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# Decoding immune low-response states in sepsis: single-cell and 3D spatial transcriptomic insights into immunoparalysis

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Sepsis remains a leading cause of critical illness worldwide. Despite advances in supportive care, durable benefit from immune-directed therapies is limited, reflecting heterogeneity with immune low-response states ('immunoparalysis') across innate and adaptive compartments. In this review we summarize advances from single-cell RNA and ATAC profiling, immune-repertoire assays and 3D spatial transcriptomics that resolve monocyte, dendritic-cell (cDC1, cDC2 and pDC), lymphocyte and NK-cell programs, and appraise translational opportunities spanning endotype-guided risk stratification, pharmacodynamic monitoring and spatial biomarkers. We also discuss enduring challenges—including assay standardization, harmonized thresholds for monocyte HLA-DR and whole-blood stimulation, and limited availability of clinically compatible spatial platforms—that temper implementation. By integrating bedside function (HLA-DR trajectories, LPS-induced cytokine capacity) with single-cell endotypes (MS1/HLA-DR^low S100A^high monocytes, dendritic-cell attrition, checkpoint-biased T cells) and host–pathogen topology from FFPE-ready spatial assays, emerging strategies aim to restore antigen presentation, reconstitute priming, disrupt inhibitory myeloid–lymphoid circuits and prevent secondary infection. Our synthesis provides an appraisal of the evolving landscape of immunoparalysis-informed precision medicine in sepsis and outlines pragmatic standards for composite biomarkers, patient selection and on-therapy decision rules. We hope these insights will assist investigators and clinicians as they endeavor to convert descriptive immune low-response states into tractable, reversible clinical entities.

KEYWORDS

**sepsis, immunoparalysis, immune low-response states, monocyte HLA-DR, endotoxin tolerance, single-cell RNA sequencing**

# 1 Introduction

Sepsis is a leading cause of critical illness and death worldwide and is characterized by organ dysfunction arising from a dysregulated host response to infection (1–4). Contemporary immunology has reframed this response as dynamic and heterogeneous, with phases of exuberant inflammation often overlapped by immune low-response states collectively termed immunoparalysis (5–7). These states are marked by impaired antigen presentation, altered cytokine production, and defects in innate–adaptive crosstalk that increase susceptibility to secondary infection and adverse outcomes, underscoring the need for precise endotyping and immune-directed interventions (8–11).

Clinically, immunoparalysis is captured by functional and phenotypic readouts that indicate reduced host defense capacity. Two complementary assays are most established: ex vivo lipopolysaccharide–stimulated cytokine production (typically diminished tumor necrosis factor-α release) and decreased expression of HLA-DR on circulating monocytes, the latter indexing an antigen-presentation deficit (12–15). Both correlate with infection risk and mortality and have been proposed for risk stratification and for selecting patients into immuno-adjuvant trials (16–18). Mechanistically, these abnormalities align with endotoxin tolerance programs in myeloid cells and checkpoint-mediated exhaustion in lymphocytes.

High-dimensional profiling now offers a path to resolve this heterogeneity. Single-cell RNA sequencing in sepsis blood defined discrete immune states—including an expanded $CD14^{+}$ monocyte state with suppressed HLA-DR and altered inflammatory signaling—that robustly distinguish patients and provide mechanistic anchors for biomarker development (19–21). Subsequent multicohort analyses reinforced that composite single-cell signatures capturing lymphopenia, dendritic-cell loss, and myeloid HLA-DR downregulation track disease trajectories and may improve diagnostics and prognostication across age groups (22–24). These findings support an endotype-based view of sepsis in which therapeutic responsiveness depends on the prevailing immune program rather than on a uniform "hyperinflammation" construct.

At the tissue level, dissociative assays incompletely represent microanatomical context, which is critical for understanding host–pathogen interfaces, vascular compromise, and compartmentalized immunoregulation in sepsis. Spatially resolved transcriptomics has therefore emerged as a complementary approach, recognized for enabling quantitative maps of gene expression within intact architecture; recent advances extend these maps into three dimensions, providing volumetric views of cellular neighborhoods and gradients relevant to barrier defense and organ injury (25–28). The integration of spatial readouts with single-cell state dictionaries creates opportunities to localize immunoparalysis niches, quantify cell–cell communication *in situ*, and nominate spatial biomarkers suitable for translation.

This review synthesizes evidence on immune low-response states in sepsis with a focus on how single-cell modalities (scRNA-seq, scATAC-seq, CITE-seq, TCR/BCR profiling) and three-dimensional spatial transcriptomics delineate innate–adaptive programs, tissue topology, and host–pathogen contact zones. The aim is to standardize concepts and measurement frameworks for immunoparalysis, highlight robust cellular and spatial biomarkers for risk stratification, and outline principles for patient selection and pharmacodynamic monitoring in trials that seek to reverse immune low-response states.

# 2 Defining immunoparalysis in sepsis: concepts, metrics, and clinical context

As shown in Figure 1, immunoparalysis denotes a clinically significant, often reversible, low-response immune state that arises

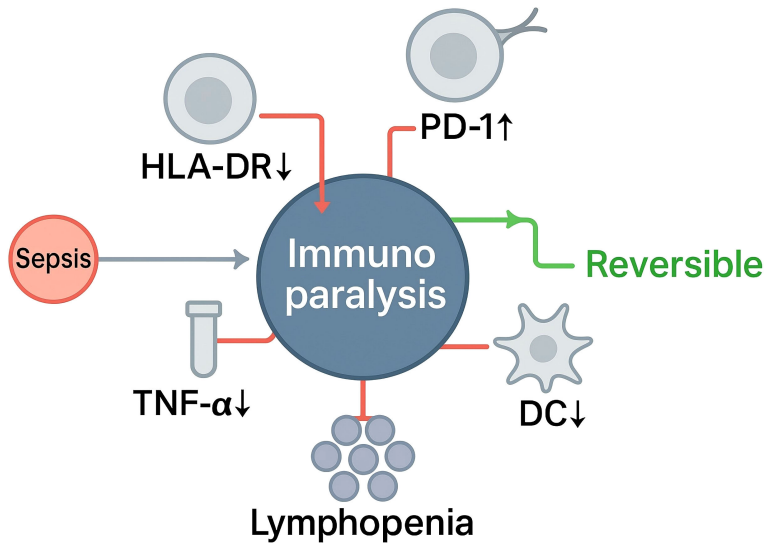


FIGURE 1
Sepsis-induced immunoparalysis: key reversible hallmarks.

during sepsis and is characterized by concurrent defects across innate and adaptive compartments, including impaired antigen presentation, blunted stimulus-induced cytokine production, apoptosis-associated lymphocyte depletion, and checkpoint-mediated T-cell dysfunction (29–31). These abnormalities reflect an adaptive reprogramming frequently described as endotoxin tolerance in myeloid cells and exhaustion or anergy in lymphocytes, and they contribute to heightened susceptibility to secondary infection and adverse outcomes. Conceptually, this state is supported by mechanistic and clinical observations of reduced HLA-DR expression on antigen-presenting cells, diminished ex vivo cytokine release after lipopolysaccharide challenge, quantitative and qualitative lymphocyte defects, and subset-specific dendritic-cell abnormalities—depletion of cDC1, functional impairment of cDC2, and contraction of pDCs (markers as listed), with the same transcriptional programs and signaling changes as previously described.

Operationalization in practice relies on complementary functional and phenotypic readouts. Monocyte HLA-DR measured by standardized flow cytometry is the most widely adopted marker of innate deactivation; persistently low expression identifies patients at increased risk of nosocomial infection and death and has been proposed as an indicator of "immune organ failure" suitable for risk stratification and for selecting candidates for immunostimulatory interventions. Longitudinal trajectories of HLA-DR during septic shock refine this risk assessment and capture recovery versus persistent suppression (32–35). Functional assays—CD107a degranulation (K562 or PMA/ionomycin) and IFN-γ release after IL-12/IL-18—map to risk (lower responses predict secondary infection) and serve as on-therapy targets with LPS-TNF and HLA-DR (36–38). Adaptive immune components provide parallel indicators: global lymphopenia and features of T-cell dysfunction, including increased PD-1/PD-L1, LAG-3 and TIM-3 signaling, align with impaired host defense and are being explored as therapeutic targets in biomarker-enriched trials (39–41). Dendritic-cell loss and dysfunction further reduce antigen presentation capacity and have been linked to secondary infection risk in septic shock, validating their inclusion within a composite definition (42, 43). In malignancy, CKD, diabetes, or prior immunosuppression, interpret 'low-response' using within-patient deltas and percentile thresholds, and require concordance across ≥2 modalities (e.g., HLA-DR$^{low}$ plus LPS-TNF$^{low}$).

A practical definition of immunoparalysis in sepsis is a time-varying syndrome of impaired innate and adaptive immunity evidenced by persistently low monocyte HLA-DR, reduced stimulus-induced cytokine production capacity, lymphocyte depletion and/or exhaustion signatures, and dendritic-cell deficiency, each associated with elevated risk of secondary infection and mortality. This framework supports standardized endotyping and provides an entry point for biomarker-guided, immune-restorative strategies in critical illness. Secondary infection' denotes a new, adjudicated infection ≥48 h after index presentation (or after initial control), assessed in day-14 and day-28 windows.

## 3 Single-cell dissection of immune low-response states: innate–adaptive programs and regulatory circuits

Single-cell modalities resolve the composite nature of immune low-response states by jointly defining cell identities, activation gradients, and regulatory dependencies at single-cell resolution. Across independent cohorts, scRNA-seq consistently identifies an expanded myeloid program characterized by HLA-DR suppression and increased S100A8/A9, RETN, VCAN, and IL1R2 expression—often referred to as the "MS1/HLA-DR$^{low}$ S100A$^{high}$" monocyte state—which is mechanistically linked to impaired antigen presentation and broad inhibitory crosstalk with lymphocytes and dendritic cells (e.g., predicted LGALS9–HAVCR2 and class I HLA–LILRB interactions) (44–46). These features recapitulate clinically recognized innate deactivation and provide a cell-state framework for immunoparalysis endotyping, as detailed in Table 1.

Adaptive compartments show concurrent low-response programs detectable by scRNA-seq and immune-repertoire profiling. Across cohorts, cDC1 depletion occurs earliest and most profoundly with down-shifted CLEC9A/XCR1 and BATF3/IRF8 regulons; cDC2 persist but exhibit dysfunction with impaired CD86/CCR7 and IL12B modules; and pDCs contract with attenuated IRF7 programs and type I interferon release—together explaining suboptimal priming and Th1 skewing. Cytokine production capacity and antigen-presentation cues decline in parallel with transcriptional features of T-cell dysfunction, including enrichment of inhibitory checkpoint transcripts and contraction of naïve/central memory pools, while dendritic-cell fractions decrease (47–49). These single-cell readouts align with clinical low-response phenotypes and reveal trajectories from activation to dysfunction that are not captured by bulk assays.

Regulatory-circuit reconstruction strengthens mechanistic inference. Chromatin accessibility profiling (scATAC-seq) and integrated analyses of endotoxin-tolerance–like states identify promoter–enhancer reconfiguration and transcription-factor programs that stabilize monocyte hyporesponsiveness, while multimodal pipelines infer active regulons and state transitions that track movement toward low-response phenotypes (50–52). Dynamic modeling (RNA velocity) and ligand–receptor inference systematically connect myeloid inhibitory signaling to T- and NK-cell dysfunction—ideally using composition-preserving permutations or cell-count offsets to control compositional confounding, zero-inflation–aware models to mitigate gene dropout, and FDR correction for multi-test burden—offering testable hypotheses for pharmacodynamic reversal.

Tissue-level sampling extends these insights beyond blood by localizing low-response programs within injured organs and host–pathogen contact zones (53–55). Time-resolved single-cell maps in septic organs reveal compartmentalized myeloid reprogramming, dendritic-cell attrition, and disrupted lymphocyte niches, supporting the view that immunoparalysis is a distributed, topology-dependent state that can now be quantified and monitored with single-cell tools.

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TABLE 1 Key single-cell–defined immune low-response programs in sepsis and how to recognize them.

| Program/Cell State | Canonical transcript or protein features (illustrative) | Single-cell readouts that establish the state | Representative inhibitory signals or network features | Functional interpretation for immunoparalysis | Analytic notes (quality and integration) |
|---|---|---|---|---|---|
| HLA-DR$^{low}$ S100A$^{high}$ monocytes ("MS1"-like) | ↓HLA-DRA/DRB1, ↓CD74; ↑S100A8/S100A9, RETN, VCAN, IL1R2, LILRB1/2 | scRNA-seq clusters with antigen-presentation deficit; CITE-seq confirms low HLA-DR surface protein | Predicted LGALS9–HAVCR2, HLA class I–LILRB, TGFB1–TGFBR pathways; myeloid suppressive regulons | Innate deactivation; antigen presentation failure; broad suppression of lymphocyte function | Guard against neutrophil contamination; harmonize ambient RNA removal; validate with flow cytometry |
| Antigen-presenting monocytes/DC with reduced capacity | ↓HLA-class II genes; ↓CCR7; ↓CD86 | Depletion of cDC1/cDC2 clusters; reduced co-stimulatory gene modules | Weak ligand delivery to T cells; impaired IL-12/IFN axes | Impaired priming and T-cell activation | Include whole-blood or DC-enriched sampling; standardize batch correction across centers |
| Exhausted/dysfunctional CD8$^+$ and CD4$^+$ T cells | ↑PDCD1, LAG3, TIGIT, HAVCR2; ↓IL7R; reduced cytotoxic module in subsets | scRNA-seq state gradients; TCR clonality skew; diminished effector gene scores | Inhibitory checkpoint circuits; reduced antigen-receipt signatures | Blunted effector responses; susceptibility to secondary infection | Pair scRNA-seq with TCR-seq; control for lymphopenia when comparing proportions |
| Atypical memory/age-associated B cells | ↑ITGAX (CD11c), FCRL5, TBX21; altered SHM/CSR patterns | BCR clonotype contraction; scRNA-seq B-cell state shift | Attenuated antigen presentation to T cells | Suboptimal antibody responses and help to T cells | Joint BCR-seq profiling advisable; remove doublets (B/T conjugates) |
| NK cell hyporesponsiveness | ↑KLRC1 (NKG2A); ↓GNLY, PRF1, NKG7 in subsets | Reduced cytotoxicity modules; altered cytokine gene scores | Inhibitory receptor dominance; reduced activating ligand inputs | Weakened early pathogen control | Include whole-blood captures; verify with degranulation markers if available |
| Myeloid progenitor bias/trained-tolerance imprint | Accessibility shifts at myeloid enhancers; ↑C/EBP/STAT motif activity | scATAC-seq peaks linked to tolerized genes; integrated GRN modules | Stabilized tolerized gene programs; diminished inflammatory transcription | Persistence of hyporesponsive myelopoiesis | Integrate scATAC-seq with scRNA-seq; use batch-aware peak calling |
| Cross-talk bottlenecks (myeloid→lymphoid) | Diminished costimulatory ligands; dominance of inhibitory ligands | Ligand–receptor networks showing low CD28/ICOS signaling and high inhibitory pairs | Net negative signaling into T/NK compartments | Propagation of low-response state across compartments | Apply curated LR databases; adjust for cell-type composition effects |

↓, decreased/downregulated expression/abundance; ↑, increased/upregulated expression/abundance.

The above cell-state dictionary supports a practical approach to endotyping: prioritize detection of the MS1/HLA-DR$^{low}$ S100A$^{high}$ monocyte expansion and dendritic-cell loss, quantify checkpoint-biased T-cell states with concurrent repertoire features, infer inhibitory myeloid–lymphoid communication, and, where feasible, corroborate stabilizing chromatin programs. These readouts provide mechanistic anchors for biomarker-guided patient selection and pharmacodynamic monitoring in trials that seek to reverse immune low-response states.

## 4 3D spatial transcriptomics of sepsis tissues: ecosystem topology and host–pathogen interfaces

Three-dimensional spatial transcriptomics enables quantitative mapping of immune activity within intact organ architecture, providing volumetric context for gradients, barriers, and interfacial zones that shape antimicrobial defense during sepsis. Organism-wide spatial profiling in experimental sepsis already demonstrates tissue-specific transcriptional programs across multiple organs, establishing the feasibility and biological value of spatially resolved readouts for systemic infection (56–58). Extending these approaches into true 3D volumes allows precise localization of low-response immune niches at epithelial, endothelial, and perivascular interfaces where host–pathogen contact and microcirculatory compromise converge.

Volumetric reconstruction is now technically routine through serial-section acquisition coupled to dedicated alignment frameworks. Open-ST registers consecutive sections into a coherent 3D representation while preserving whole-transcriptome coverage, enabling reconstruction of cellular neighborhoods and long-range axes within diseased tissue. Computational toolkits such as Spateo further support 3D gradient modeling and inference of intercellular interactions across entire organs (59–61). Slice-to-slice and cross-modality alignment at scale can be performed with STalign, which maps sections to a three-dimensional common coordinate framework using diffeomorphic metric mapping, and with SLAT, a graph-based algorithm that robustly aligns heterogeneous spatial slices across technologies (62–64). Together, these methods permit organ-level assemblies in which immune

programs can be quantified as continuous fields rather than isolated 2D snapshots.

Within such volumes, ecosystem topology can be defined by combining cell-type deconvolution with spatial statistics that test whether ligand–receptor signaling is non-randomly organized in space (58, 65, 66). Approaches like SpatialDM use bivariate spatial autocorrelation to score interaction hotspots, allowing *in situ* quantification of immunoregulatory circuits (for example, inhibitory myeloid–lymphoid signaling) and their relationship to distances from vasculature, airspaces, or tubular lumina (28, 50, 67). This framework operationalizes "immunoparalysis niches" as measurable 3D entities that co-vary with tissue gradients relevant to sepsis pathophysiology.

Resolving host–pathogen interfaces requires simultaneous detection of microbial and host transcripts. Two sequencing-based strategies now enable this. Spatial host–microbiome sequencing (SHM-seq) co-captures polyadenylated host mRNA and 16S rRNA from bacteria on the same array, accurately mapping bacterial biogeography against host programs (68–70). Spatial metatranscriptomics (SmT) broadens this to include fungal taxa by jointly sequencing 16S and 18S/ITS, while explicitly addressing contamination risk by leveraging spatial patterns to separate true tissue-embedded microbial signals from environmental noise (71–73). For clinical material, dual spatial transcriptomics in FFPE sections achieves unbiased co-detection of human and viral RNAs, offering a practical route to archived sepsis tissues and high-containment samples. Collectively, these methods transform microbe–host colocalization from proxy histology into transcriptome-wide, spatially explicit measurements.

Platform choice influences detection at the host–pathogen boundary. Oligo-dT capture alone is suboptimal for many microbes and can be hindered by FFPE-induced 3′ tail modification, motivating total-RNA or targeted capture schemes in infected tissue (74, 75). Recent array chemistries using random primers extend spatial capture to total RNA in FFPE while retaining high spatial resolution, improving the likelihood of detecting microbial transcripts alongside host responses in clinically processed sepsis samples (76–78). High-field-of-view platforms such as Stereo-seq additionally support wide-area mapping at near-single-cell granularity, facilitating reconstruction of large infected regions and their immunoregulatory gradients.

Integration with single-cell atlases strengthens mechanistic interpretation. Projecting single-cell–defined immune states into 3D spaces using tools such as scHolography reconstructs cellular neighborhoods and refines estimates of cell–cell communication in volumes, complementing spot-level deconvolution and enabling hypothesis testing on how myeloid deactivation, dendritic-cell attrition, or checkpoint-biased T cells are spatially organized relative to pathogen density and tissue microanatomy (79–81). These analyses can be standardized across cohorts by aligning multi-slice datasets into common coordinate systems with STalign or SLAT, supporting comparative studies of therapeutic modulation of low-response states.

A practical sepsis workflow therefore acquires serial sections from infected organs, reconstructs 3D transcriptomic volumes with Open-ST or Spateo, overlays host–pathogen colocalization via SHM-seq or SmT (FFPE-compatible where necessary), and quantifies spatially significant signaling using methods such as SpatialDM. The resulting volumetric maps delineate immunoregulatory niches at barrier and vascular interfaces that are likely to determine secondary-infection risk and therapeutic responsiveness, providing a foundation for spatial biomarkers and pharmacodynamic readouts in trials seeking to reverse immune low-response states.

## 5 Clinical translation and outlook: biomarkers, risk stratification, and therapeutic reversal strategies

Clinical translation of immune low-response states in sepsis should converge on composite, serially measurable biomarkers that couple circulating function, single-cell endotypes, and spatial context to decision-making. A pragmatic framework links three tiers. First, bedside-accessible functional and phenotypic readouts quantify host-defense capacity and its recovery dynamics; thresholds and slopes over the first week should inform risks of secondary infection, prolonged organ support, and death, and gate entry into immune-restorative interventions. Second, single-cell state dictionaries—capturing expansion of HLA-DR$^{low}$ S100A$^{high}$ monocytes, dendritic-cell attrition, and checkpoint-biased T cells—can be operationalized as reduced-gene RNA or protein panels with predefined quality controls; these signatures provide mechanistic enrichment for trials and pharmacodynamic anchors to confirm on-target immune reversal. Third, spatially aware metrics from routine histology–compatible platforms (including FFPE-adapted spatial assays) should localize immunoparalysis niches at epithelial, endothelial, and perivascular interfaces and quantify adjacency rules (e.g., macrophage–endothelium interface length, lymphoid aggregate burden) that modify risk beyond cell fractions (24, 82–84). Integration across tiers yields an "immunoparalysis index" with calibrated cut points, validated in prospective cohorts, and accompanied by minimal-surrogate surrogates (e.g., monocyte HLA-DR plus reduced-gene myeloid signature) for resource-limited settings.

Therapeutic reversal strategies should be biomarker-guided and time-sensitive: candidates include antigen-presentation upregulation and dendritic-cell reconstitution, targeted disruption of inhibitory ligand–receptor circuits, and context-aware checkpoint modulation; for PD-1/PD-L1, LAG-3 or TIM-3 trials, enroll checkpoint-high patients (e.g., PD-1$^{hi}$ CD8$^{+}$ T cells and/or PD-L1$^{hi}$ monocytes in the top cohort quartile together with HLA-DR^low), and predefine pharmacodynamic reversal as increased TCR- or cytokine-stimulated IFN-γ/TNF with higher CD107a degranulation/cytotoxic scores and a fall in checkpoint MFI or gene scores toward reference ranges (85–88). Trial designs should embed adaptive enrichment, early futility based on target engagement, and safety monitoring for hyperinflammatory breakthroughs; we recommend primary endpoints of infection-

free and organ-support–free days to day-28 (composite ventilator-, vasopressor- and renal-replacement–free days), with secondary endpoints including 28/90-day mortality, new secondary infection by day-14, ΔSOFA to day-7, ICU-free days to day-28, and patient-reported outcomes. Immunostimulatory therapies should be withheld when hyperinflammatory markers suggest a MAS-like phenotype (e.g., very high ferritin or rapidly rising IL-6/CRP).

Over the near term, we anticipate: analytical standardization of monocyte HLA-DR and whole-blood stimulation assays; translation of single-cell signatures into CLIA-ready panels; extension of spatial readouts to archived tissues using total-RNA–compatible chemistries; and composite risk models that outperform single biomarkers and enable allocation of immunoadjuvants to the subset most likely to benefit. These steps can convert heterogeneous, descriptive low-response states into tractable clinical entities with measurable entry criteria, reversible targets, and reproducible outcome gains.

## Author contributions

YY: Writing – original draft. YZ: Writing – original draft. JW: Writing – original draft. YL: Writing – original draft. XL: Writing – review & editing, Writing – original draft.

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## Conflict of interest

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