Immunity & Inflammation

BRIEF REPORT

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Single-cell spatiotemporal mapping reveals a spleen-to-lung neutrophil axis in antiviral defense

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Initiation of antiviral innate immunity is critical for host defense [1]. While the coordinated action of multiple organs is known to be essential to launch the efficient systemic immune response, recent studies highlight the critical role of inter-organ immune cell migration in shaping immune response and inflammation and determining disease outcomes. For instance, gut-derived immune cells, such as IgA-producing plasma cells [2] and IFNγ$^+$ NK cells [3], can traffic to the central nervous system and exacerbate neuroinflammation in multiple sclerosis and Parkinson's disease.

In contrast to the well-characterized gut–brain axis [4], the cellular dynamics between peripheral immune reservoirs and sites of primary infection remain poorly mapped. This is particularly true for neutrophils, the key frontline responders to respiratory viruses. Following pulmonary viral infection, the lung experiences massive neutrophil infiltration, a hallmark of antiviral defense and immunopathology [5, 6]. However, whether these neutrophils are solely derived from local proliferation or are substantially supplemented from distant immune organs has remained an open question. Specifically, the spleen, a major hematopoietic and immune regulatory organ, has not been systematically investigated as a potential reservoir for pulmonary neutrophils during viral infection.

Here, by integrating single-cell RNA velocity, transcriptomic profiling, and spatial deconvolution in the golden hamster model of SARS-CoV-2 infection, we reveal that splenic neutrophils can migrate to lung during the peak of antiviral response. This previously unrecognized spleen-to-lung neutrophil axis demonstrates that splenic neutrophils replenish pulmonary neutrophil pools at various differentiation stages and contribute to innate immune defense. Our study not only redefines the systemic origin of lung neutrophils but also positions the spleen as a key extramedullary hub for neutrophil deployment during respiratory viral infection, a finding with broad implications for understanding and therapeutically modulating inflammatory responses in pneumonia and beyond.

To demonstrate the cellular dynamics between spleen and lung, we first performed RNA velocity analysis [7] of single-cell data from the lungs of golden hamsters at Day 7 post-SARS-CoV-2 infection to explore the differentiation patterns of various immune cells [8, 9]. In monocyte and macrophage subpopulations, an activation-proliferation cycle was found between the activated macrophage subpopulation (Mac_Slamf9) and the proliferative subpopulation (Mac_Slamf9_Mki67) (Fig. S1a). In contrast, neutrophil subpopulations exhibited a complete unidirectional differentiation chain that started from proliferative subpopulations, progressed through immature and mature states, and culminated in activated cells (Fig. 1a). Cell cycle analysis revealed that early immature

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neutrophils were mainly in the G2M phase (Fig. S1b). However, in monocyte and macrophage subpopulations, Mac_Slamf9_Mki67 cells were in the G2M phase, while other macrophage subpopulations including Mac_Slamf9 were mainly in the S-phase (Fig. S1b). These results indicated that after viral infection, macrophages/monocytes and neutrophils exhibited distinct proliferation-differentiation patterns. Macrophages showed a simultaneous activation and proliferation cycle, whereas the proliferation of neutrophils was separated from activation, potentially originating from other organs.

The spleen is an important immune organ that plays a crucial role in antiviral immune responses. The number of neutrophils in the spleen increased significantly at 5-7 days post-viral infection (Fig. S2), a phenomenon that matched the massive infiltration of neutrophils in the lung. We compared the transcriptomic expression profiles of neutrophils in the spleen and lung of hamsters. The results showed that neutrophils in both spleen and lung could be mainly classified into three groups: proliferative, non-activated, and activated. Moreover, same neutrophil subpopulations from spleen and lung exhibited highly similarity in gene expression levels (Fig. 1b). We calculated the differentially expressed genes (DEGs) in highly similar neutrophil subpopulations between the spleen and lung, and found that spleen neutrophil subpopulations showed increased expression of *Fos, Egr1, Rgs2*, and *Cd5l,* while showing decreased expression of *Sod2* and *Isg15* (Fig. 1c). We further interrogated the phenotypic similarity between spleen and lung neutrophils according to supervised machine learning algorithms implemented in Seurat [10] (Fig. 1d). The results showed that the Neu01_Mpo_Mki67 subpopulation in the spleen was closely aligned with the preNeu and preNeu_Mki67 subpopulations in the lung; the Neu02_Mki67 in the spleen was closely related to the preNeu_Mki67 and immNeu_Mki67 subpopulations in the lung; the Neu03_Ltf was most similar with immNeu_Mmp8lo subpopulation in lung, and Neu04 subpopulations in the spleen were phenotypically close to the corresponding mature neutrophil subpopulations in the lung, and the spleen Neu05_1_Ccl4lo was close to the lowly activated subpopulation acNeu_lo in the lung, respectively (Fig. 1d). These results indicated that the neutrophil subpopulations enriched in the spleen at 5–7 days may migrate into lung and differentiate into the corresponding subpopulations.

To further verify the potential infiltration of splenic cells into the lung, we utilized spatial transcriptomics data and performed deconvolution analysis using the Redeconve [11] package to determine the distribution of different cell subpopulations in lung spatial chips, with single-cell data from both spleen and lung as the reference. The results suggested that among immune populations in lung, only a proportion of neutrophils exhibited a splenic origin, which approached or even exceeded the proportion of lung-originated neutrophils at 5-7 days post-viral infection (Fig. 1e, f, Fig. S3, S4a and b). However, for other immune cell subpopulations, the proportion of lung-originated cells was significantly higher than that from the spleen. Notably, for macrophage-monocyte cells of lung in the same period, the proportion of spleen-derived cells was much lower than that of lung-originated cells (Fig. 1f and Fig. S3). Additionally, the proliferative SP_Neu01_Mpo_Mki67 subpopulation in the early differentiation stage was enriched in the lung at Day 5, while the immature SP_Neu02_Mki67 subpopulation and the mature/mildly activated Neu05_1_Ccl5lo subpopulation were enriched in the lung at Day 7 (Fig. 1e and Fig. S3). Furthermore, we detected the dynamic changes of chemokine expression in the lung at different time points post-infection. We found that a variety of well-characterized chemokines with neutrophil chemotactic potential [12] were highly expressed in the distinct lung cell subpopulations: *Cxcl5* was prominently expressed in the LU_Epi_Club; *Cxcl12* was highly enriched in LU_Mac_Spp1_Il10, LU_Mac_Spp1_Il10_Mki67 and LU_Mac_Cd38, which also exhibited high expression of a panel of chemokine ligand (Ccl) family molecules; additionally, *Ccl11* showed elevated expression in the fibroblast subpopulations (Fig. S5a). Notably, all these chemokines reached peak expression levels in the lungs at 5–7 days post-infection (Fig. S5b). We further examined the expression of receptors for these chemokines in

(See figure on next page.)

Fig. 1 Splenic neutrophils replenish pulmonary neutrophils during SARS-CoV-2 Infection. **a** RNA velocity analysis of neutrophils in lung of hamster at Day 7 post-infection. Arrows indicate the potential direction of state transitions. **b** Heatmap of gene expression similarity among neutrophils in spleen and lung. **c** Volcano plots of differentially expressed genes of SP_Neu01_Mpo_Mki67 vs LU_preNeu, SP_Neu03_Ltf vs LU_immNeu_Mmp8lo, SP_Neu04_1_Mmp9hi vs LU_immNeu_Mmp8hi and SP_Neu04_3_Mmp9hi_Pf4 vs LU_maNeu_Mmp9h. (cutoff: ($|\log_2(\text{fold change})| > 0.5$, p. adj < 0.05)). **d** Phenotypic linkage between neutrophil subpopulations in spleen and lung from hamster. **e** Spatial distribution pattern of different neutrophil subpopulations at different timepoints. **f** Boxplot showing the proportion of lung- and spleen-originated major immune subpopulations at Day 5 and Day 7 post-infection according to deconvolution analysis. **g** Work model demonstrated a spleen-to-lung neutrophil axis in antiviral defense through single-cell spatiotemporal mapping. Neu, Neutrophil; SP, spleen; LU, lung

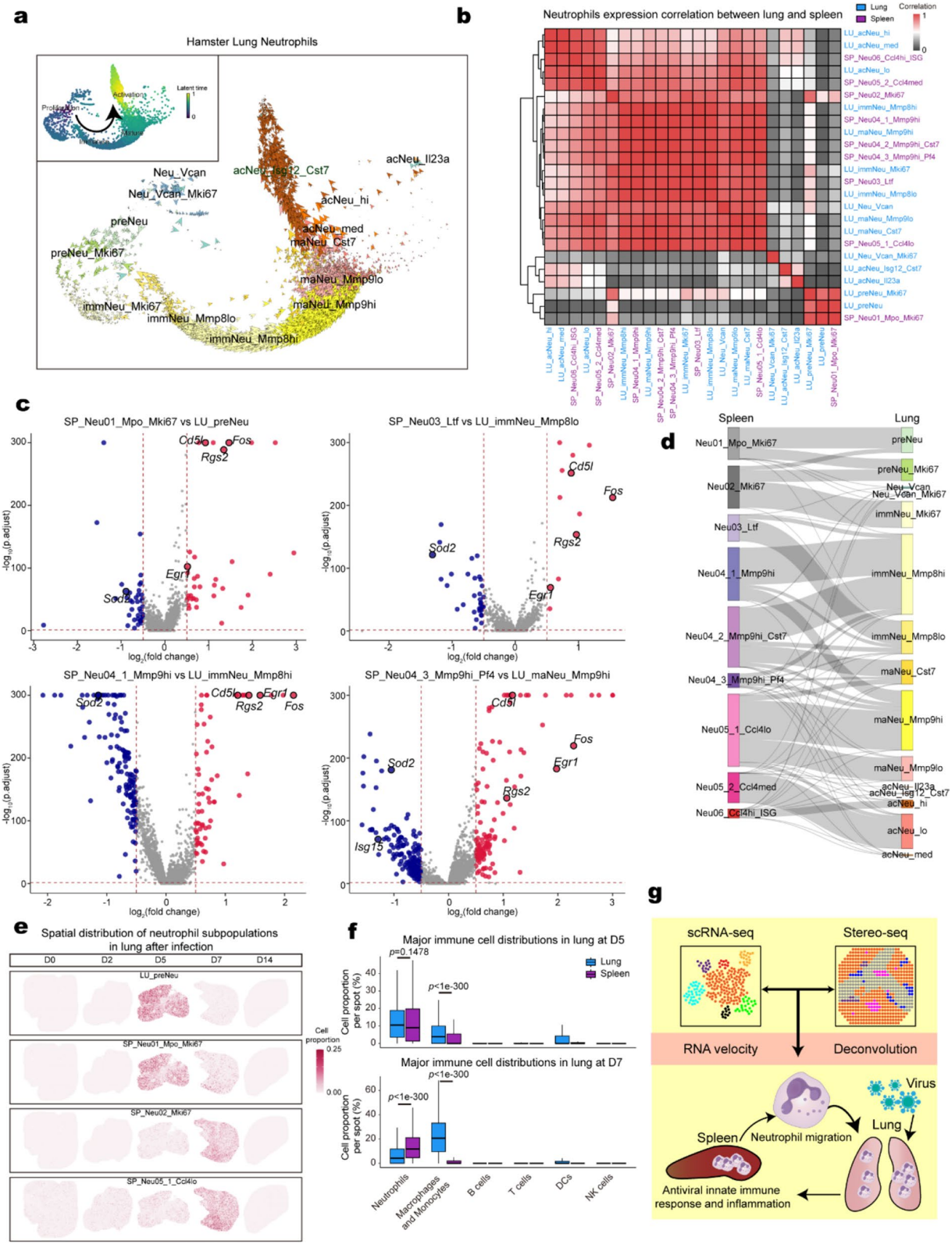

Fig. 1 (See legend on previous page.)

splenic neutrophil subpopulations. Our results revealed that immature neutrophils highly expressed *Cxcr4*, which may mediate their chemotaxis toward *Cxcl12*; in contrast, mature neutrophils expressed *Cxcr2* as well as *Ccr1* and *Ccr3*, rendering them responsive to other chemokines that were upregulated at 5–7 days post-infection (Fig. S5c). These results indicated that splenic neutrophils may migrate to the lung at different stages of differentiation to replenish pulmonary neutrophils and exert antiviral immune responses.

In conclusion, by integrating single-cell RNA velocity, transcriptomic profiling, and spatial deconvolution, we demonstrated that the massive neutrophil infiltration in the lung during SARS-CoV-2 infection was primarily originated from the spleen, and spleen served as an important organ for neutrophil proliferation during pulmonary viral infection. However, the contributions of bone marrow, the canonical site for neutrophil production, to this process remain to be elucidated. Additionally, the direct functional correlation between the quantity, function of lung neutrophils derived from spleen, and viral clearance, disease severity awaits experimental validation in the future. For instance, splenectomy or adoptive transfer may provide more straightforward evidence for linking spleen-mediated neutrophil maturation to the antiviral immune function in lung. Despite this, we identify a spleen-to-lung neutrophil axis that might be critical to the initiation of immune response and inflammation and provide therapeutical targets for treating pneumonia and other pulmonary inflammatory diseases.

Supplementary Information

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Supplementary Material 1.

Authors' contributions

X.C. designed and supervised the research; Z.Y., B.C. and X.R. performed bioinformatics analysis; B.W., S.L. and Y.L. provided reagents and helpful discussion; Z.Y., X.R. and X.C. analyzed the data and wrote the manuscript.

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Data availability

The hamster lung scRNA-seq data and Stereo-seq data analyzed in this study were deposited in CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) under accession number CNP0002742 and CNP0002978.

The hamster spleen scRNA-seq data analyzed in this study were deposited in CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) under accession number CNP0007511.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing of interests

The corresponding author Xuetao Cao is the Editor-in-Chief of the journal Immunity & Inflammation. The authors Bingjing Wang and Shuo Liu are the editors of Immunity & Inflammation. However, they were not involved in the peer-review or decision-making process for this manuscript. The authors declare no other competing interests.

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