alveolar epithelial cells in the lung), tissue-resident immune cells and newly recruited immune cells, that could sense and display effector mechanisms that further alter organ milieu and function. Yellow boxes indicate either paucity of data or lack of explicit framing in the current sepsis immunobiology models of the following concepts: scaling of microbial threat, resistance, disease tolerance, resilience, resolution/repair and compartmentalisation of immune responses. We discuss these concepts in main text to inform the proposed definition of dysregulated immune responses.
Figure-2: Hypothetical overlap of subphenotypes reported in sepsis and potential challenges

Current sepsis subtyping is often done as a single domain (clinical data or one of the omics) focused analysis, which largely ignores the functional interconnections between different biological domains and are unlikely to capture the entire immunological complexity of sepsis biology. Summary descriptors highlight apparent similarities between molecular subphenotypes and between clinical subphenotypes. For example, there are similarities between MARS-2 vs SRS-1 vs inflammopathic and between MARS-3 vs SRS-2 vs adaptive molecular subphenotypes. Similar subphenotypes are represented with an overlap in the figure. There are numerous challenges with the current approach to subphenotyping. These include (but not limited to) different data inputs, different dimensionality reduction analytic approaches, limited use of integrated information from two more biological data domains and uncertainty around differential biological mechanisms linked to each subphenotype, probabilistic assignments, unique targetable mechanisms with functional relevance in a subphenotype, surrogate markers or end points or treatment response features at a biological level, reproducibility in multiple independent datasets and uncertainty around feasibility of implementation globally including in resource limited settings.

Figure-3: Reframing of dysregulated immune responses in sepsis informs potential treatments

The degree of immunopathology in sepsis is related to the magnitude and duration of abnormalities in resistance, disease tolerance, resilience, resolution and repair mechanisms. If future studies could identify patients with one or more of these mechanisms dominating and explaining the sepsis state, then these can be targeted with specific treatments within clinical trials. The proposed treatments are examples and do not represent an exhaustive list. A patient may require more than one treatment, based on their dominant mechanisms. These dominant mechanisms may vary over time, when assessed with longitudinal sampling. The dominant mechanism could also differ between blood and one or more tissue compartments and is likely to vary by sepsis subtypes.

Figure-4: Research to identify the biological variations and classify sepsis using systems immunology

Data generation is required for multiple domains in patients with infections, sepsis and acute illnesses, as all omics dimensions contribute towards observed heterogeneity in sepsis. Genotyping
gives information for ‘Past’ population selection and Drift. Epigenetic changes accounts for lifetime exposures prior to sepsis, intergenerational effects. Variation within biological data occur in genomics data from (e.g., single-nucleotide polymorphisms (SNP), copy number variation (CNV)); epigenomic data (e.g., DNA methylation, histone modification, chromatin accessibility, transcription factor binding, long noncoding (Inc)RNAs); transcriptomic data (e.g., gene expression, alternative splicing); proteome (protein levels, post translational modifications); and at metabolome levels (e.g., metabolites). Proteins and metabolites are the effector molecules with biological activity. Biologically, the generation of protein coding mRNAs and metabolites are complex processes. When transcription factors and RNA polymerase can access DNA and initiate transcription, protein coding pre-mRNAs are produced. Subsequent generation of mature mRNA is essential for nuclear export, stability and translation. Only a portion of such mRNA transcripts (including splice variants) are translated into proteins. Protein levels and biological activity are affected SNPs in regions of genes coding for amino acids and post-translational modifications (PTMs). Thus, information flow between these biological domains and combinatorial variations across domains generate heterogenous sepsis clinical phenotypes. Systems immunology refers to the study of interactions within the immune system, their regulatory functions and the emergent properties of immune responses. Analysis of multi-domain data to enable subtyping can be dominant mechanism-based or knowledge based, with or without data integration analytic approaches used in systems immunology studies.
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Declaration of interests

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<table>
<thead>
<tr>
<th>Concept</th>
<th>Knowledge gaps</th>
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| Abnormal resistance           | • Diagnostic tests for scale of microbial threat  
• Differences between sterile inflammations and sepsis-related inflammation  
• Inter relationship between hyperinflammation and immunosuppression.  
• Features and diagnostic criteria for hyperinflammation, and for immunosuppression.  
• Identifying subsets of hyperinflammation, and for immunosuppression.  
• What happens to immunosuppression pathways when anti-inflammatory therapies are used for hyperinflammation?  
• How closely do the measurements of immune state in blood reflect vital organ immune state?  
• What are the modifiable mechanisms for resistance, disease tolerance, resilience, resolution, and repair affected in sepsis?  
• Will outcomes improve if we test different immunomodulation strategies over the illness course with time-series analyses of immunological data?  
• New drug targets                                                                 |
| Impaired tolerance - resilience| • Pathways involved in disease tolerance in humans with sepsis.  
• Prevalence of impaired resilience in sepsis cohorts  
• Mechanisms contributing to impaired resilience during sepsis.  
• Relationship of impairments disease tolerance and resilience pathways to sepsis illness trajectory to enabling timing of interventions  
• Prevalence of impaired immune resilience during the pre-sepsis period to enable primary prevention.  
• New drug targets                                                                 |
| Impaired resolution - repair   | • Pathways involved in impaired resolution in humans with sepsis.  
• Mechanisms contributing to impaired repair in humans with sepsis.  
• Relationship of impairments in resolution - repair pathways to sepsis illness trajectory to enabling timing of interventions  
• New drug targets                                                                 |

<table>
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<th>Measurements and analytic approaches</th>
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| • Longitudinal blood sampling in cohort studies  
• Before and after treatment sampling in clinical trials  
• Standardization of clinical sampling procedures, and data sharing  
• Standardised immunophenotyping as per the Human Immunology Project guidance  
• Multi-layer immunomics  
• Cytokine networks  
• Interactome for sepsis  
• Time-series analyses  
• Systems immunology principles  
• Integration of clinical and immunological data based on current knowledge to highlight pathways involved in resistance, disease tolerance, resilience, resolution, and repair affected in sepsis.  
• Network medicine principles  
• Drug repurposing and novel discoveries using information from pathway analyses specific for sepsis  
• Enhanced target discovery  
• In silico medicinal chemistry  
• Diagnostics for impaired disease tolerance, resilience, resolution, and repair in patients with sepsis |
| Sepsis immune states/ subtyping (Mechanism / knowledge based/) | Construct personalized perturbation profiles for cell–cell regulatory mechanisms across individual subjects  
Agreement on minimum standard or framework for sepsis subtyping  
Multiomic integration to generate novel sepsis subtypes  
Features and diagnostic criteria sepsis subtypes  
Diagnostic tests for subtypes  
Refined therapeutic approaches based on reframed sepsis subtyping data  
Construct personalized perturbation profiles for cell–cell regulatory mechanisms across individual subjects  
Agreement on minimum standard or framework for sepsis subtyping  
Multiomic integration to generate novel sepsis subtypes  
Features and diagnostic criteria sepsis subtypes  
Diagnostic tests for subtypes  
Refined therapeutic approaches based on reframed sepsis subtyping data | Systems immunology |
Microbial threat
- Inflammation triggers, Sensors, Effectors (PAMPs, DAMPs, HAMPs), (PRRs), (Leukocytes)

Sepsis ‘state’ = Dysregulated host (immune and non-immune) responses + infection
- I.e., Resistance mechanisms (and altered homeostasis)

Inflammation signals (IS) in blood
- e.g., Effector vs Homeostasis

Alteration of tissue homeostasis (ATH) → organ dysfunction + organ cross talk

Inflammation signals within organs
- Organ cell alterations
  - e.g. nephron, hepatocyte, alveolar epithelium, neurons, cardiomyocytes

Supporting cells within organs (normally resident or newly recruited)
- e.g. macrophages, neutrophils, endothelial cells, stromal cells, fibroblasts

Inflammation sensors

Inflammation effectors

Organ milieu alterations
- e.g. Interstitial inflammation, exudate, disrupted extra-cellular matrix

Tolerance (and resilience) mechanisms

Resolution (and repair) mechanisms

Sepsis state
- Information from blood level assessments
- Information from organ level assessments
- i.e., Resistance mechanisms (and altered homeostasis)

Compartimentalisation
Examples of transcriptome-based subpopulations

- **MARS-1**: Enhanced haem biosynthesis with impaired PRR, cytokine and lymphocyte signalling, impaired antigen presentation
- **MARS-2**: Enhanced PRR and cytokine signalling with raised NF-kB and IL-6 signalling
- **MARS-3**: Enhanced adaptive immune functions (T cell pathways)
- **MARS-4**: Enhanced PRR and cytokine signalling with raised interferon signalling

**SRS-1**: Immunosuppressed phenotype with features of endotoxin tolerance, T-cell exhaustion, and downregulation of HLA class II
**SRS-2**: SRS-1 description is relative to SRS-2

**Inflammopathic**: Innate immune activation with higher mortality
**Adaptive**: Adaptive immune activation with lower mortality
**Coagulopathic**: Higher mortality, older, and with clinical and molecular evidence of coagulopathy

Examples of clinical ± biomarkers-based subpopulations

- **Cluster-1**: Shock with elevated creatinine
- **Cluster-2**: Minimal MODS
- **Cluster-3**: Shock with hypoxaemia and altered mental state
- **Cluster-4**: Hepatic dysfunction

**Alpha**: Few abnormal laboratory values, less organ dysfunction
**Beta**: Older, comorbid population, higher renal dysfunction
**Gamma**: More inflammation with pulmonary dysfunction
**Delta**: Liver dysfunction, with septic shock

**Class-1**: Uncomplicated septic shock profile with few organ dysfunctions
**Class-2**: Pneumonia with ARDS requiring mechanical ventilation with few other organ dysfunctions
**Class-3**: Post-operative abdominal sepsis, older with relatively low organ dysfunction scores
**Class-4**: Severe septic shock with high severity scores, high positive blood culture, high lactate, low platelets
**Class-5**: Pneumonia with ARDS and MODS, similar to Class-2, but with higher SOFA, and higher nosocomial infections
**Class-6**: Late septic shock, features like long time between ICU admission to start of vasopressors for septic shock

**Subphenotype-1**: Subphenotype-2 description is relative to this subphenotype
**Subphenotype-2**: Higher inflammation, higher endothelial injury, higher mortality compared with Subphenotype-1

**Subphenotype-1**: Subphenotype-2 and 3 descriptions are relative to this subphenotype
**Subphenotype-2**: Intermediate phenotype
**Subphenotype-3**: Subphenotype with highest inflammation, endothelial injury, and mortality compared with Subphenotype-1
Infection

Transition to sepsis state

Sepsis state

Abnormal resistance

Excessive inflammation

Therapy = Inhibit Hyperinflammation
- Glucocorticoids
- IL-6 inhibitors
- JAK/STAT kinase inhibitors

Early deaths = Progressive organ failure

Late deaths = Smouldering inflammation

Immunosuppression

Therapy = Reverse immunosuppression
- Recombinant IL-7
- GM-CSF
- Anti PD-1
- IFN-γ

Deaths = From primary infection

Late deaths = From secondary infections

Impaired tolerance - resilience

Therapy = Enhance tolerance/ resilience
- Anthracyclines
- Tetracyclines, Metformin
- Microbiome derived metabolites

Uncertain association to early or late deaths

Impaired resolution - repair

Therapy = Pro-resolution/ pro-repair
- Low dose aspirin
- Glucocorticoids
- Lipid mediators

Possible association with late deaths = from persistent inflammation
**Data generation**

Cohort studies and clinical trials generate multi-domain immunology data in patients clinically diagnosed as infection, sepsis, or acute inflammatory illnesses.

**Genotype and Epigenetic changes**

- Pre-sepsis health
- Infection
  - Scale of microbial threat

**Genome level**
- epigenetics; CNVs; SNPs; IncRNAs

**Transcriptional level**
- mRNA
- Transcription factors
- Cytokines
  - Proteome
  - Metabolites
  - Lipids

**Translational level**

**Post translational level**

**Sepsis state**

- Abnormal resistance
- Impaired tolerance - resilience
- Impaired resolution - repair

**Clinical features**

**Systems immunology**

Assessments of dysregulated responses with multi-domain immunology data in patients with sepsis to identify known and to-be-discovered mechanisms.

**Dominant mechanism-based subtyping**
- Abnormal resistance (hyperinflammation)
- Abnormal resistance (immunosuppression)
- Impaired tolerance - resilience
- Impaired resolution - repair

**Knowledge-based subtyping**
- Clinical
- Endotypes
- Treatment responsive
- Clinically important