Neutrophils

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Charting granulopoietic disturbances in sepsis

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A blood single-cell atlas of human sepsis identifies subsets of immune-suppressive neutrophils in people at high risk.

A life-threatening organ dysfunction caused by a dysregulated host response to infection, sepsis affects millions of people each year and is a leading cause of death worldwide[1]. The pathophysiology and clinical manifestations of sepsis are heterogenous, highlighting the need for biomarkers of patient subgroups that could benefit from targeted therapies. In this issue of *Nature Immunology*, Kwok et al.[2] combine single-cell genomics with clinical information to map aberrant granulopoiesis in human sepsis. The authors uncover subsets of neutrophils with immunosuppressive capacities, and delineate specific molecular signatures in individuals with poor outcomes. These findings shed light on the contribution of neutrophils to immune dysregulation in sepsis, and underscore the power of mapping cellular heterogeneity for treating and diagnosing severe infections.

At the root of sepsis is a maladaptive immune response to microbes, with concomitant elicitation of tissue-destructive inflammatory reactions with immune paralysis that enables pathogen replication. Myeloid cells are known to contribute to the immunopathology of sepsis, with neutrophils — the most abundant leukocytes in the blood — being implicated in both hyperinflammation and immunosuppression phenotypes through ill-defined mechanisms[3]. However, a comprehensive analysis of granulocytes in sepsis has been lacking, largely because these cells are poorly represented in the widely used mononuclear cell fraction of peripheral blood samples. Furthermore, transcriptome studies of neutrophils are notoriously challenging owing to their low RNA content and their tendency to release granules containing hydrolytic enzymes. Nonetheless, recent studies have shown the feasibility and power of single-cell RNA sequencing (scRNA-seq) using human neutrophils, highlighting an unanticipated degree of diversity and plasticity at steady state and in disease[4–6].

Kwok et al.[2] generate an atlas of both scRNA-seq and surface protein expression for more than 250,000 blood leukocytes from septic or convalescent individuals, as well as healthy donors or people undergoing cardiac surgery. The authors detected a broad increase in neutrophil abundance and diversity in sepsis, reflecting heightened synthesis and release into the circulation of cells lacking terminal differentiation markers during emergency granulopoiesis. Of note, four clusters of immature neutrophils—termed *IL1R2*$^+$ Neu, *PADI4*$^+$ Neu, *MPO*$^+$ Neu and *MKI67*$^+$ *CYP1B1*$^+$ Neu—were expanded in people with sepsis but not in those with sterile inflammation caused by surgery, highlighting disease-specific granulopoietic disturbances (Fig. 1a).

The identified sepsis-associated neutrophils displayed transcriptional features of so-called granulocytic myeloid-derived suppressor cells (G-MDSCs), and they were able to inhibit the proliferation and activation of CD4$^+$ T cells in vitro (Fig. 1b). However, caution should be taken when extending these results to an in vivo setting, because suppression assays are often influenced by technical aspects—number, purity and viability of isolated cells—that are hard to control when working with scarce patients' material[7]. For instance, co-culture experiments with T cells required the use of bulk populations of CD66b$^+$ neutrophils, potentially obscuring the effect of each subset on the observed phenotype. Nevertheless, the authors[2] provide correlative evidence that sepsis-associated neutrophils broadly contributed to immune suppression, implying pervasive functional alterations of the granulocyte pool. They found that the eicosanoid prostaglandin E_2 (PGE_2) partially mediated the suppression of CD4$^+$ T cells by neutrophils from individuals with sepsis, as an antagonist of the cognate receptor restored the expression of the lymphocyte-activation markers CD69 and PD-1, but not the proliferation of CD4$^+$ T cells[2]. This finding is exciting and deserves further testing, also considering the multifaceted immunological functions of PGE_2 in tumor-associated neutrophils[8,9]. However, many other suppressive mechanisms are at play, such as the release of immune-modulating cytokines, reactive oxygen species (ROS), granules or neutrophil extracellular traps (NETs)[7].

The authors then investigated the persistence and cellular determinants of neutrophil alterations in convalescent patients — that is, individuals who had recovered from a sepsis episode and were analyzed between one and six months after hospital discharge (Fig. 1c). Although the frequencies of sepsis-associated neutrophil subsets progressively returned to baseline, neutrophils from convalescent individuals continued to display reduced expression of inflammatory and interferon response genes, and they maintained a partial capacity to inhibit CD4$^+$ T cells. These data indicate that circulating neutrophils retain some form of memory of sepsis-associated alterations, months after recovery.

Acute inflammatory events elicit persistent chromatin and/or metabolic changes in differentiated myeloid cells and their progenitors — a process termed 'trained immunity' — which underlies long-term skewing of hematopoiesis[10]. It turns out that this process occurs in human sepsis, as revealed by single-cell transcriptome and chromatin analyses of CD34$^+$ hematopoietic stem cells (HSCs) isolated from the blood of patients. The authors identify two sepsis-associated clusters of circulating HSCs (termed C5 and C7) that show high expression levels of neutrophil genes (*MPO*, *RNASE2*, *ELANE* and *FKBP2*) and accessibility of binding sites for transcription factors of the CEBP family. Future studies that link the observed genomic alterations of HSCs in sepsis to the molecular properties of specific neutrophil subsets — generated during and upon resolution of sepsis — will provide key insights into the determinants and persistence of granulopoiesis disturbances.

Whole-blood gene-expression data have previously been used to derive quantitative sepsis-response signatures (SRSs) that efficiently stratify patients according to clinical outcomes[11]. The authors focus on a subgroup of high-risk individuals, termed SRS1, with features of immune suppression and high granulocyte counts[2]. Notably, neutrophils from people with SRS1 suppressed T cell activation more efficiently than neutrophils from other groups. People with SRS1 also showed markedly increased blood frequencies of a particular subset of sepsis-associated neutrophils—that is, immature *IL1R2*$^+$ Neu (Fig. 1a,b). Cell deconvolution analyses revealed a selective enrichment of gene signatures corresponding to the latter population in high-risk

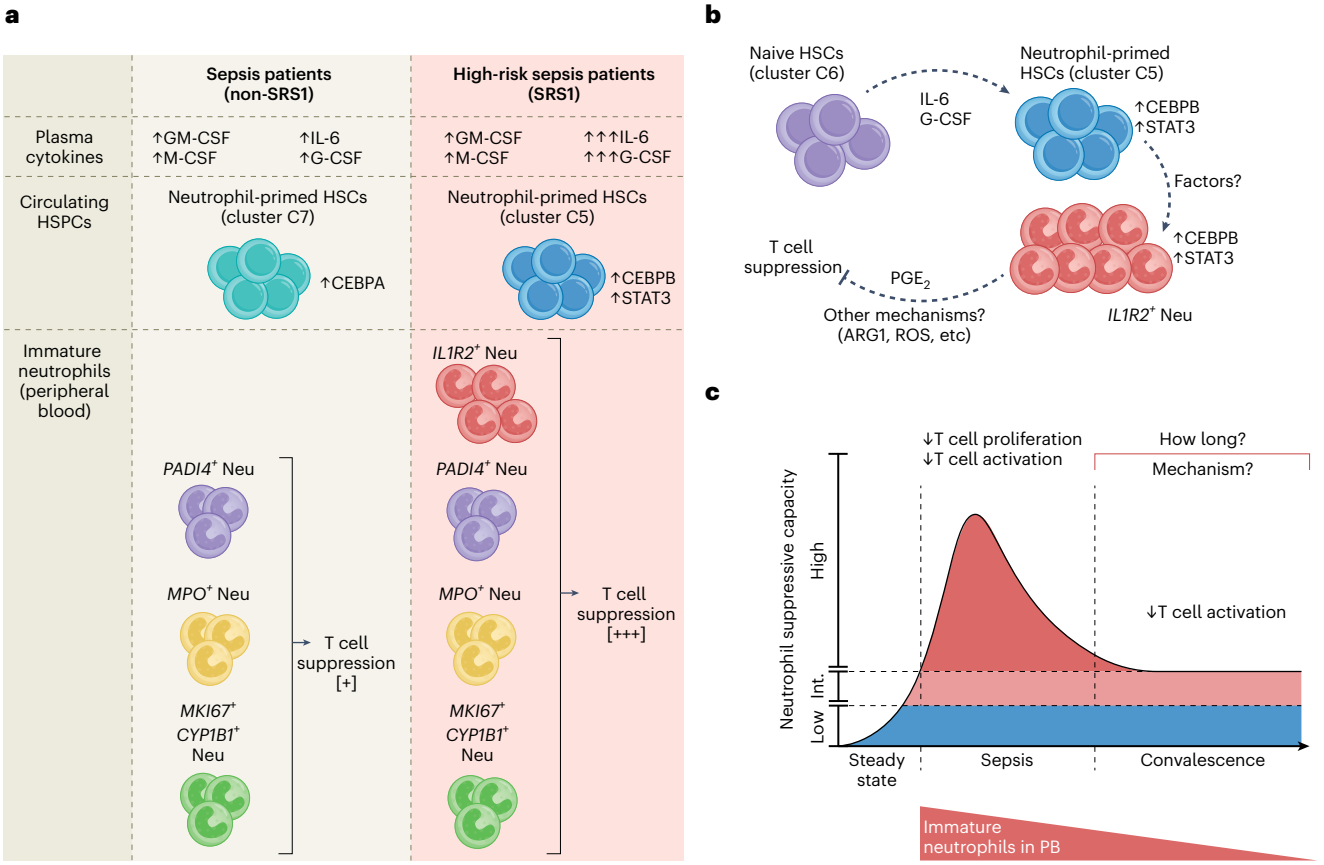

Fig. 1 | Granulopoietic disturbances in high-risk and convalescent sepsis patients. Kwok et al.[2] have generated a combined atlas of scRNA-seq and surface protein expression for leukocytes from septic and convalescent individuals, healthy donors and people undergoing cardiac surgery. **a**, Multiple subsets of immature neutrophils (*PADI4*$^+$, *MPO*$^+$ and *MKI67*$^+$*CYP1B1*$^+$ Neu) with immunosuppressive capacity are found in the blood of people with sepsis (both non-SRS1 and SRS1), concomitant with the emergence of a neutrophil-primed cluster (defined as C7) of HSCs with a CEBPA signature. In addition, a population of immature *IL1R2*$^+$ Neu is detected in high-risk (SRS1) sepsis, possibly underlying increased T cell suppression. SRS1 patients are also characterized by a distinct neutrophil-primed HSC cluster (C5) with a CEBPB and STAT3 signature, and by elevated levels of plasma IL-6 and G-CSF. **b**, Increased levels of G-CSF and IL-6 in high-risk sepsis are proposed to elicit neutrophil-primed HSCs that fuel the emergence of immunosuppressive *IL1R2*$^+$ Neu. The precise differentiation dynamics, mechanistic control and functional properties of these cells remain to be elucidated. **c**, Although the frequency of immature granulocytes returns to steady-state levels when people recover, the immunosuppressive capacities acquired by neutrophils are partially maintained in convalescence. Future work should define the functional implications, long-term persistence and association of such alterations with epigenetic and/or metabolic reprogramming of hematopoietic progenitors. ARG1, arginase 1; CEBPA or CEBPB, CCAAT enhancer binding protein alpha or beta; GM-CSF: granulocyte–macrophage colony-stimulating factor; HSCs, hematopoietic stem cells; IL-1R2, interleukin-1 receptor type II; MPO, myeloperoxidase; PADI4, protein-arginine deiminase type 4PB; peripheral blood; STAT3, signal transducer and activator of transcription 3.

subgroups of individuals with viral (SARS-CoV2 or influenza) or bacterial (fecal peritonitis) infections, community-acquired pneumonia, acute respiratory distress syndrome (ARDS) or pediatric sepsis.

Although their specific functional properties remain to be determined, immature *IL1R2*$^+$ Neu appear promising as biomarkers of high-risk sepsis or infection. A population of immature IL-1R2^{hi} monocytes has been described in septic patients with infections of the urinary tract[12], raising the possibility that both cell types may emerge from a common developmental program in sepsis. Computational analyses[2] suggested a key role for CEBPB and STAT3 — two transcription factors previously associated with emergency granulopoiesis — in *IL1R2*$^+$ Neu and other neutrophil subsets from SRS1 groups. By integrating scRNA-seq, chromatin accessibility analyses and in silico perturbation experiments, the authors provide evidence that CEBPB and STAT3 control the development of a neutrophil-primed subset of HSCs (previously defined as cluster C5) that is selectively expanded in SRS1 patients. They further show that two STAT3-activating cytokines, granulocyte colony-stimulating factor (G-CSF) and interleukin-6 (IL-6), are increased in the plasma of individuals with SRS1, highlighting possible therapeutic targets (Fig. 1a,b).

The findings of Kwok et al.[2] raise several exciting questions. It will be interesting to elucidate how, mechanistically, the identified networks of cytokines and transcription factors orchestrate the phenotypic, molecular and functional (immunosuppressive) properties of neutrophils in people with sepsis; whether there are dedicated differentiation programs connecting sepsis-elicited subsets of HSCs and IL-1R2$^+$ neutrophils in high-risk individuals; what is the fate of sepsis-associated neutrophils upon their exit from the circulation and

entry into tissues; whether these cells contribute to organ dysfunction, and how; and whether these data can ultimately be translated into clinical applications. By showing how elucidating neutrophil heterogeneity can assist in patient stratification and inform the development of personalized medicine for sepsis, this study should prompt immunologists to embrace the challenges of comprehensive characterization of patient samples as a way to discover new biology, to generate mechanistic hypotheses and to find clinical correlates of neutrophil behaviors in humans.

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References

1. Singer, M. et al. *J. Am. Med. Assoc.* **315**, 801–810 (2016).
2. Kwok, A. J. et al. *Nat. Immunol.* https://doi.org/10.1038/s41590-023-01490-5 (2023).
3. van der Poll, T., Shankar-Hari, M. & Wiersinga, W. J. *Immunity* **54**, 2450–2464 (2021).
4. Zilionis, R. et al. *Immunity* **50**, 1317–1334 (2019).
5. Schulte-Schrepping, J. et al. *Cell* **182**, 1419–1440 (2020).
6. Montaldo, E. et al. *Nat. Immunol.* **23**, 1470–1483 (2022).
7. Bronte, V. et al. *Nat. Commun.* **7**, 12150 (2016).
8. Veglia, F. et al. *Nature* **569**, 73–78 (2019).
9. Gong, Z. et al. *Sci. Immunol.* **8**, eadd5204 (2023).
10. Netea, M. G. et al. *Nat. Rev. Immunol.* **20**, 375–388 (2020).
11. Cano-Gamez, E. et al. *Sci. Transl. Med.* **14**, eabq4433 (2022).
12. Reyes, M. et al. *Nat. Med.* **26**, 333–340 (2020).

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Competing interests

The authors declare no conflicts of interest.