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TRANSLATIONAL REVIEW

The Complex Immune Cell Composition and Cellular Interaction in the Alveolar Compartment of Patients with Acute Respiratory Distress Syndrome

Shiqi Zhang[1], JanWillem Duitman[2,3], Antonio Artigas[5], and Lieuwe D.J. Bos[1,4]

[1]Department of Intensive Care Medicine, [2]Department of Pulmonary Medicine, [3]Department of Experimental Immunology, and [4]Laboratory of Experimental Intensive Care and Anesthesiology, Amsterdam University Medical Center, Location University of Amsterdam, Amsterdam, the Netherlands; and [5]Corporacion Sanitaria Universitaria Parc Taulí, Institut d'Investigació i Innovació Parc Taulí (I3PT-CERCA), CIBER Enfermedades Respiratorias, Universitat Autónoma de Barcelona, Sabadell, Spain

ORCID IDs: 0009-0007-5011-0952 (S.Z.); 0000-0003-4254-3380 (J.D.); 0000-0003-2911-4549 (L.D.J.B.).

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Abstract

Acute respiratory distress syndrome (ARDS) is characterized by protein-rich edema due to alveolar–capillary barrier dysfunction caused by inflammatory processes. Currently, our understanding of the inflammatory response in patients with ARDS is mainly based on assessment of the systemic compartment and preclinical studies. Investigations into the intricate network of immune cells and their critical functions in the alveolar compartment remain limited. However, with recent improvements in single-cell analyses, our comprehensive understanding of the interactions between immune cells in the lungs has improved. In this review, we summarize the current knowledge about the cellular composition and interactions of different immune cell types within the alveolar space of patients with ARDS. Neutrophils and macrophages are the predominant immune cells in the alveolar space of patients with ARDS. Yet, all immune cells present, including lymphocytes, participate in complex interactions, coordinate recruitment, modulate the lifespan, and control apoptosis through various signaling pathways. Moreover, the cellular composition of alveolar immune cells is associated with the clinical outcomes of patients with ARDS. In conclusion, this synthesis advances our understanding of ARDS immunology, emphasizing the crucial role of immune cells within the alveolar space. Associations between cellular composition and clinical outcomes highlight the significance of exploring distinct alveolar immune cell subsets. Such exploration holds promise for uncovering novel therapeutic targets in ARDS pathophysiology, presenting avenues for enhancing clinical management and treatment strategies for patients with ARDS.

Keywords: ARDS; immune cells; alveolar; immunology; single cell

Acute respiratory distress syndrome (ARDS) is a life-threating condition characterized by severe exudative pulmonary edema resulting in acute respiratory failure (1). Alveolar edema is secondary to widespread damage to the alveolar–capillary barrier and infiltration of inflammatory cells (1, 2). Based on available data, ARDS affects approximately 10% of patients admitted to the ICU and approximately 33% of patients hospitalized with coronavirus disease (COVID-19) during the peak of the pandemic (3, 4). Despite advancements in supportive care strategies, the mortality and morbidity rates of ARDS remain high (2). Hyperinflammation is recognized as a significant contributor to the pathogenesis of ARDS, whereas traditional antiinflammatory strategies have generally failed to improve the outcomes in unselected patients with ARDS; however, benefits have been described in

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Correspondence and requests for reprints should be addressed to Lieuwe D.J. Bos, Ph.D., Intensive Care Medicine, Amsterdam UMC, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands. E-mail: l.d.bos@amsterdamumc.nl.

This article has a data supplement, which is accessible at the Supplements tab.

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ARDS due to COVID-19 (1, 5). A better understanding of the underlying mechanisms driving dysregulation of immune responses is crucial for the identification of subphenotypes and the development of novel personalized therapeutic strategies.

The host response in ARDS is compartmentalized, and differential responses are observed in the systemic and alveolar compartment. The local, alveolar, immune response in ARDS is a complex process involving various immune cell populations and intricate interactions among them. Neutrophils are the first and predominant immune cells recruited to the site of inflammation and release an array of cytotoxic substances that mediate the propagation of lung injury and are the source of neutrophil extracellular traps (NETs) (6, 7). Alveolar macrophages (AMs), also present in high numbers in part of the ARDS population, possess significant phenotypic plasticity and play diverse roles throughout different stages of the disease, including initiation and maintenance of inflammation and lung tissue repair (8). Additionally, emerging evidence suggests that other immune cell types such as lymphocytes and dendritic cells (DCs), albeit in the minority, also contribute significantly to the biological mechanisms involved in ARDS (9, 10). Extensive studies into myeloid cells *in vivo* have shown that cell–cell interplay exists and is important for inflammation modulation, immune responses, and tissue repair, influencing the progression of the disease (11–15). To comprehend dysregulated immune responses in lungs of patients with ARDS, it is imperative to gain insight into the dynamic interplays and functional characteristics of alveolar immune cells.

Novel single-cell analysis tools have emerged as powerful techniques for identifying and characterizing specific leukocyte subpopulations, enhancing our understanding of biological processes in health and disease. These techniques have been applied to patients with ARDS, whereby immune cells from the systemic and alveolar compartment are extensively analyzed. By providing a higher-resolution view of cellular heterogeneity, enabling detailed investigations of individual cells, these advanced tools could uncover previously unrecognized cellular subsets and their contributions to the pathogenesis of ARDS.

The present review summarizes the available literature on the composition of leukocyte populations and their interactions in the alveolar space of patients with ARDS. We aimed to provide an overview of our current understanding of the complex cellular milieu in the alveolar space of patients with ARDS and to identify areas of recent progress in pathophysiological understanding, as well as obvious gaps in our current knowledge that should be the focus of future research. *See* data supplement for more detailed information on our search methods.

Overview of Leukocyte Composition of Bronchoalveolar Space

Neutrophils are the predominant cell type in the bronchoalveolar space of patients with ARDS across nearly all included studies (Tables 1 and E2 in the data supplement). The second most common cell type in ARDS are AMs, which comprise recruited AMs and resident AMs. Recruited AMs are the most prominent macrophage population in ARDS and exhibit increased expression of markers indicative of the proinflammatory macrophage (M1) phenotype, particularly during the early stages of ARDS (16, 17). As the disease progresses, there is a shift in polarization toward the antiinflammatory macrophage (M2) phenotype, and M2 become the prevalent macrophage subset in the alveolar space (18–20). The lymphocyte population is extremely heterogeneous and dynamic because it contains various cell types with immune properties. It is worth noting that lymphocytes are more abundant in the early stage of COVID-19–related ARDS patients compared with non–COVID-related ARDS (Table 1) (17, 21, 22). In patients with ARDS, T cells are the predominant cell type within the total alveolar lymphocyte population, with tissue-resident memory T cells and effector memory lymphocytes representing the major T-cell subsets (20, 23, 24). In contrast, other lymphocytes, including B cells, natural killer T (NKT) cells, and NK cells, are detected in very limited numbers during the acute phase (20, 22–25). DCs, primarily comprising conventional DCs and a small population of plasmacytoid DCs, are observed in the alveolar space of patients with ARDS, making up $<1\%$ of leukocytes (19, 21, 24).

In the next sections, we will discuss the molecular signatures of each cell type, evaluate their interactions, and provide the clinical implications of these findings.

Alveolar Neutrophils in Human ARDS

The Identifiable Molecular Signatures of the Neutrophils

Neutrophils in the alveolar space are typically identified by the presence of surface markers CD66b and CD11b, although other markers are also reported (summarized in Table E3). The majority of studies reported that the neutrophils recruited to the lungs are in an immature state ($CD16^+$, *CEACAM8*, *LTF*, and *DEFA3*) (21, 22, 25, 26). Clinically, immature neutrophils, also called band cells, are associated with bacterial infection (27, 28). In one study, however, recruited neutrophils in Black patients were predominantly mature ($CD10^+$) at the time of recruitment from the circulation, with an increased expression of an immature gene (*CXCR4*) upon airway recruitment (29). Yet, this observation was not directly compared with other populations of patients within the same study. Although the maturity status of most recruited neutrophils remains elusive, a recurrent observation is the increased expression of activation and adhesion markers, including CD63, CD18, and L-selectin, on the recruited neutrophils, suggesting a persistent active inflammatory microenvironment of the alveolar space in ARDS (29–31). Overall, most studies support that immature neutrophils recruited to the alveolar space will be activated by the microenvironment, and varying states of maturity can be observed during ARDS.

The Cell–Cell Cross-Talk of Immune Cells and the Involved Soluble and Cell Surface Markers

The cellular interactions between neutrophils and other immune cells in the alveolar space are well investigated. The cross-talk between neutrophils and monocytes/macrophages involves pathways related to cell migration and immune regulation, including cytokine families such as the chemokine (C-X-C motif) ligand (CXCL), chemokine (C-C motif) ligand (CCL), IL-1, FLT1 (fms-related receptor tyrosine kinase 1), and ILT (immunoglobulin-like transcript) (19, 29, 32, 33). Specifically, increased expression of CCR1 and CXCR2 on AMs facilitates neutrophil recruitment

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Table 1. Summary of the Studies with Data on Immune Cell Composition in the Alveolar Compartment of Patients with ARDS

Study, Year	Population	Control Group	Sample Collection Timing	Counting Method	Groups (Object Number)	Total Cells/ Concentration	Granulocytes/PMNs		Mono-/Macrophages	Lymphocytes
							Neutrophils	Eosinophils		
Hashimoto *et al.* (64), 2000	10 septic ARDS, 7 septic non-ARDS	Nonsmoking, normal lung function	Day 1, Day 7	Cytospin	ARDS acute phase	$24.5 \pm 3.5 \times 10^4$/ml	$59.4 \pm 4.0\%$	—	$29.7 \pm 4.3\%$	$7.7 \pm 1.1\%$
					ARDS late phase	$21.9 \pm 5.6 \times 10^4$/ml	$41.0 \pm 6.2\%$	—	$49.7 \pm 5.7\%$	$6.7 \pm 1.0\%$
					Control group			—		
Matute-Bello *et al.* (44), 2000	45 ARDS	Healthy volunteers	Days 1, 3, 7, 14 post-ARDS	Not mentioned	Acute phase	48.7×10^6 [$2.4–511.7 \times 10^6$]	78% [4–95%]		—	—
					Late phase	21.4×10^6 [$6.2–38.1 \times 10^6$]	15.5% [1–43%]		—	—
Rosseau *et al.* (56), 2000	16 ARDS, 33 ARDS/pneumonia	Healthy volunteers	Day 1 postintubation	Hemocytometer and cytospin	ARDS	$24.9 \pm 4.6 \times 10^4$/ml	$9.7 \pm 2.6 \times 10^4$/ml	$0.03 \pm 0.006 \times 10^4$/ml	$14.3 \pm 3.1 \times 10^4$/ml	$0.9 \pm 0.1 \times 10^4$/ml
					ARDS/pneumonia	$34.3 \pm 3.2 \times 10^4$/ml	$14.3 \pm 2.4 \times 10^4$/ml	$0.3 \pm 0.12 \times 10^4$/ml	$17.2 \pm 1.7 \times 10^4$/ml	$2.5 \pm 0.2 \times 10^4$/ml
					Control	$7.5 \pm 1.8 \times 10^4$/ml	$0.01 \pm 0.002 \times 10^4$/ml	$0.01 \pm 0.003 \times 10^4$/ml	$6.6 \pm 1.4 \times 10^4$/ml	$0.8 \pm 0.1 \times 10^4$/ml
Horowitz *et al.* (84), 2006	13 ARDS	—	First week post-ARDS	Hemocytometer and cytospin	Nonresolving ARDS	—	$72.2 \pm 19.8\%$	$0.25 \pm 0.1\%$	$26.7 \pm 19.4\%$	$1.0 \pm 0.4\%$
					Resolving ARDS	—	$66.1 \pm 12.6\%$	$0.1 \pm 0.1\%$	$32.8 \pm 12.4\%$	$1.2 \pm 0.6\%$
Conway Morris *et al.* (85), 2009	21 ARDS/ALI	Healthy volunteers	—	Cytospin	ARDS/ALI	—	9.4×10^5/ml ($2.7–19 \times 10^5$/ml)	—	1.8×10^5/ml ($0.89–3.6 \times 10^5$/ml)	—
					Control group	—	0.03×10^5/ml ($0.01–0.09 \times 10^5$/ml)	—	2.6×10^5/ml ($1.9–6.24 \times 10^5$/ml	—
Quesnel *et al.* (86), 2012	62 ARDS, 30 ALI	Ventilated patients without ALI/ARDS	Around Day 6 post-MV	Cytospin	ARDS	32.7 (10–147.1) (10^4/ml)	87% (70–92%)		13% (8–30%)	—
					ALI	46.1 (8.9–65.2) (10^4/ml)	79% (63–90%)		21% (10–37%)	—
					Control group	17.2 (5.0–67.6) (10^4/ml)	53% (13–90%)		47% (10–87%)	—
Juss *et al.* (83), 2016	ARDS	Non-ARDS patients	Within 48 h after ARDS	FC	Mild	—	$69.7 \pm 2\%$		—	—
					Moderate	—	$73.7 \pm 11.1\%$		—	—
					Severe	—	$59.8 \pm 2\%$		—	—
					Control group	—	$6.5 \pm 3.2\%$		—	—
Morrell *et al.* (63), 2018	6 ARDS in cyTOF experiments, 30 ARDS in gene expression experiments	Healthy volunteers	—	Cytospin	ARDS cohort 1	—	$54 \pm 27\%$	—	$43 \pm 26\%$	—
					ARDS cohort 2	—	$41 \pm 33\%$	—	$54 \pm 31\%$	—
					Control group	—	$1 \pm 0\%$	—	$90 \pm 4\%$	—
Garnier *et al.* (18), 2018*	33 Moderate to severe ARDS	—	Day 1–3 post-ARDS	FC	ARDS	4×10^4/ml ($1.7–8 \times 10^4$/ml)	—		$34.8 \pm 2\%$	—
Grunwell *et al.* (30), 2020	19 Pediatric ARDS	ICU non-ARDS patients	—	Cytospin	PARDS	—	87.0% (69.2–95.8%)	—	—	—
					Control group	—	87.2% (79.2–93%)	—	—	—
Bendib *et al.* (58), 2021	70 ARDS	—	Within 48 h after ARDS	FC	Survivor	4.15×10^4/ml ($2.24–9.62 \times 10^4$/ml)	69% (38–83%)	—	25% (12–49%)	3% (1–6%)
					Nonsurvivors	5.65×10^4/ml ($2.5–17 \times 10^4$/ml)	72% (38–90%)	—	26% (8–51%)	5% (1–9%)
Bendib *et al.* (57), 2021	10 ARDS	ICU non-ARDS patients	Within 48 h after ARDS	FC	ARDS	5.67×10^4/ml ($3.65–7.2 \times 10^4$/ml)	80.5% (61.2–89.5%)	—	15.5% (8.8–85.5%)	3.2% (2–6.1%)
					Control	1.3×10^4/ml ($0.77–1.37 \times 10^4$/ml)	1.0% (0.7–1.0%)	—	66.0% (66.0–85.5%)	30.0% (12.5–33.0%)
Dentone *et al.* (61), 2021	64 COVID-19	—	At admission to ICU	—	Survivors	—	71% (46–90.5%)	—	20% (7–42%)	1% (0.3–2.25%)
					Nonsurvivors	—	63% (36–83.5%)	—	35% (16.5–56.7%)	1% (1–4%)
Holliday *et al.* (75), 2021	28 COVID-19	—	Within 7 d post-MV	FC	—	26.67×10^4/ml [$0.79–177.65 \times 10^4$/ml]	67.3% [5–99%]		15.6% [0–51%]	—
Kim *et al.* (25), 2022	2 COVID-19–related ARDS	—	Day 3 post-ARDS	H&E staining	Patient 1	—	45%*		35%	20%
					Patient 2	—	35%*		45%	20%
Voicu *et al.* (50), 2022	12 COVID-19–related ARDS with ECMO	—	Around 6 d postintubation for weaned group and 15 d for nonweaned group	FC	All ARDS	4.5×10^4/ml ($0.91–2.56 \times 10^4$/ml)	73% (24–82%)	0% (0–0%)	14% (7–24%)	11% (7–17%)
					Weaned from ECMO/ventilator	18×10^4/ml ($9.02–25.6 \times 10^4$/ml)	11% (2–34%)	0% (0–0%)	23% (18–36%)	42% (10–75%)
					Nonweaned	45×10^4/ml ($1.11–6.66 \times 10^4$/ml)	77% (60–83%)	0% (0–0%)	14% (7–28%)	8% (5–13%)

Definition of abbreviations: ALI = acute lung injury; ARDS = acute respiratory distress syndrome; COVID-19 = coronavirus disease; cyTOF = cytometry by time of flight; ECMO = extracorporeal membrane oxygenation; FC = flow cytometry; H&E = hematoxylin and eosin; IQR = interquartile range; MV = mechanical ventilation; PMN = polymorphonuclear neutrophil.

Data including a parenthetical range is denoted as median (IQR); otherwise, data is presented as mean ± SD.

*Eosinophils accounted for >25% of the PMNs; macrophages, 139/μl (70–310/μl).

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into the lungs, whereas neutrophil-secreted CCL3/4 enhance the recruitment of circulating monocytes/macrophages (Figure 1) (19, 29). Another positive feedback mechanism exists between Th17 (T-helper 17) cells and neutrophils in the airways, where Th17 cells induce the recruitment of neutrophils to the lung and prolong their survival through IL-17A, and neutrophils, in turn, instruct Th17 differentiation of naive T cells via IL-6 secretion (Figure 1) (21, 34–36). Additionally, T cells could extend the lifespan of lung neutrophils through the production of IL-1β, which enhances the expression of neutrophils' antiapoptotic genes (29). However, this mechanism has not been established to be of clinical relevance in ARDS, and future studies should elucidate whether this mechanism plays a role in ARDS.

When neutrophils have been recruited to the alveoli, they employ self-recruitment mechanisms to facilitate the ongoing influx of neutrophils into the lungs. The mature neutrophils are governed by the IL-8/CXCR2 axis, which is synergistic with CCL2/7, whereas CXCR4 is associated with immature neutrophil recruitment (29, 37–39). Moreover, NETs released by neutrophils may promote M1-like macrophage polarization,

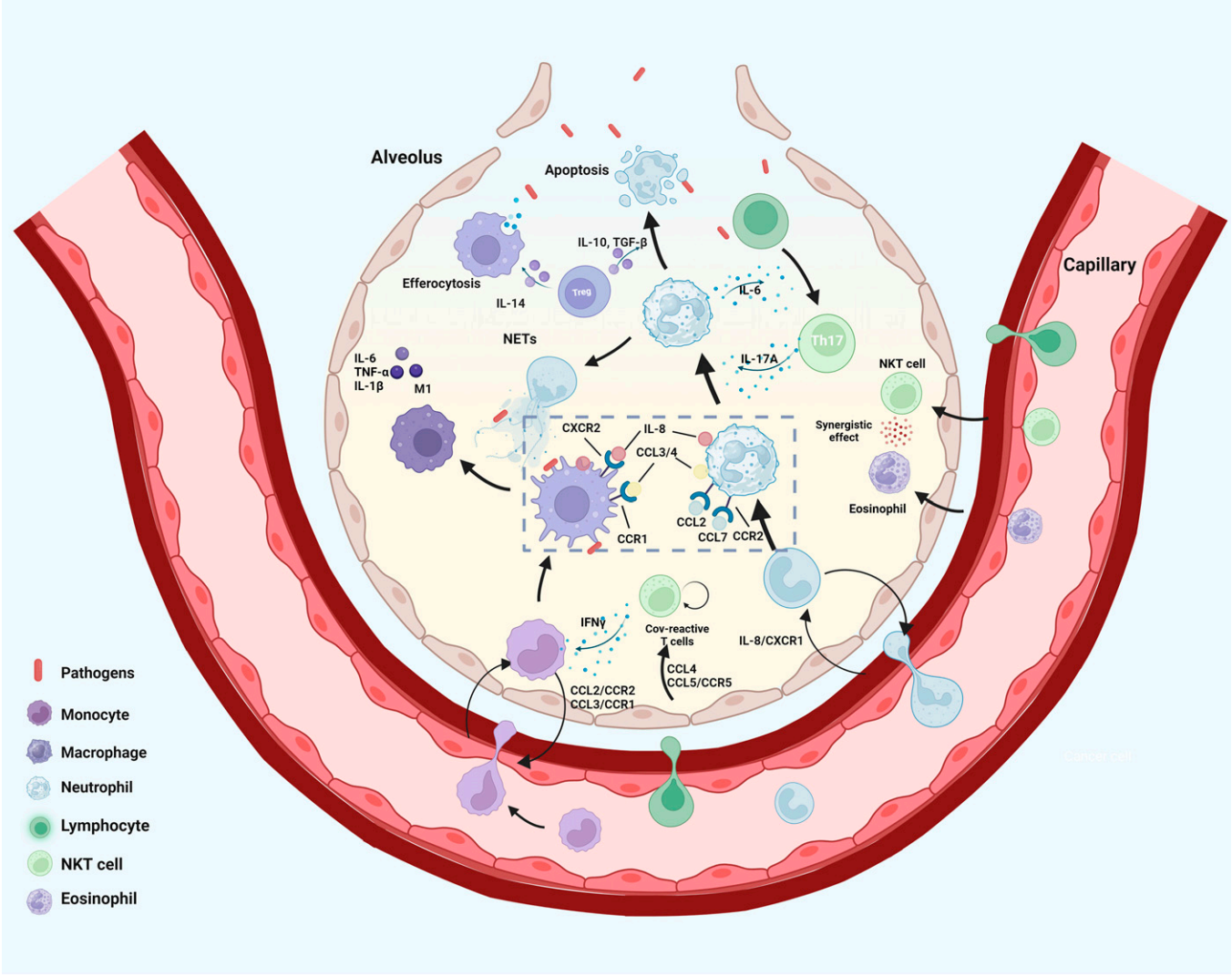


Figure 1. Schematic representation of immune cell recruitment and cellular interaction in the alveolar compartment of patients with acute respiratory distress syndrome (ARDS). In patients with ARDS, neutrophils migrate into the alveoli through self-recruitment, primarily guided by IL-8, along with CCL2/7 (chemokine [C-C motif] ligand). The recruitment of neutrophils is further stimulated by CXCR2 (C-X-C chemokine receptor) and CCR1 (C-C chemokine receptor) upregulated on macrophages. In turn, neutrophils promote monocyte/macrophage recruitment through the secretion of CCL3/4. In addition, circulating monocytes are recruited in the lungs through the CCL2/CCR2 and CCL3/CCR1 axes. Neutrophils also release neutrophil extracellular traps that stimulate macrophage polarization toward the proinflammatory M1 macrophage phenotype. T cells play a role in stimulating efferocytosis by macrophages and guiding the apoptosis of neutrophils. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–infected alveolar macrophages (AMs) could produce T-cell chemoattractants while activated T cells can produce IFN-γ, leading to AM apoptosis and subsequent monocyte recruitment. Additionally, neutrophils can direct naive T cells to develop into Th17 (T-helper 17) cells, and the production of IL-17A by Th17 cells can, in turn, stimulate the recruitment of neutrophils. Furthermore, natural killer (NK) cells and eosinophils have a synergistic effect on each other during the recruitment process. NET = neutrophil extracellular trap; NKT = natural killer T cells.

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as indicated by increased M1-reflecting cytokines (IL-6, TNF-α, IL-1β) in BAL fluid supernatants, implying that NETs may play a role in driving a proinflammatory state and aggravating lung tissue damage within the lungs of patients with ARDS (Figure 1) (16). In addition, NETs prime T cells by reducing their activation threshold, at least *ex vivo* (40). Taken together, neutrophils play an important role in sustaining the inflammatory immune response by secretion of inflammatory mediators, instruction of inflammatory macrophages, and NET formation during ARDS.

Composition and Cellular Interactions in Relation to the Clinical Condition of Patients with ARDS

As mentioned above, excessive accumulation of neutrophils and the release of NETs in the lungs are prominent features of ARDS and are closely linked to disease severity and clinical outcomes (7, 23, 29, 41). In the early stages of ARDS, increased neutrophil infiltration and increased NET formation in the alveolar space are associated with improved survival (Figure 2) (42). Conversely, sustained neutrophilia in the alveolar compartment indicates a poor prognosis (Figure 2) (23, 43). In pediatric ARDS, however, a high level of NETs in the airway fluid at the early stage of disease is linked to fewer ventilator-free days and longer ICU stays, highlighting a distinct immune response in the lungs of children (30). During ARDS, mechanical ventilation was shown to activate neutrophils, and the subsequent elastase release by these neutrophils was associated with the extent of organ failure in these patients (31). An *in vitro* study demonstrated that BAL fluid supernatant from patients with early-stage ARDS exerts a potent antiapoptotic effect on human neutrophils, which diminishes as the disease progresses, although the underlying mechanism remains elusive (44). Interestingly, increasing clinical data indicates that the higher neutrophil-to-lymphocyte ratio in peripheral blood can predict worse outcomes in patients with severe COVID-19, but its validity in the alveolar space has yet to be confirmed (45–49). Corticosteroid administration is associated with a decrease of neutrophil counts and neutrophil-related cytokines in BAL fluid (50–52). Persistently increased levels of neutrophil activation biomarkers in the alveolar space have been linked to higher mortality rates (52). However, the extent to which this reduction in neutrophils and neutrophil-related cytokines contributes to corticosteroid-induced improvement in survival remains elusive.

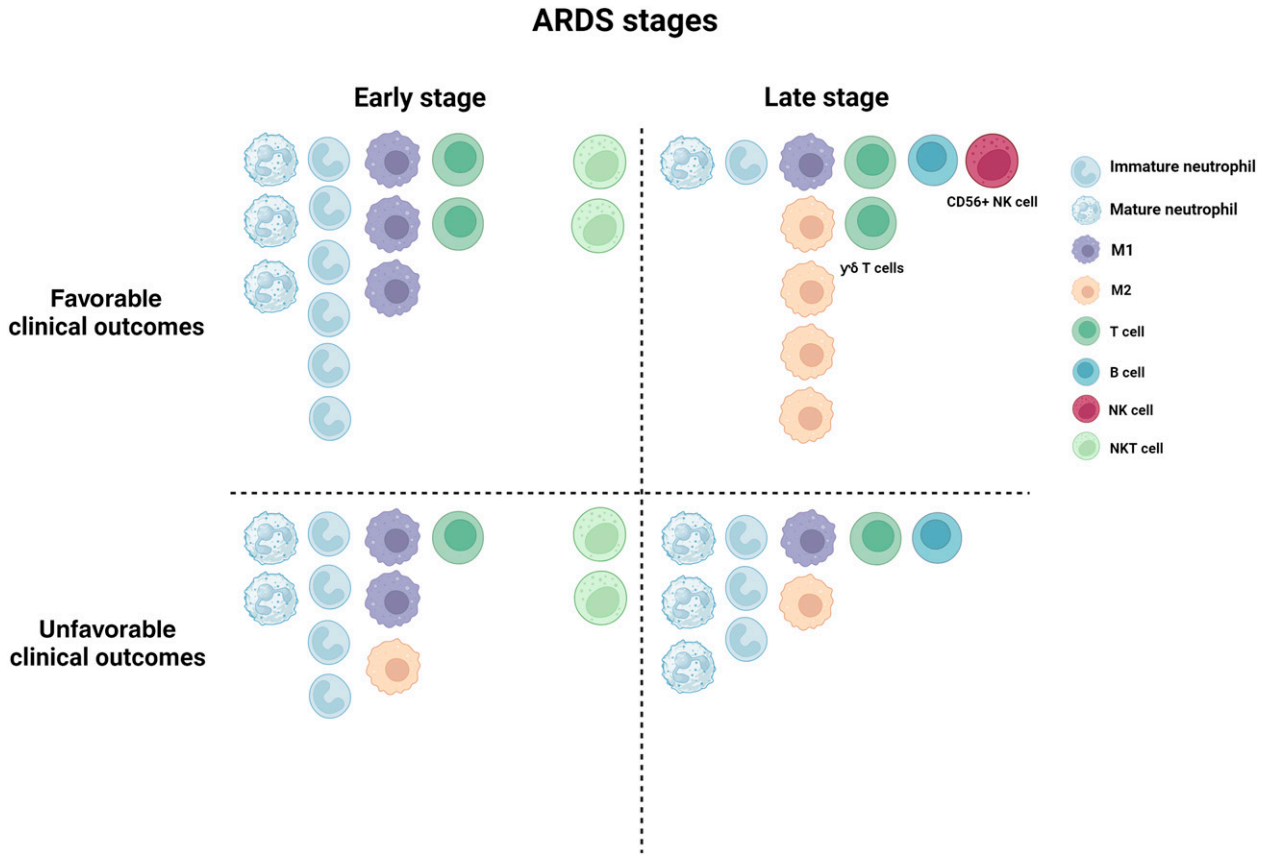


Figure 2. Alveolar cellular features of patients with ARDS with different severities and disease stages. In patients with favorable outcomes, the alveolar space is filled with newly recruited neutrophils in the early stage and with a higher proportion of AMs, especially of the antiinflammatory M2 phenotype, in the late stage. Conversely, in patients with worse outcomes, the neutrophils persist into the late stage of the disease with fewer macrophages present. Higher levels of overall T cells in the early stage are associated with favorable clinical conditions. NK cells and γδ T cells are less abundant in fatal cases of ARDS, and $CD56^{+}$ NK cells were detected in patients with fatal outcomes. During the disease, B-cell counts are very low in the early stage and increase in the late stage, and NKT cells are the opposite. The relative abundance of each cell type under different conditions is indicated by the numbers of cells in the image.

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Clinical Implications of Alveolar Neutrophils in ARDS

Interventions aimed at modulating neutrophil activity hold promise as a therapeutic strategy for ARDS, but this treatment strategy has largely failed so far. The complex dynamics of neutrophil populations in the dynamic space may explain the lack of a successful treatment strategy. Based on the available data, it can be speculated that early neutrophil recruitment in the lungs should, in general, be considered an appropriate response while sustained alveolar neutrophilia is maladaptive. This would have important consequences for the timing of antineutrophil interventions in the alveolar space as well as the recruitment of patients who are more likely to respond to such treatments. Thus, it is essential to discern the circumstances under which neutrophil activation is beneficial, with the aim of shifting the balance toward therapeutic benefit rather than harm.

Alveolar Monocytes/Macrophages in Human ARDS

The Identifiable Molecular Signatures of Macrophages

The identifiable and functional markers for alveolar monocytes/macrophages are intricately attributable to the differentiation process and polarization of AMs. CD14 serves as a general marker for monocytes/macrophages, whereas CD68 and CD169 are key identifiers that distinguish macrophages from monocytes within the alveolar space (*see* Table E3). Nonclassical monocytes ($CD14^{+}CD16^{2+}$) were the predominant monocyte phenotype observed in alveoli, and differentiated into AMs among ventilated patients with acute lung injury (53, 54). AMs in ARDS studies were usually categorized into M1 and M2 phenotypes, with the M1 phenotype identified by the expression of CD80 and CD86, whereas CD206 marks the M2 phenotype (55). AMs identified in patients with ARDS exhibited a highly activated status with increased expression of CD64, HLA-DR, CD11b, or CD69 (19, 21, 56). For alveolar monocytes in pneumonia-related ARDS, however, a decrease in activation characterized by the downregulation of HLA-DR expression and reduced phagocytosis activity was observed (57, 58). These findings suggest that, upon recruitment into the lungs, monocytes experience a transition, losing their typical monocyte features while acquiring macrophage characteristics and differentiating into activated macrophages.

The Cell–Cell Cross-Talk of Immune Cells and the Involved Soluble and Cell Surface Marker

Alveolar monocytes/macrophages play an important role in recruitment of other inflammatory immune cells. Circulating monocytes are recruited into the lung by AMs through the CCL2/CCR2 and CCL3/CCR1 axes (Figure 1) (20, 29, 59). After recruitment, monocytes differentiate into macrophages and engage in interactions with lymphocytes, alongside their previously discussed interactions with neutrophils. During COVID-19–induced ARDS, a positive feedback loop exists whereby severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–infected AMs produce T-cell chemoattractants while activated T cells produce IFN-γ, leading to AM apoptosis and further monocyte recruitment, perpetuating a persistent proinflammatory state in the lungs (Figure 1) (17). In addition, different subsets of T helper cells have been demonstrated to influence the polarization of macrophages toward the M1 or M2 category by regulating various chemokines like IFN-γ, IL-4, and IL-13, but this theory has been validated only in animal experiments (9, 12, 60). Furthermore, the potential immune-stimulatory interactions involving *CTLA4*, *CD28/CD80*, and *CD86* were observed among T cells and monocytes/macrophages in single-cell gene analysis (33). However, the exact mechanistic implications of these interactions require further investigation.

Composition and Cellular Interactions in Relation to the Clinical Condition of Patients with ARDS

The abundance of the monocyte/macrophage subpopulation in the lungs is predictive of the clinical outcomes of patients with ARDS. Throughout the course of ARDS, there is a decrease in AM levels during the acute phase, followed by an increase during the recovery phase (Figure 2) (25). HLA-DR expression indicative of antigen-presenting capacity on AMs at the early stage is linked to lower Sequential Organ Failure Assessment scores in patients with ARDS, suggesting that the number of organ failures was associated with macrophage inhibition in the lungs, even though this change was not associated with hospital mortality (57, 58). AM polarization plays an important role in the initiation and resolution of inflammation in ARDS. Indeed, the early-stage enrichment of the proinflammatory phenotype (i.e., M1) was correlated with improved survival outcomes in patients with ARDS, whereas, intriguingly, the total AM percentage was linked to higher ICU mortality rates (Figure 2) (8, 22, 61).

In the later stages of ARDS, individuals with a higher proportion of AMs within the alveolar space, particularly those of the antiinflammatory phenotype (i.e., M2), exhibited improved oxygenation, reduced mechanical ventilation duration, and better survival outcomes, suggesting a favorable trajectory in lung recovery (Figure 2) (8, 18, 23, 56). Indeed, the continued presence of monocytes and immature macrophages in the alveolar and lung parenchyma has been associated with the subsequent fibrotic responses and recovery in the lungs of patients with ARDS (Figure 2) (18, 20, 62). Another subset of IL-10–overexpressing AMs that exhibit a strong phagocytic function was shown to accelerate the repair of alveolar epithelial injury through the secretion of hepatocyte growth factor and was more abundant in ARDS survivors compared with nonsurvivors (18).

Functional marker genes such as *PD-L1*, a well-recognized indicator of cellular exhaustion, were found to show lower expression on AMs in mechanically ventilated individuals compared with their healthy counterparts, implying a potential maintenance of macrophage function in mechanically ventilated patients (63). The expression level of the PD-L1/PD-1 pathway–associated genes were also significantly decreased in AMs from patients with ARDS who died or underwent prolonged mechanical ventilation (63). However, the absence of phenotype information in this study (63) highlights the need for further exploration to elucidate the specific roles of AMs in the pathophysiology of ARDS.

Clinical Implications of AMs in Patients with ARDS

Collectively, AMs exhibit significant phenotypic plasticity and undergo diverse functional changes throughout different stages of the disease. The polarization of AMs could serve as a predictor of the progression of inflammation resolution

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during ARDS, suggesting that personalized monitoring and modulation of lung macrophage polarization may prove beneficial in resolving ARDS. Furthermore, the association between AMs and lung fibrosis enables the identification of patients at risk of developing this condition, facilitating the early implementation of intervention strategies such as antifibrotic therapies. Surface biomarkers reflecting various activities of AMs, such as phagocytic activity, could aid in identifying novel subsets of AMs to be considered as future targets of treatment.

Alveolar T and B Lymphocytes in Human ARDS

Identifiable Molecular Signatures of the Involved Cells

T cells are identified as $CD3^+$ cells and further subdivided into $CD4^+$ and $CD8^+$ subpopulations. Additionally, they could be classified into conventional α-β T cells and γ-δ T cells based on T-cell surface receptor (24). In patients with ARDS, regulatory T cells (Tregs), identified by CD25 and FoxP3, have been the subject of extensive investigation. Alveolar T cells exhibited upregulated activation markers/genes associated with antigen recognition (HLA-DR), cytokine production (*IFNG*, *CCL2,* and *CCL4*), immune regulation (CD38, CD152, LAP, CXCR6), and apoptosis (Fas, PD-L1, perforin, granzyme A, granzyme B), all indicative of their sustained activated state. Moreover, a persistent recruitment of activated lymphocytes to the sites of inflammation is shown during the course of ARDS (20, 24, 34, 58, 64–66). Furthermore, the observed downregulation of CD57, a marker associated with late-stage T-cell exhaustion, suggests a prolonged and sustained T-cell activation in ARDS (24). B cells are typically identified by the presence of CD19 (naive B cells) or CD20 (*MS4A1*; plasma cells) on their cell surface, whereas IL-10 expression is used as a marker for regulatory B-cell function (19, 20, 25, 67, 68). Overall, diverse lymphocyte subsets are present in the alveolar compartment of patients with ARDS in an activated state.

The Cell–Cell Cross-Talk of Immune Cells and the Involved Soluble and Cell Surface Marker

In addition to previously mentioned immune-stimulatory interactions involving T cells and AMs, as well as the positive feedback loop between Th17 cells and neutrophils, the recruitment of T cells to the inflamed site can be influenced by AMs through the *CCL5/CCR5* axis as shown by single-cell RNA sequencing (Figure 1) (33). Alveolar Tregs play a crucial role in resolving lung injury through various mechanisms, including the induction of neutrophil apoptosis, macrophage efferocytosis, and reduction of fibrocyte recruitment (21, 69, 70). On the contrary, the recruitment of Tregs in the alveolar compartment was shown to be facilitated by IL-10–producing B cells, implying that regulatory B cells possess the ability to modulate inflammation (68). Indeed, the early recruitment of IL-10–producing B cells to the alveolar space of patients with ARDS suppressed Th1 inflammation, but whether this is mediated by Treg recruitment needs to be elucidated (68). A distinct IL-10–expressing subset of Tregs was shown to downregulate inflammation by suppressing IFN-γ production in $CD8^+$ T cells and promoting lysis of monocytes and DCs during ARDS onset (71). T cells could also interact with other antigen-presenting cells, including NK cells, via the costimulatory pathway, characterized by an overexpression of CTLA-4/CD152 on cell surfaces, which is yet to be validated in patients with ARDS (34, 72, 73).

The Composition and Cellular Interactions in Relation to the Clinical Condition of Patients with ARDS

In the early stage of disease, the levels of total $CD4^+$ and $CD8^+$ T cells in the lung compartment are higher in survivors than in nonsurvivors (66, 74). This observation may be attributed to the accumulation of tissue-resident memory T cells and effector memory lymphocytes, the predominant T cell subsets in the lungs, which was also shown to be associated with favorable outcomes, as reflected by lower Sequential Organ Failure Assessment scores (20, 22). However, activated and naive T cells are conversely associated with favorable clinical conditions and outcomes (22, 61, 66, 75). Interestingly, during the initial phase of ARDS, airway T-cell numbers are increased in younger individuals and patients with preexisting diabetes and obesity (20, 75).

In the later stages of ARDS, a depletion of activated T cells and a notable increase in the percentage of γδ T cells were observed, particularly in fatal cases (Figure 2) (22, 24, 61). This suggests that a reduced antiviral response in the lungs may contribute to the unfavorable outcomes observed. Nevertheless, the γδ T-cell percentage was lower in patients with ARDS who died compared with those who survived (24).

Conflicting findings regarding Tregs with respect to their presence and abundance in patients with ARDS and their influence on clinical outcomes have been reported. Most studies demonstrated that alveolar Tregs increased significantly during ARDS and could affect clinical outcomes (23, 71, 76, 77). A higher percentage of Tregs at the early stage of ARDS was associated with accelerated recovery from the disease (23). However, some studies showed that the Treg percentage was similar or even lower in patients with ARDS than in those without ARDS (34, 65). Similar to AMs, overexpression of IL-10 by Tregs contributed to decreased inflammation and better survival (71). Indeed, increased levels of IL-10 in the lungs are linked to a shorter duration of mechanical ventilation, which supports its potential use as a therapeutic agent (23, 78).

B cells were detected at low levels in the lungs during the acute phase of ARDS, and their numbers increased during the convalescent phase, but the overall B-cell counts does not seem to be associated with the clinical outcomes, including the duration of mechanical ventilation (Figure 2) (23, 25). Notably, a previous study indicated that patients with ARDS with lung infiltration of IL-10–producing B cells within the initial 2 days after ARDS onset exhibited improved survival, but whether this improvement is associated with the suppression of Th1 inflammation or is a result of the suppressive effect of IL-10 on inflammation in general still requires further investigation (68). Importantly, a limitation of the study of the role of B cells in the setting of ARDS is their low abundance, making it difficult to obtain sufficient material for functional analysis (19, 20, 29).

Clinical Implications of Alveolar Lymphocytes in ARDS

Alveolar lymphocytes perform multifaceted regulatory functions in modulating immune cell activity, and lymphocytes are therefore valuable targets for the development of novel therapeutic agents for the treatment of patients with ARDS. For example, Tregs may contribute to the resolution of alveolar hyperinflammation, thereby restoring

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balance toward recovery. Furthermore, comprehensive exploration of lung-localized immune responses, particularly T cell–mediated immunity in patients with ARDS, could prove advantages in vaccine development. Emerging evidence indicating the reliability of T cell–mediated immunity as a correlate of vaccine protection, especially in elderly populations, strongly supports the integration of T-cell responses into COVID-19 vaccine design (79, 80).

Alveolar NKT Cells and NK Cells in Human ARDS

The Identifiable Molecular Signatures of the Involved Cells

NKT cells and NK cells, as distinct subsets of lymphocytes, share functional similarities that enable rapid responses to infections and regulation of immune reactions. NKT cells are identified as $CD3^+CD16^+$ cells, whereas NK cells are mainly characterized by the presence of CD56 and CD16, with a further distinction in their classification of two subsets: $CD56^{dim}$ and $CD56^{bright}$ NK cells (25, 66). $CD56^{dim}$ NK cells are characterized by their cytotoxic activity, whereas $CD56^{bright}$ NK cells exhibit lower cytotoxicity but play a prominent role in cytokine production. Activated NK cells show increased expression of Ki-67, HLA-DR, and CD69 compared with naive NK cells (81).

The Cell–Cell Cross-Talk of Immune Cells and the Involved Soluble and Cell Surface Marker

The investigation of NKT cells and NK cells is not as comprehensive as that of the aforementioned immune cell types, and limited studies showing specific cell–cell cross-talk of NKT or NK cells in the setting of ARDS were found. A single study investigating lung inflammation during COVID-19–induced ARDS showed that infiltration of NKT cells into infected lungs has a synergistic effect together with eosinophils, potentially contributing to eosinophilic lung inflammation (Figure 1) (25). For NK cells, a single study was found in which the investigators suggest that NK cells influence T-cell function at different stages of the disease, including T-cell activation, immune response magnitude, immune memory establishment, and interactions with Treg cells (82). However, these functions have not yet been validated in studies focused on patients with ARDS.

The Composition and Cellular Interactions in Relation to the Clinical Condition of Patients with ARDS

The impact of NKT cells and NK cells on disease progression has been investigated only in patients with severe COVID-19. In this population, pulmonary NKT cell counts were increased during the acute phase and constituted the majority of $CD3^+$ T cells, but decreased during the convalescent phase (Figure 2) (25). On the contrary, the dynamic changes of NK cells during ARDS remain unclear. Nevertheless, previous studies demonstrated that, in the late stage of the disease, severe cases exhibited significantly lower NK cell counts in the alveolar space compared with moderate cases, yet total NK cell levels showed no difference between survivors and nonsurvivors (Figure 2) (19, 29). Interestingly, increased levels of $CD56^{bright}$ NK cells in the lungs were associated with fatal outcomes, whereas low levels were associated with survival of patients with COVID-19 (66) (Figure 2), which could be related to the low cytotoxic activity and attribution to inflammation of $CD56^{bright}$ NK cells, thus preventing elimination of the infected cells and promoting inflammation.

Clinical Implications of Alveolar NKT Cells in ARDS

Despite their lower abundance relative to other immune cells, changes in the levels of alveolar NK cells and NKT cells could serve as indicators of treatment response, aiding in the assessment of intervention efficacy and proving evidence for therapeutic decision-making.

Compartmentalization and Phenotyping

It is important to acknowledge that BAL findings primarily reflect the alveolar space and do not capture inflammatory processes in the interstitial tissue, which play a key role in ARDS pathophysiology. Similarly, the literature indicates that patients with ARDS exhibit compartmentalized immune cell profiles, with some shared features between blood, tracheal aspirate, and BAL, but also various important differences (Table E4). For example, neutrophils are highly prevalent and hyperactive in both compartments, with a pronounced increase in the lungs (23, 42, 83). Moreover, AMs in the lungs display higher activation compared with blood monocytes (58). The heightened activation of neutrophils and AMs reflects a robust localized immune response. In contrast, lymphopenia is sometimes observed in both compartments, but alveolar samples show higher levels of Tregs and Th17 cells with pronounced exhaustion markers compared with blood, highlighting an attempt to regulate excessive inflammation at the site (23, 65, 76). These findings emphasize the importance of analyzing BAL samples, as they provide critical insights into the localized immune processes that cannot be fully understood through blood analysis alone.

This review identifies different subtypes of ARDS based on their etiology, each exhibiting notable differences in alveolar cellular response. For example, COVID-19–related ARDS exhibits a higher presence of T lymphocytes in the lungs compared with non-COVID ARDS, indicating a more pronounced adaptive immune response on site (17, 21, 24, 66). Besides, COVID-19–related ARDS features unique cell types such as low-density neutrophils and γ-δ T cells that are less commonly reported in non–COVID-19–related ARDS, which are associated with hyperinflammation and tissue damage (20, 24, 26). Additionally, compared with non–pneumonia/infectious ARDS, pneumonia/infectious–related ARDS is characterized by higher neutrophil and lymphocyte counts in BAL fluid, reflecting a more pronounced acute inflammatory response (34, 42, 56, 76). Interestingly, within infectious ARDS, the microbiologic etiology of pneumonia-evoked ARDS did not influence the ratio of Tregs to CD4 or the macrophage phenotype (56, 76). Moreover, direct ARDS is characterized by an early, robust proinflammatory response (M1-like gene sets) in AMs that correlates with better outcomes (8). This response diminishes over time in survivors, indicating a controlled resolution of inflammation. Indirect ARDS, however, lacks this distinct early proinflammatory signature and temporal pattern (8). These differences underscore the diverse immune response mechanisms based on the etiology of ARDS.

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Conclusions and Perspectives

In conclusion, neutrophils and macrophages are pivotal in driving the progression of ARDS, whereas all immune cells collaborate in orchestrating the recruitment, lifespan modulation, and apoptosis among themselves. The intricate dysregulation of alveolar immune cells during ARDS is affected by cytokine production and varying disease stages (Figure 1). Inappropriate or excessive activation of immune cells can significantly impact the clinical outcomes of patients with ARDS (Figure 2). Integrating the host immune response within alveolar space into pathophysiologic concepts of acute respiratory failure will enhance our understanding of ARDS etiology and propel advancements in therapeutic design. Therefore, a clear knowledge of the local host immune response at different stages of the disease is critical for the successful implementation of novel therapeutic interventions aimed at intervening therapeutically and improving outcomes in this clinical context. However, it is important to note that the association observed between BAL cellular profiles and clinical outcomes should be interpreted with caution, as these associations are potentially confounded by varying treatment approaches, underlying etiologies, patient demographic characteristics, and other clinical factors.

Future research could focus on the identification of the functional distinct subsets of immune cells in patients with ARDS of different etiologies, investigating the dynamic changes of intercellular interplays alongside the analysis of cytokine production throughout different stages of the disease and their potential impacts on the long-term outcomes of the patients. Moreover, human studies should delve into the exploration of less common cell types, their origins, and subtle changes within the microenvironment of ARDS. Furthermore, scant data are available concerning the role of alveolar immune cells in the development of fibrosis and the influence of prior immune modulation, for example through chemotherapy, on the alveolar immunological response. ■

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