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UNDERSTANDING THE DISEASE

Compartmentalisation of immune responses in critical illness: does it matter?

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Critical illness is pragmatically defined as an acute illness resulting in organ dysfunction, necessitating organ support in specialised settings (intensive care units), to survive [1]. Despite current best care, ~ 30% of patients die within 30-days from onset of critical illness. Over the last 50-years, > 500 randomised clinical trials (RCTs) involving common critical illnesses, such as sepsis syndromes [2], and acute respiratory distress syndrome (ARDS) [3], have failed to show tangible benefit with pharmacological interventions, despite most of the interventions tested being grounded in detailed understanding of the biological mechanisms involved [1]. This lack of success is thought to arise from differences in biological features and in outcome risk between critically ill patients (i.e. loosely framed as heterogeneity). Hypotheses that are being pursued to improve the success of future RCTs include grouping patients based on observable clinical and or biological features (termed enrichment, vs subphenotyping) [4], incorporate attributable risk of critical illness during trial design, innovative trial designs (adaptive RCTs), and redefine critical illness (such as treatable traits) [5]. In this editorial we explore another hypothesis: compartmentalisation of immune responses during critical illness syndromes such as sepsis could explain the lack of benefit with immunomodulatory treatments? Compartmentalisation refers to the process whereby immune responses are confined to, or differ between, anatomical compartments within the body. Examples of compartments include the lungs, peritoneum, urinary tract and peripheral blood. Although we mostly focus on infectious insults, the canonical nature of immune responses implies that compartmentalised responses will occur in sterile insults such as trauma [6] and need to be considered in all critical illness-associated immune profiling.

Broadly, the core argument with compartmentalisation of immune responses could be summarised by considering two extreme immune states ('excess inflammation' vs 'immunosuppression') and two opposing immunomodulation strategies ('anti-inflammatory' vs 'immunostimulants'). As our window to determining immune states is most often blood, we seldom consider whether the tissue immune state at the time of blood sampling is similar or different to blood? In the two discordant scenarios, immunomodulation based only on systemic immune state could harm patients. Furthermore, mechanisms which predominate in one tissue space, may not dominate or even be relevant in other tissue spaces [7] (Fig. 1A).

Mechanisms of compartmentalised responses

There are multiple mechanisms which underpin this compartmentalisation of immune responses [8]. Even within the blood vessels, sampling the circulating cells gives an incomplete picture of current immune status. Circulating immune cells, most notably neutrophils, may marginate, loosely attaching to the endothelial wall. This subpopulation is largely unmeasured in blood samples. Immune responses within the tissues themselves are effected by a combination of resident and recruited immune cells. The resident cells, by definition, are not found in the circulation and yet play a vital role in shaping local immune responses. In streptococcal pneumonia models, inflammation is driven primarily by tissue resident macrophages, at least early on in the disease process, and remains highly

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Immune state in blood	Immune state in tissues	Overall 'immune' state	*Impact of immunosuppression	*Impact of immunostimulants
Inflammation	Inflammation	Concordant	Benefit	Harm
Immunosuppression	Immunosuppression	Concordant	Harm	Benefit
Inflammation	Immunosuppression	Discordant	Uncertain	Uncertain
Immunosuppression	Inflammation	Discordant	Uncertain	Uncertain

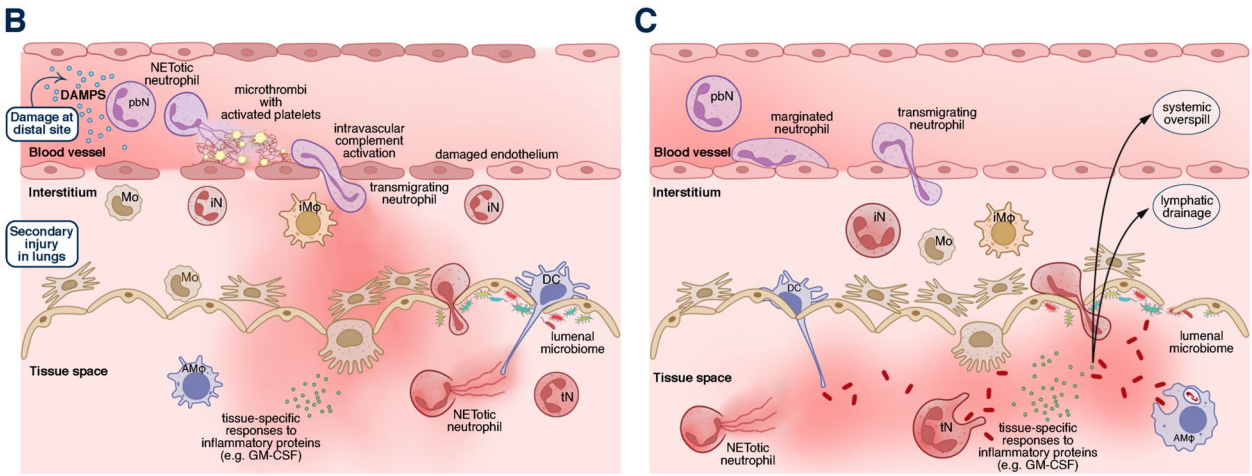

Fig. 1 Diagrammatic representation of some of the mechanisms which lead to compartmentalised inflammatory responses in critical illness. **A** indicates the summary of the hypothesis that discordant immune responses in blood and tissue compartments may lead to inappropriate therapies. *Refers to the immune state assessment based on either blood or tissue-based biomarker. **B** illustrates how distant insults and systemic spill-over of damage-associated molecular patterns (DAMPS) can lead to inflammatory and thrombotic changes in the blood, which damage the endothelium and lead to secondary damage at distal sites such as the lungs. **C** illustrates how direct tissue injury, in this example from pneumonia, produces systemic overspill of inflammatory mediators into both the lymphatic and blood compartments. In both situations, cross-talk between tissue resident and infiltrating immune cells, stromal and epithelial cells influences tissue responses and makes them qualitatively distinct from responses in circulating cells. Within luminal organs with communication with the external environment the added complexity of the microbial inhabitants (microbiome) further influences mucosal immunity. Conversely, soluble plasma proteins, such as complement, coagulation cascade components and colloidal proteins, may be largely restricted to, or in far higher abundance, in the blood compartment. Notably, in both compartments, the presence of inflammatory mediators does not automatically indicate cellular hyper-function, and indeed such mediators may drive impaired antimicrobial functions [7, 15]. *pbN* peripheral blood neutrophil, *iN* interstitial neutrophil, *tN* tissue neutrophil, *iMφ* interstitial macrophage, *AMφ* Alveolar macrophage, *Mo* infiltrating monocyte, *DC* dendritic cell

compartmentalised within the lung until bacteraemia supervenes [9]. Tissue resident memory can be demonstrated in isolated lung lobes when they are selectively exposed to antigen [10]. Infiltrating immune cells undergo changes in their phenotype, underpinned by changes in transcriptional signatures, as they infiltrate an inflamed area. Even moving between the interstitium and site of inflammation produces demonstrable changes [11], with upregulation of pathways associated with antimicrobial functions [11]. Immune signalling molecules, such as cytokines and growth factors, may have divergent and tissue-specific functions. Granulocyte-colony stimulating factor (GM-CSF) appears to have a significant role in driving autoimmune disease such as rheumatoid arthritis, and yet it is also critical to the health of alveolar macrophages and subsequent pulmonary antimicrobial responses [12]. Indeed, the potential for compartmentalised responses has led to the rationale for testing of both systemic GM-CSF blockade and inhaled recombinant GM-CSF in COVID-19 [12]. However, despite evidence of compartmentalised responses in coronavirus disease 2019 (COVID-19) [12, 13], studies have neither targeted compartmentalised endotypes nor ascertained differences in responses to immunotherapies in patients with COVID-19.

Evidence for compartmentalisation in critically ill patients

The evidence for compartmentalisation of inflammatory responses is increasingly well described amongst critically ill patients. In ventilated patients with lung infiltrates, alveolar cytokine patterns can differentiate bacterial infection from non-infectious causes whilst circulating cytokine levels are unrevealing [14]. Such effects are not unique to the lungs, and other tissue sites of infection, such as leptomeninges, peritoneum and urinary tract, demonstrate tissue-restricted inflammation [8], further details of these compartments are provided in supplemental table 1. Differential phenotypes are noted in neutrophils from the blood and lungs of patients with ARDS, with the alveolar neutrophils showing greater priming, resistance to apoptosis and with hyper-segmented nuclei relative to those isolated from autologous peripheral blood [15]. In patients with COVID-19, obesity has been found to alter immune pathway activation with suppression of type I and II interferons and tumor necrosis factor (TNF) alpha signalling in pulmonary cells, but an increase in TNF alpha pathway signalling in peripheral blood cells [13]. Even when phenotypes are similar, they may be driven by different mediators. For example, whilst peripheral blood neutrophil phagocytic dysfunction is driven by complement component 5a (C5a), different mediators drive phagocytic dysfunction in the alveolar space [7]. In these contexts, simply sampling the peripheral blood could give a misleading impression as to the pathophysiological state of the patient, and potentially be used to rationalise a therapy which may not target pathways active in tissue spaces. Figure 1B, C illustrates how immune responses may be provoked by direct or indirect injury, and some of the mechanisms which lead to compartmentalised responses.

Compartmentalised immune responses in practice.

Understanding how we may exploit the insights arising from the growing understanding of compartmentalised, tissue-specific responses is crucial to developing clinically applicable tools and therapies. Although many tissue spaces can be challenging to access, the lungs provide an important window due to both their comparative accessibility and critical importance as sites of infection and inflammation. The paired sampling of blood and lung fluid (by lavage or aspiration) allows for the detection of processes which may be distinct between these two spaces [14]. A growing range of in-vivo photonic and radioactive probes can extend this to *in-situ* phenotyping [16]. A further advantage of the lungs is its accessibility for topical or locally active therapies.

To consider a practical, albeit hypothetical, scenario arising from the insight that molecules such as GM-CSF may have divergent roles in different tissue spaces and different illness phases [12] Profiling the immune status in both the peripheral blood and lungs could allow for site-specific modulation. Using either neutralising antibodies, to inhibit signalling, or recombinant proteins to augment it at the appropriate time and in the appropriate space. Such apparently opposing interventions could be used sequentially, or even potentially simultaneously, but would require the ability to reliably assess immune status in each compartment. Although other tissue spaces are more challenging to access, in certain situations indwelling micro-dialyser catheters may allow for localised instillation of therapeutic agents.

In conclusion, we highlight a hypothesis supported by data. Prevalence of concordant vs discordant immune state between blood and tissues (at the very least lung), and the feasible surrogates of such a classification would be a useful step forward to achieve successful immunomodulation in critically ill adults.

Supplementary Information
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References

1. Shankar-Hari M, Wunsch H, Rowan K, Singer M, Rubenfeld GD, Angus DC (2021) Reflections on critical care's past, present, and future. Crit Care Med 49(11):1855–1865
2. Marshall JC (2014) Why have clinical trials in sepsis failed? Trends Mol Med 20(4):195–203
3. Saha R, Assouline B, Mason G, Douiri A, Summers C, Shankar-Hari M (2021) Impact of differences in acute respiratory distress syndrome randomised controlled trial inclusion and exclusion criteria: systematic review and meta-analysis. Brit J Anaesth 127(1):85–101
4. Shankar-Hari M, Rubenfeld GD (2019) Population enrichment for critical care trials: phenotypes and differential outcomes. Curr Opin Crit Care 25(5):489–497
5. Pinder EM, Rostron AJ, Hellyer TP et al (2018) Randomised controlled trial of GM-CSF in critically ill patients with impaired neutrophil phagocytosis. Thorax 73(10):918–925
6. Xiao W, Mindrinos MN, Seok J et al (2011) A genomic storm in critically injured humans. J Ex Med 208(13):2581–2590
7. Morris AC, Kefala K, Wilkinson TS et al (2009) C5a mediates peripheral blood neutrophil dysfunction in critically ill patients. Am J Resp Crit Care 180(1):19–28
8. Cavaillon JM, Annane D (2006) Compartmentalization of the inflammatory response in sepsis and SIRS. J Endotox Res 12(3):151–170
9. Bergeron Y, Ouellet N, Deslauriers AM, Simard M, Olivier M, Bergeron MG (1998) Cytokine kinetics and other host factors in response to pneumococcal pulmonary infection in mice. Infect Immun 66(3):912–922
10. Bice DE, Muggenburg BA (1996) Pulmonary immune memory: localized production of antibody in the lung after antigen challenge. Immunology 88(2):191–197
11. Khoyratty TE, Ai Z, Ballesteros I et al (2021) Distinct transcription factor networks control neutrophil-driven inflammation. Nat Immunol 22(9):1093–1106
12. Lang FM, Lee KMC, Teijaro JR, Becher B, Hamilton JA (2020) GM-CSF-based treatments in COVID-19: reconciling opposing therapeutic approaches. Nat Rev Immunol 20(8):507–514
13. Guo SA, Bowyer GS, Ferdinand JR et al (2022) Obesity associated with attenuated tissue immune cell responses in COVID-19. Biorxiv. https://doi.org/10.1101/2022.01.14.475727
14. Morris AC, Kefala K, Wilkinson TS et al (2010) Diagnostic importance of pulmonary interleukin-1β and interleukin-8 in ventilator-associated pneumonia. Thorax 65(3):201–207
15. Juss JK, House D, Amour A et al (2016) Acute respiratory distress syndrome neutrophils have a distinct phenotype and are resistant to phosphoinositide 3-kinase inhibition. Am J Resp Crit Care 194(8):961–973
16. Akram AR, Chankeshwara SV, Scholefield E et al (2018) In situ identification of gram-negative bacteria in human lungs using a topical fluorescent peptide targeting lipid A. Sci Trans Med. 10(464):eaal0033