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REVIEW

Macrophage Phenotypic Plasticity and Inflammatory Mechanisms in Hyperoxia-Induced Lung Injury

Wei Fu[1], Yawei Yao[2], Tingyang Meng[1], Yulan Li[2]

[1]The First School of Clinical Medical, Lanzhou University, Lanzhou, Gansu, People's Republic of China; [2]Department of Anesthesiology, The First Hospital of Lanzhou University, Lanzhou, Gansu, People's Republic of China

Correspondence: Yulan Li, Department of Anesthesiology, The First Hospital of Lanzhou University, Lanzhou, Gansu, People's Republic of China, Email liyul@lzu.edu.cn

Abstract: Hyperoxia-induced lung injury is a prominent inflammatory complication encountered in neonatal and adult critical care, contributing to acute lung injury and bronchopulmonary dysplasia. Although oxidative stress is a primary initiating factor, accumulating evidence suggests that dysregulated immune responses, particularly those mediated by macrophages, critically influence disease progression and resolution. Macrophages exhibit remarkable phenotypic plasticity in response to hyperoxic stress, extending beyond the conventional pro-inflammatory and anti-inflammatory polarization framework. Recent advances, including single-cell transcriptomic analyses, have revealed substantial heterogeneity among macrophage subsets, highlighting inflammatory, metabolically reprogrammed, senescent, and pyroptotic phenotypes in hyperoxic lung injury. These phenotypic shifts are tightly regulated by inflammatory signaling pathways, immunometabolic alterations, and cellular stress responses. In this review, we summarize current evidence regarding macrophage phenotypic plasticity in hyperoxia-induced lung injury, with a focus on key inflammatory pathways, metabolic reprogramming, inflammasome activation, and emerging cell fate programs such as pyroptosis and cellular senescence. We further discuss macrophage-mediated intercellular communication with epithelial and endothelial cells and its contribution to persistent inflammation and impaired lung repair. By integrating these findings, this review aims to provide updated insights into macrophage-driven inflammatory mechanisms and to highlight potential avenues for therapeutic modulation in hyperoxic lung injury.

Keywords: acute lung injury, macrophage plasticity, single-cell transcriptomics, immunometabolism, cellular crosstalk

Introduction

Oxygen is essential for the survival of aerobic life forms. Respirator therapy with high fractions of inspired oxygen (FiO_2 ≥50% or more) is still the treatment regimen for patients with hypoxemia which can be found in intensive care unit, neonatal ward and anaesthesia for surgery.[1,2] Despite inhibiting the growth of various bacterial pathogens, prolonged exposure to hyperoxic ambient air can lead to oxidative stress of lung tissues. Further, this will lead to rapid and massive accumulation of Reactive Oxygen Species (ROS). When the accumulation of ROS surpasses the ability of the body's antioxidant system to scavenge them, it causes apoptosis of the pulmonary epithelial cells and disruption of the endothelial barrier, leading to the infiltration of inflammatory cells and subsequent pulmonary edema, which is better known as Hyperoxic Acute Lung Injury (HALI).[3,4] HALI leads to diffuse alveolar damage during the acute phase in neonates, but, especially in those with extremely low birth weight, it also affects alveolar secondary septation and microvascular development. This causes alveolar simplification and decrease in lung function which may lead to BPD.[5] Although it is generally accepted that oxidative stress plays an important role in HALI, antioxidant therapies alone have not shown adequate efficacy in clinical trials.[1,6] This indicates that the evolutionary process of HALI is not just created by chemical oxidation but rather a biological cascade that involves the immune system internally.

Macrophages refer to the most abundant and functionally active type of immune cells resident in the lung. Macrophages are instrumental in the recognition as well as clearance of pathogens associated with molecular patterns

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that are also known as PAMPs. Furthermore, macrophages also help in the clearance of damage-associated molecular patterns or DAMPs. Moreover, by secreting cytokines, growth factors and extracellular vesicles (EVs), they communicate with other cell types (epithelial cells, endothelial cells, fibroblasts).[7,8] Alveolar macrophages (AMs) are considered to make up about 55% of pulmonary immune cells. They are located at the air–liquid interface at the alveoli. There, they are exposed to the external environment and high concentrations of inhaled oxygen.[9] Under conditions of hyperoxia, AMs are both targets of ROS assault as well as key inducers of the cytokine storm. Hyperoxic signals can trigger AMs rapidly, leading to their shift from a resting state to a highly activated pro-inflammatory state. As a result, cytokines, including TNF-α, IL-1β, and IL-6, as well as chemokines, including MIP-2 and MCP-1 that recruit neutrophils and monocytes, are released in a massive amount into the alveolar space.[10–12] If left unchecked, this excessive inflammatory response induces secondary exacerbation of tissue injury to the lung tissues.

Researchers have demonstrated that under hyperoxia, the macrophage functional state extends beyond the M1 (pro-inflammatory) and M2 (anti-inflammatory) spectrum. Studies using single-cell RNA sequencing (scRNA-seq) revealed high heterogeneity of hyperoxia-induced macrophages and identified unique subsets associated with senescence, pyroptosis and specific metabolic states.[13] Under hyperoxic stress, macrophage mitochondrial metabolism, endoplasmic reticulum unfolded protein response and epigenetic regulatory networks are subjected to reprogramming. An understanding of these complicated molecular events holds major clinical translational value to inhibit the progression of lung injury and facilitate tissue regeneration. A study of the molecular mechanism of macrophage phenotypic plasticity in hyperoxic environment will help not only to clarify fundamental pathophysiological mechanisms of HALI but also to highlight potential therapeutic avenues for discovering new therapeutic targets to remodel immune microenvironment and facilitate lung repair (Figure 1).

Evolution of Macrophage Subsets in Hyperoxic Environments

HALI's primary pathology is immune response. According to biological process and pathway enrichment analyses, immune-response-related genes' expression patterns were upregulated in HALI.[14] Recent advancements in single-cell RNA sequencing (scRNA-seq) and lineage tracing technology have confirmed that the macrophages in a HALI do not constitute a homogeneous population, but a complex system comprised subsets with distinct origins and functions.[15] The spatiotemporal manifestation of hyperoxic lung injury is reflective of the waxing and waning, as well as the replacement of subsets, during the course of injury.

It is biologically imperative to distinctly delineate macrophage heterogeneity between neonatal and adult hyperoxia models, as they govern fundamentally different pathophysiological trajectories. In adult ARDS models, macrophage responses primarily revolve around acute sterile inflammation followed by fibrotic resolution of a mature lung architecture. Conversely, in neonatal models—which exquisitely simulate preterm bronchopulmonary dysplasia during the critical developmental window of alveolarization (eg., P1–P14 in mice)—hyperoxia programs devastating disruptions in organogenesis.[16] ScRNA-seq reveals that neonatal hyperoxia-activated macrophages robustly dictate the developmental fate of alveolar type 2 (AT2) cells. Through aberrant IL-1R and TGF-β signaling, these macrophages force AT2 cells into a developmentally arrested, stem-cell-like "damage-associated transient progenitor" (DATP) state, thoroughly aborting normal AT1 differentiation and alveolar septation. Furthermore, macrophage heterogeneity in neonatal hyperoxia is uniquely characterized by profound sexual dimorphism. Transcriptomic mapping illustrates that male-derived lung immune cells exhibit significantly heightened expressions of pro-fibrotic and epithelial-to-mesenchymal transition (EMT) signals compared to their female counterparts. This sexually dimorphic macrophage programming provides critical mechanistic insight into why male premature infants suffer higher incidences of severe BPD, a distinct developmental feature rarely emphasized in adult lung injury paradigms.[17]

Tissue-Resident and Recruited Alveolar Macrophages in Hyperoxic Lung Injury

Pulmonary macrophage populations are functionally heterogeneous and dynamically regulated by developmental origin and local microenvironment. In the alveolar compartment, macrophages can be broadly categorized into tissue-resident alveolar macrophages (TR-AMs), monocyte-derived alveolar macrophages (Mo-AMs), and interstitial macrophages

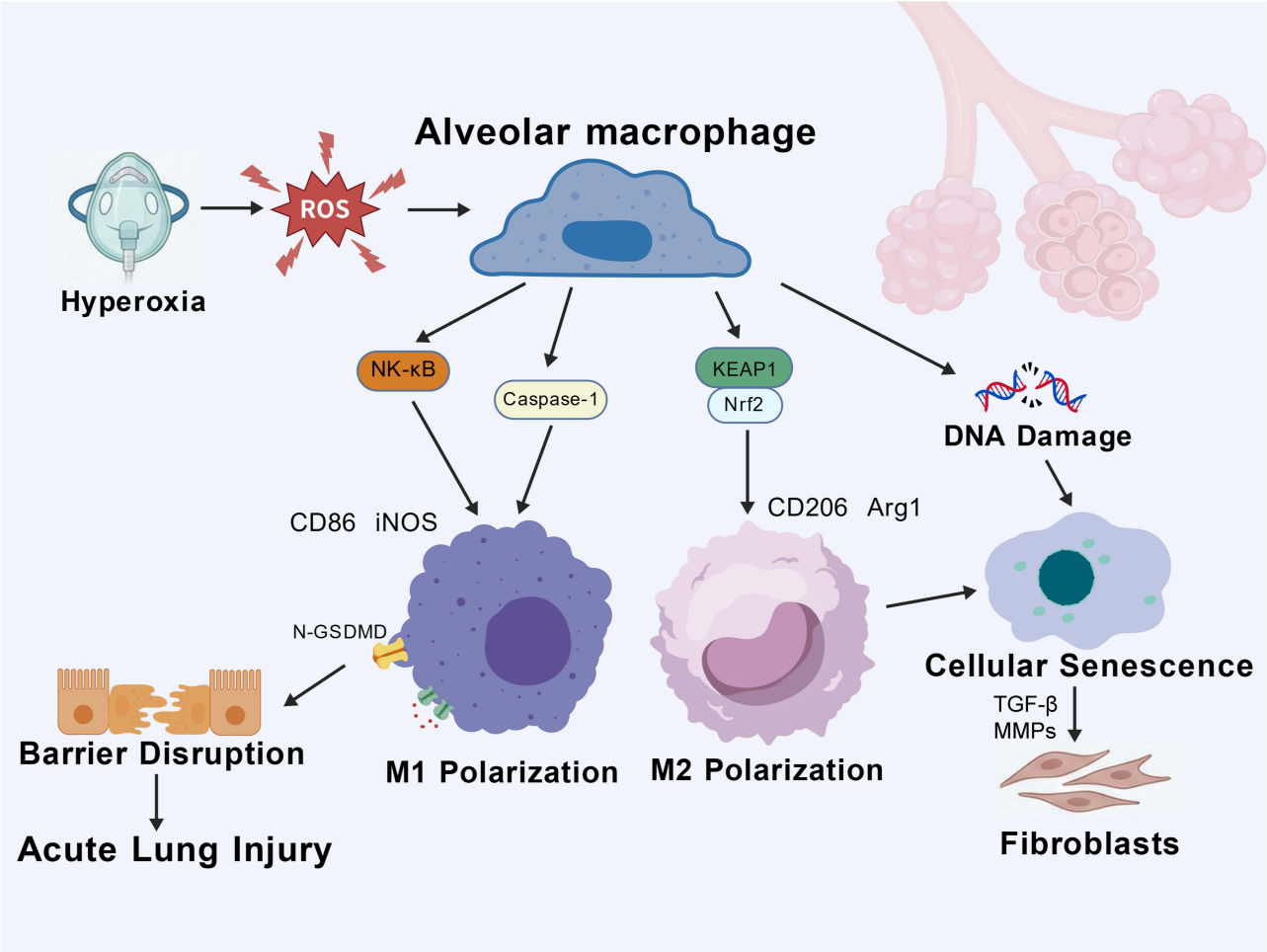

Figure 1 Phenotypic Plasticity and Molecular Mechanisms of Macrophages (Created with bioRender.com).

residing within the lung interstitium.[18] Among these populations, TR-AMs and Mo-AMs play distinct yet interconnected roles in shaping inflammatory responses during hyperoxic lung injury.

TR-AMs: TR-AMs mainly arise from the yolk sac or fetal liver during the embryonic period and continue to self-renew postnatally. They are enriched in Siglec-F, CD11c, and MerTK, which are the primary defenders of alveolar homeostasis endowed with potent phagocytosis and anti-inflammatory capacity.[19–21] In the initial phases of hyperoxic conditions, TR-AMs become functionally impaired (eg., impaired phagocytosis and metabolic alterations) and are substantially depleted through ROS-induced apoptosis or pyroptosis.[18,22] Studies have revealed that exposure to hyperoxic levels less than 90% results in a marked reduction in viability along with a halt in proliferation of primary AMs within three days.[10] The loss of TR-AMs disrupts the balance of immune response in the lungs, making the host more prone to opportunistic infections.[20]

Mo-AMs: Chemokines such as CCL2 (MCP-1), CXCL1 are released from damaged cells in the alveolar epithelial barrier from hyperoxia. The rapid recruitment of Ly6Chi monocytes from peripheral blood to cross the vessel wall and enter the lung interstitium and alveolar space for differentiation into Mo-AMs.[23] Exogenous macrophages exhibit pro-inflammatory and expresses high levels of CD11b, Ly6C, and iNOS in the early phase of injury.[19] In spite of being in the same environment, Mo-AMs and TR-AMs have different transcriptomic, metabolic and epigenetic profiles. Mo-AMs display a tendency to utilize glycolytic metabolism and show reduced anti-apoptotic functions. They are usually eliminated through Fas-mediated apoptosis during the resolution of inflammation. Alternatively, they may partly mature into resident-like cells to establish the TR-AM population. Although Mo-AMs help to replenish the macrophage pool,

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their differentiation and maturation take time. Moreover, their homeostatic regulatory capacity is less developed than that of TR-AMs and, hence, their inflammatory response is more easily dysregulated.

Interstitial Macrophages (IMs)

The IMs present in the lung interstitium, beyond the alveolar space, also play an important role in HALI. IMs can be divided in two subsets, MHC-II high and MHC-II low. They are involved in antigen presentation and immune regulation. Hyperoxia-induced vascular remodeling and fibrosis might involve interactions between IMs, endothelial cells, and fibroblasts, through the release of VEGF or TGF-β.[24,25] IMs physically reside within the lung interstitium and exert direct, spatially restricted paracrine control over pulmonary vascular remodeling. Mechanistically, IMs are anatomically sequestered into functional subsets based on the expression of folate receptor β (FRβ).[26] Under homeostatic conditions, $FR\beta^+$ IMs localize tightly to the vascular adventitia, actively maintaining the perivascular fibroblast pool and niche stability through the targeted, high-level secretion of Insulin-like Growth Factor 1 (IGF-1).[27] However, under severe hyperoxic injury, rapid influx of recruited monocyte-derived IMs dramatically disrupts this delicate vascular niche. These recruited IM populations—specifically the expanded $SPP1^+$ subsets—orchestrate profound pathological vascular remodeling via defined ligand-receptor signaling axes. First, they secrete massive quantities of PDGFB, which directly engages PDGFRα on pulmonary smooth muscle cells, driving pathogenic smooth muscle hyperplasia and medial wall thickening. Simultaneously, IM-derived THBS1 forcefully engages SDC4 receptors on general capillary endothelial cells. This specific THBS1-SDC4 interaction actively suppresses endothelial cell proliferation and survival, precipitating the devastating microvascular rarefaction and arrested angiogenesis that serve as histopathological hallmarks of bronchopulmonary dysplasia.

Subpopulation Analysis from the Perspective of Single-Cell Transcriptomics

The conventional flow cytometry markers like CD68 and F4/80 can no longer dissect the macrophage heterogeneity under hyperoxia at a 50-μm resolution. In recent times, scRNA-seq technology has defined a more detailed atlas of macrophage subsets.[13] Macrophage clusters were identified by scRNA-seq in mouse models of hyperoxia-induced Bronchopulmonary dysplasia and acute inflammation. Clusters 1 and 2 (homeostatic clusters), which predominate in healthy mice, highly expresses conventional AM markers. Clusters 3 and 4 have appeared to be newly recruited macrophages observed with the maximum strength under hyperoxia or LPS stimulation. There is a specifically strong increase in pro-inflammatory genes and in metabolism-related genes in these cells. Cluster 5, known as the Resolution Cluster, comes in during the resolution phase. Likely it is a transitional population undergoing cell death or moving toward a reparative phenotype.

Exposure to hyperoxia causes significant changes in macrophage gene expression profile.[15] In AMs, Inhba and Marco are upregulated by hyperoxia, while Ear1 is downregulated. Immunofluorescence assays confirm that Inhba and Marco expression is increased in leukocytes morphologically resembling alveolar macrophages ($Ptprc^+/CD45^+$) in lung tissue sections from hyperoxic mice and BPD patients. The induction of Ccr2 and Csf1r in IMs by hyperoxia, and by extension their ligands CCL2 and CSF1, suggest the involvement of the CCL2-CCR2 axis in inflammation.

Recent scRNA-seq data reveals a macrophage subset exhibiting typical senescent features in lungs of hyperoxia-exposed neonatal mice. The population displays high expression of cell cycle inhibitory genes (Cdkn2a/p16, Cdkn1a/p21). Moreover, this population possesses unique metabolic traits and secretes many different SASP factors such as IL-6, TGF-β, and MMPs. The buildup of these cells is linked to inhibited alveolarization and insufficient tissue repair capacity.[28]

In a hyperoxia-induced pulmonary hypertension model, IMs display characteristic spatiotemporal heterogeneity. The $MHCIIhiCCR2^+EAR2^+$ subset dominates the acute inflammatory phase, while the second phase of vascular remodeling involves a shift to the $TLF^+VCAM1hi$ subset. Enriched in the perivascular region, the latter promotes smooth muscle cell proliferation and vascular wall thickening by secreting vascular remodeling factors.[29]

Crucially, the robustness and cross-species reproducibility of these high-resolution macrophage subset classifications have been extensively validated through the integration of murine and human scRNA-seq datasets.[30] Profibrotic monocyte-derived macrophage subsets characterized by specific transcriptomic signatures—such as the pronounced

enrichment of Gpnmb and SPP1 (Secreted Phosphoprotein 1)—are highly conserved across species and consistently emerge irrespective of the specific injury model, manifesting in both hyperoxia and bleomycin paradigms.[31] While the fundamental transcriptomic identities defining tissue-resident versus recruited macrophages exhibit profound cross-species homology, the exact topological proportions of specific transitional states can fluctuate dynamically in response to the specific intensity and chronicity of the hyperoxic challenge. This demonstrates that scRNA-seq mapping provides a biologically robust yet dynamically responsive framework for cataloging macrophage heterogeneity.[32,33]

Macrophage Phenotypic Plasticity

The ability of macrophages to adapt to changes in their environment is central to their plasticity. In the strong environmental stimulus of hyperoxia, macrophages have different fate decisions, besides the classical M1/M2 polarization, such as pyroptosis and senescence. The phenotypes together govern the result of lung injury.

Classical M1/M2 Polarization in Hyperoxia Injury

During the early phase of hyperoxia, the lung tissue experiences acute oxidative stress. As effective damage-associated molecular patterns (DAMPs), ROS engage in TLR4-and RAGE-mediated M1 polarization in AMs. AMs at this stage express high levels of surface markers such as iNOS, CD86, and CD80, together with the enormous secretion of pro-inflammatory cytokines and chemokines.[34] In experiments combining LPS with hyperoxia, hyperoxia significantly enhances the M1 polarization effect caused by LPS, leading to a doubling of pro-inflammatory factor levels and the inhibition of M2-related gene expression.[35] In mouse models, there is a marked increase in M1 macrophages 24 hours after hyperoxic exposure which highly correlates with increased pathological lung injury scores.[34] M1 macrophages in sterile hyperoxic injury lead to oxidative damage and apoptosis in the alveolar epithelial cells (AECs) through the release of high concentrations of NO and ROS, and disrupt the alveolar-capillary barrier to cause protein-rich pulmonary edema.[21,36,37]

As the injury continues, host damage repair mechanisms try to push AMs to change phenotypes towards M2 phenotypes. M2 macrophages classically express Arginase 1 (Arg1), Mannose Receptor (CD206), Fizz1 and Ym1, while secreting substances such as IL-10, TGF-β, and IGF-1.[9] However, exposure to prolonged hyperoxia often prevents transition from M1 to M2. In neonatal BPD models, high levels of M1 markers remain detectable even late in the injury process, with an impaired capacity to form alveoli.[2] This ongoing inflammatory milieu in M1 prevents stem cell differentiation and alveolar septation. In HALI complicated by fibrosis, the pro-fibrotic M2 subtype (M2a-like) emerges. These cells produce TGF-β and PDGF in excess that causes fibroblast to myofibroblast conversion.[12] Research has shown that collagen is deposited within the lung tissue on day 14 of hyperoxic exposure. This deposition is related to aberrant macrophage repair functions.[2] This phenotype reflects the syncretism of pro-inflammatory and reparative signals within the microenvironment which shows failure of tissue repair and persistence of chronic inflammation (Table 1).

Importantly, recent lineage-tracing and transcriptomic studies reveal a direct transdifferentiation phenomenon known as Macrophage-Myofibroblast Transition (MMT). During the chronic remodeling phase of hyperoxia-induced injury, a specific subset of bone marrow-derived monocyte/macrophages can directly transform into collagen-producing myofibroblasts. This MMT process is fundamentally driven by the robust activation of the TGF-β1/Smad3 signaling axis. These transitional cells uniquely co-express macrophage lineage markers (eg., CD68, F4/80) alongside myofibroblast functional markers (eg., α-smooth muscle actin). Through MMT, macrophages transcend their traditional role as mere paracrine regulators of fibroblasts and directly contribute to extracellular matrix deposition and the fibrotic obliteration of the alveolar architecture.[38,39]

While the traditional M1/M2 dichotomy has historically provided a simplified conceptual framework for understanding early pro-inflammatory versus late reparative shifts, it is fundamentally inadequate for capturing the multi-dimensional plasticity of macrophages in vivo. Recent high-resolution single-cell transcriptomics have conclusively demonstrated that the strict M1/M2 classification is a severe oversimplification derived largely from extreme in vitro stimulation paradigms. Under the complex, dynamic biochemical milieu of hyperoxic lung injury, macrophages frequently adopt highly complex hybrid states, simultaneously co-expressing canonical M1 (eg., iNOS, CD86) and M2 (eg., Arg1, CD206) transcriptomic signatures within the very same cell. Rather than existing as discrete phenotypic endpoints,

Table 1 Temporal Summary of Phenotypic Plasticity

Polarization State of Macrophage	M1 (Pro-Inflammatory)	M2 (Profibrosis)
Microenvironment	Excess ROS produced by hyperoxia, DAMPs released by damaged cells (eg. HMGB1), endotoxin (LPS), abnormal hypoxic signals	Tissue damage repair signaling, phagocytosis of apoptotic cells, IL-4/IL-13, lipid mediators
Core Transcription Factors and Molecular Markers	iNOS, CD86, CD80, MHC-II, IL-1R	CD206, Arg1, Ym1, Fizz1, SPP1
Major Secreted Cytokines and Metabolites	TNF-α, IL-1β, IL-6, MIP-2, CXCL1, ROS, NO	TGF-β, IL-10, PDGF, IGF-1, VEGF
Pathophysiological consequences in HALI	Intense recruitment of neutrophils; induction of apoptosis of alveolar epithelial cells; disruption of alveolar-capillary tight junction barrier leading to protein-rich pulmonary edema and diffuse alveolar damage	Early responsible for debris removal and inflammation resolution; but under chronic hyperoxia exposure, persistent TGF-β drives fibroblast activation and MMT, resulting in abnormal collagen deposition, alveolar arrest, and microvascular dysplasia
Core Mechanism and Signal Pathway	NF-κB; HIF-1α; NLRP3 inflammasome hyperactivation; aerobic glycolytic reprogramming	TGF-β1/Smad3; OXPHOS; Nrf2/HO-1

macrophage activation unfolds across a continuous transcriptomic and functional spectrum dynamically contoured by local microenvironmental cues, metabolic availability, and ontogeny. Relying solely on the rigid M1/M2 paradigm fundamentally obfuscates the identification of novel, distinct functional subsets—such as specific vascular-remodeling IMs or highly specialized lipid-associated macrophages (LAMs)—which are functionally indispensable to the pathogenesis and resolution of hyperoxic injury.[40]

Pyroptosis

Pyroptosis is a type of programmed cell death that is usually accompanied by massive release of pro-inflammatory factors. Recent studies show its role in HALI in great detail. The NF-kB pathway upregulates the transcription of NLRP3 & Pro-IL-1β caused by DAMPs TLR recognition.[41] The overproduction of mitochondrial ROS, the release of mitochondrial DNA, and the potassium efflux caused by the P2X7 receptor stimulate the recruitment of NLRP3 to the multiprotein complex consisting of the adaptor protein ASC and the effector protein Pro-Caspase-1.[42–44] Caspase-1 activity is indicated by the cleavage of precursors IL-1β and IL-18 to mature form. Moreover, activated Caspase-1 cleaves Gasdermin D (GSDMD) to release its N-terminal domain (GSDMD-N). The cell membrane has pores formed by GSDMD-N, which leads to cell swelling, rupture, and release of intracellular substances, causing a very strong inflammatory response.[2] In a neonatal model of hyperoxia in mice, pulmonary macrophages display a significant increase in NLRP3 and Caspase-1 and GSDMD-N expression, which is associated with IL-1β release. A therapeutic approach utilizing the Caspase-1 specific small-molecule inhibitor VX-765 significantly inhibits this pathway, which blocks macrophage pyroptosis along with lung alveolarization and pulmo vascular development. Inhibiting Map3k7 enhances the resistance of AT2 cells to hyperoxia and inhibits the pyroptosis of macrophages, indicating that macrophage pyroptosis is an important link in hyperoxia-induced BPD.[45]

The pathological significance of macrophage pyroptosis extends far beyond mere cellular depletion; it serves as a massive, catastrophic amplifier of intercellular crosstalk. The rapid, mechanically disruptive expulsion of mature IL-1β, IL-18, and intracellular DAMPs (eg., HMGB1) through GSDMD membrane pores directly assaults adjacent alveolar endothelial and epithelial cells. This concentrated cytokine barrage drastically downregulates critical tight junction proteins (eg., Claudins), thoroughly compromising alveolar-capillary barrier integrity. Simultaneously, this pyroptotic

expulsion functions as an overpowering chemoattractant gradient that hyper-recruits neutrophils to the injury site, establishing a devastating feed-forward loop of tissue destruction.[46]

Cellular Senescence

Hyperoxia induces acute cell death and incites senescence in surviving macrophages. Senescent macrophages show an irreversible arrest of the cell cycle with high p16INK4a (Cdkn2a) and p21CIP1 (Cdkn1a) expression plus SA-β-gal positivity.[47,48] These cells secrete the SASP factors IL-6, IL-1α, MMP-12, and TGF-β on a continuous basis. Through paracrine signalling, these factors recruit secondary senescence in the neighbouring AT2 cells, deplete the stem cell pool, and hinder the regeneration of damaged alveoli.[28] Senescent macrophages in lung cancer research have even been shown to have immunosuppressive properties that create a tumor-promoting microenvironment, suggesting that senescent macrophages may also play a similar role in abnormal long-term tissue remodeling by hyperoxia.[49]

Critically, senescent macrophages dictate the pathological fate of the surrounding alveolar niche through the sustained elaboration of a highly active Senescence-Associated Secretory Phenotype (SASP). Comprising overwhelmingly high levels of IL-6, TGF-β, and MMP-12, this robust paracrine signaling cascade induces paracrine (secondary) senescence in previously healthy neighboring AT2 epithelial cells, thereby prematurely exhausting the regenerative stem cell progenitor pool. Concurrently, SASP components drive pathological, unchecked fibroblast activation, functionally linking the senescence of the macrophage compartment to the definitive arrest of alveolarization and the fibrotic structural remodeling characteristic of prolonged hyperoxic injury.[50]

Relevant Molecular Mechanisms

The intracellular signaling networks of macrophages orchestrate a complex phenotypic change. In HALI, the connected web of signaling pathways involving oxidative stress, inflammasomes, hypoxia sensing and ER stress all regulate macrophage fate.

The Nrf2/KEAP1 Pathway

Nuclear factor erythroid 2-related factor 2 (Nrf2) is the master regulator of the cellular antioxidant response. Nrf2 is prolylhydroxylated at C-terminal by KEAP1 enzyme, which gets ubuiquitinated and is degraded by proteasome. The hyperoxia-generated reactive oxygen species (ROS) modifies the cysteine residues such as Cys151 on KEAP1 inducing a conformational change that releases Nrf2 allowing it to translocate into the nucleus. Consequently, Nrf2 attaches to ARE and leads to the transcription of antioxidant enzymes like Heme Oxygenase-1 (HO-1) and NQO1.[51–53] Jia et al demonstrated that etomidate effectively mitigates pulmonary injury in HALI due to its property of modulating Nrf2/HO-1 signalling.[54] In hyperoxic conditions, the infiltration of macrophages is more severe in Nrf2-knockout mice, and their expression of the antioxidant enzyme HO-1 is significantly suppressed. Yet, Nrf2 pathway activation is not enough to ameliorate excessive ROS and redox unbalance in a severe HALI. This shortcoming promotes the activation of pro-inflammatory pathways including NF-κB, which consequently induces M1 polarization and pyroptosis in macrophages.

The NF-κB Pathway

NF-κB is a well-studied transcription factor that modulates M1 macrophage polarization and SASP secretion. Oxidative stress leads to an activation of the IκB kinase (IKK) complex causing phosphorylation and degradation of the inhibitory protein IκBα and releases NF-κB p65/p50 dimer for nuclear translocation.[55] Research indicates that the pattern of NF-κB activation in lungs of neonatal hyperoxia differs from that of adults. Neonates display a greater degree of nuclear translocation and more sustained activation, primarily of the p50 subunit, accompanied by swift proteolytic degradation of IκBα and enhanced levels of the ubiquitin E3 ligase β-TrCP.[56] The hyperactivation that occurs in a stage-specific manner may represent the molecular basis for the neonate susceptibility to chronic inflammation associated with BPD. Drugs like celecoxib can alleviate hyperoxia-induced macrophage inflammation by inhibiting COX-2 and blocking the nuclear translocation of NF-κB. This also down-regulates Aquaporin 1 (AQP1) and pulmonary edema.[57]

The HIF-1α Pathway

Hypoxia-inducible factor-1α (HIF-1α) is commonly stabilized in a hypoxic environment, but a distinction in HIF-1α's accumulation in hyperoxic lung injury, dubbed functional hypoxia.[58,59] Prolyl Hydroxylases (PHDs) hydroxylate HIF-1α for its degradation and it can only use PHDs in (O2, Fe^{2+}, α-ketoglutarate) cofactors. Mitochondrial reactive oxygen species (ROS) during hyperoxia can inhibit PHD activity by oxidizing Fe^{2+} to Fe^{3+}. HIF-1α therefore does not degrade with oxygen and stays stable HIF-1α's unusual stabilization is an important driver of metabolic reprogramming toward glycolysis in macrophages. It can upregulate genes related to glycolytic metabolism (eg, GLUT1, HK2) and directly binds to the promoter region of pro-inflammatory genes like IL-1β to promote M1 polarization and the continuous release of inflammatory cytokines.[60,61]

Endoplasmic Reticulum Stress (ERS)

The oxidative protein damage caused by excessive oxygen begins the accumulation of misfolded proteins in the ER lumen, leading to ER stress (ERS) and UPR. The signalling inside the UPR takes place through the pathways PERK, IRE1 and ATF6. Under hyperoxia, the PERK-eIF2α-ATF4 axis activates which inhibits translation of proteins and reduces ER load, however, overstimulation activates CHOP expression which directly induces macrophage apoptosis.[62,63] Subjection to hyperoxia also causes IRE1 to splice XBP1 mRNA, with generation of XBP1s This pathway regulates chaperone expression in macrophages and induces the transcription of inflammatory factors IL-6 and TNF-α. According to research, 4-phenylbutyric acid (4-PBA) is able to relieve hyperoxia-induced ERS, inhibits NF-κB activation, decreases macrophage inflammatory cytokines production, and protects lung epithelial tight junction protein Claudin-4, ameliorating hyperoxic lung injury.[64]

Metabolic Reprogramming

In recent years, a new area of research known as immunometabolism has developed to understand macrophage function. In immunology, a major shift is the understanding that cellular metabolism is not simply a mechanism of energy generation but also an instructive factor, which regulates immune cell phenotypes and functions.

Glycolysis and M1 Polarization (the Warburg Effect)

In hyperoxic environments, M1 macrophages preferentially switch to glycolysis for energy production, which is the Warburg effect.[65,66] HIF-1α is stabilized because it is unable to be degraded in hyperoxia, which makes it translocate to the nucleus. There it brings about transcription of glycolytic enzymes, such as Hexokinase 2, Pyruvate Kinase M2, Lactate Dehydrogenase A, and IL-1β.[67] During a signal process involved in inflammation, TCA cycle gets truncated (break) leading to the accumulation of citrate and succinate. Citrate has a variety of physiological and therapeutic roles. It acts both as a metabolic pathway intermediary and a molecular signal. Prostaglandin synthesis (as inflammatory mediator) and fatty acid synthesis require a pool of citrate. In addition to that, citrate is also a substrate for nitric oxide (NO) and ROS, build up. In addition, improved glycolysis diverts glucose carbon into the Pentose Phosphate Pathway (PPP) to make NADPH. NADPH is important for biosynthesis but also serves as a necessary cofactor for NADPH oxidase to produce ROS, which maintains the high levels of oxidative stress that are necessary for killing the pathogen.

Oxidative Phosphorylation and M2 Polarization

M2 macrophages mainly depend on oxidative phosphorylation (OXPHOS) in addition to fatty acid oxidation (FAO) to drive their sustained energy sustaining tissue repair and anti-inflammatory functions.[66] Notwithstanding, hyperoxic encounters cause a great deal of toxicity on the mitochondria. When excessive ROS attacks the Δψm of mitochondria, it impairs the efficiency of ATP synthesis by disrupting the electron transport chain complexes. This mitochondrial malfunction prevents macrophages from sustaining an efficient OXPHOS, and metabolically inhibits the transition to the M2 phenotype.[68] Furthermore, ROS released from damaged mitochondria activates the NLRP3 inflammasome to induce a metabolic-inflammatory vicious cycle.

Signaling Functions of Metabolic Intermediates

In M1 macrophages, there is a considerable accumulation of succinate due to varied activity of succinate dehydrogenase. Succinate serves as a signaling molecule that inhibits PHD activity, stabilizes HIF-1α, and induces the transcription of IL-1β, forming a metabolic-inflammatory feedback loop.[66] In contrast, the compound itaconate, which is a product of the conversion of the substance cis-aconitate into a chemical structure of the same name by the enzyme immune-responsive gene 1 (IRG1) exhibits powerful anti-inflammatory and antioxidant effects by promoting the activation of the Nrf2 pathway. This helps to curtail excessive inflammation. Within the context of the aforementioned, itaconate, or any derivative thereof, supplementation represents a potential therapeutic approach.[67]

Metabolic Crosstalk with the Alveolar Microenvironment

Macrophage metabolic reprogramming entirely transcends intrinsic cell regulation, establishing profound metabolic crosstalk with the broader stromal and immune microenvironment. During the acute hyperoxic phase, the massive upregulation of aerobic glycolysis in hyperactive M1-like macrophages rapidly and greedily depletes local glucose reservoirs, creating a severely nutrient-deprived alveolar niche.[69] This fierce nutritional competition forces neighboring alveolar epithelial cells to completely pivot their energy infrastructure toward alternative metabolic pathways, primarily relying on fatty acid oxidation, to maintain survival and barrier integrity amidst the stress. Furthermore, specific metabolites expelled by these metabolically altered macrophages act as highly potent extracellular signaling molecules. For instance, macrophage-derived lactate and succinate are actively taken up by surrounding lung fibroblasts. Once internalized, these metabolites directly and potently inhibit prolyl hydroxylase domain (PHD) enzymes. This paracrine metabolic signaling stabilizes HIF-1α within the fibroblasts entirely independently of actual oxygen tension, thereby locking the fibroblasts into a hyper-secretory, pro-fibrotic state that incessantly exacerbates pathological extracellular matrix deposition and structural fibrosis.[70]

Epigenetic Regulation

Environments with elevated oxygen levels may not only change signal transduction but also permanently imprint the genome via epigenetic modification which alters macrophage behaviour.

lncRNAs and circRNAs

lncRNAs and circRNAs frequently act as ceRNAs or sponges of miRNAs that release target genes from repression (Table 2). Concrete, high-impact mechanistic examples underscore the extraordinary regulatory power of ncRNAs in governing hyperoxia-induced macrophage plasticity. The prominent long non-coding RNA MALAT1, for instance, is robustly upregulated under hyperoxic and endotoxin stress.[71] Mechanistically, MALAT1 meticulously sustains classical pro-inflammatory activation by actively repressing Clec16a expression and directly inhibiting mitochondrial pyruvate carrier (MPC)-driven oxidative phosphorylation, thereby starving the cell of the metabolic prerequisites necessary for transitioning to a reparative M2 state.[71] Paradoxically, in vivo lineage studies reveal that while MALAT1 ablation

Table 2 Key ncRNA Regulatory Axes in Hyperoxic Lung Injury

ncRNA Type	Name	Target miRNA/ Protein	Downstream Effect/ Target Gene	Functional Outcome	References
LncRNA	MALAT1	Sponge for miR-206	Fibronectin 1 (FN1)	Promotes epithelial-mesenchymal transition (EMT); exacerbates inflammation	[71, 72]
LncRNA	TUG1	Sponge for miR-29a-3p	Elastin (ELN)	Regulates elastin expression; alleviates apoptosis	[73]
CircRNA	CircABCC4	Sponge for miR-663a	PLA2G6	Promotes phospholipase expression; exacerbates BPD progression	[74]
CircRNA	CircABPD1	Sponges miR-330-3p	HIF-1α	Inhibits the HIF-1α pathway; attenuates lung injury	[75]
CircRNA	CircANKRD36	Sponge for miR-330	ROCK1	Promotes macrophage inflammatory response	[76]

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successfully attenuates initial acute pulmonary inflammation, it severely and catastrophically exacerbates subsequent fibrotic remodeling, highlighting its highly context-dependent, dual-edged role in delicately balancing acute defense and chronic repair.[77] In the realm of circular RNAs, circABCC4 undergoes massive upregulation in macrophages exposed to neonatal hyperoxia. It acts as an exceptionally effective competitive endogenous RNA (ceRNA) sponge specifically targeting miR-663a. By sequestering this microRNA, circABCC4 completely unleashes the translation of PLA2G6, an enzyme that aggressively synthesizes pro-inflammatory lipid mediators, thereby profoundly amplifying the pathological progression and tissue destruction characteristic of bronchopulmonary dysplasia.[78]

miRNAs

miR-21 is a pro-inflammatory microRNA that is expressed in the lungs of individuals subjected to hyperoxia. It promotes M1 polarization by targeting and inhibiting the anti-inflammatory gene SATB1.[25]

miR-210, the hypoxiamiR, is paradoxically and strongly induced in macrophages during hyperoxia. It impairs mitochondrial function that regulates necroptosis and metabolism.[60]

Research shows that exosomes secreted from M2 macrophages are enriched with miR-25-5p. When alveolar epithelial cells internalize this miRNA, this miRNA can reciprocally target CDKN1C or the NRBP2/PI3K/AKT axis, thereby suppressing epithelial pyroptosis and promoting lung development. This reveals a new mechanism of macrophage-epithelial intercellular communication by miRNAs.[79]

Exosomes

Macrophage-derived exosomes (Exos) are important mediators of communication within the microenvironment. Hyperoxia prompts macrophages to distribute Exos containing pro-inflammatory miRNAs (such as miR-221, miR-320). When epithelial cells take up these EXO, this leads to an increased release of pro-inflammatory factors which reactivate macrophages, causing a vicious cycle.[80] On the contrary, studies show that Lipoxin A4 treatment promotes the secretion of miR-25-5p-enriched Exos from M2 macrophages. These exosomes act on the epithelial cells to hamper their pyroptosis, through inhibition of the NRBP2/PI3K/AKT axis, thereby significantly improving lung injury.[79]

Mechanistic Integration of Immunometabolism and Epigenetics

The phenotypic plasticity of macrophages under hyperoxia is intricately and fundamentally governed by the mechanistic crosstalk between metabolic reprogramming and epigenetic remodeling. Metabolic intermediates do not merely serve as passive energetic byproducts; they act as critical obligate substrates and allosteric regulators for epigenetic enzymes, effectively bridging the cytosolic metabolic state with nuclear chromatin architecture.[81] For instance, the hyperoxia-induced shift toward glycolysis significantly alters the intracellular pool of acetyl-CoA. This metabolite functions as the essential acetyl donor for histone acetyltransferases (eg., p300/CBP), facilitating the widespread histone acetylation necessary to maintain an open chromatin conformation at the promoter regions of pro-inflammatory cytokines, such as IL-1β.[82,83] Concurrently, alterations in amino acid metabolism influence the generation of S-adenosylmethionine (SAM). As the primary methyl donor for DNA methyltransferases (eg., DNMT3A), SAM dictates the DNA methylation-dependent epigenetic silencing of anti-inflammatory and tissue-repair gene networks. Furthermore, the massive intracellular accumulation of succinate—resulting from the hallmark truncation of the TCA cycle in inflammatory macrophages—profoundly and competitively inhibits JmjC domain-containing histone demethylases, structurally cementing a pro-inflammatory epigenetic landscape. This immunometabolic-epigenetic integration establishes a rigid, self-sustaining feedback loop that locks macrophages into maladaptive phenotypes during prolonged hyperoxic stress.[84]

Intercellular Crosstalk Between Macrophages and the Lung Microenvironment

HALI is a whole-system phenomenon characterized by the disruption of intercellular communication rather than the pathology of single cells. Macrophages are at the center of this network and influence alveolar epithelial cells, vascular endothelial cells, and fibroblasts through paracrine mechanisms and contact.

Macrophage-Epithelial Interactions

Under homeostatic conditions, the alveolar epithelium exerts the resting state of macrophages through the expression of CD200, which binds to the CD200R on the macrophage. Exposure to hyperoxia leads to epithelial injury and a downregulation of CD200 expression that releases the macrophage "brake" signal. As a result, activated macrophages secrete IL-1β, TNF-α, and TRAIL that directly induces apoptosis and necrosis of epithelial cells leading to the breakdown of the alveolar–capillary barrier.[7]

Macrophage-Fibroblast Interactions

Macrophage and fibroblast interaction during the late phase or tissue repair inflammatory process of BPD influences fibrotic outcome. Macrophages have been shown to elicit IL-6 secretion from fibroblasts through P2RX4 in recent studies. The expression of Arginase 1 (Arg1) in macrophages is upregulated by IL-6. According to Arginase 1, which catalyzes the hydrolysis of arginine, arginine is converted to ornithine by arginase 1, which fibroblasts take up and convert to proline. Proline which is key for collagen formation directly drives pulmonary fibrosis pathogenesis.[85]

Macrophage-Endothelial Interactions

A prominent feature of BPD is impaired pulmonary vascular development. Macrophages exposed to hyperoxia transfer miR-23a-3p to endothelial progenitor cells (EPCs) via exosome. In EPCs, miR-23a-3p inhibits proliferation and angiogenic capacity, causing pulmonary microvessel rarefaction. In vivo experiments have demonstrated that blocking this pathway with AntagomiR-23a-3p significantly restores pulmonary vascular density and alveolarization.[11] Additionally, according to reports, neonatal exposure to hyperoxia does not only impede the process of alveolarization and escalate the count of pulmonary macrophages but also correlates with specific shortcomings in neurological development. These include defects in myelination, cerebrovascular hyper-vascularization, and malformations in cerebral microglia. This indicates a possible immune communication along the lung-brain axis.[86]

The Impact of Macrophages on Other Immunocytes

Beyond stromal interactions, hyperoxia-activated macrophages robustly orchestrate the recruitment and activation of other immune populations. Under hyperoxic stress, activated alveolar macrophages establish potent chemokine gradients by hypersecreting macrophage inflammatory protein-2 (MIP-2), KC (CXCL1), and LIX, which are essential for driving the massive influx of neutrophils into the alveolar space. Concurrently, macrophage-lymphocyte crosstalk shifts the local milieu toward a profound pro-inflammatory state. Macrophages significantly upregulate costimulatory molecules including CD40, CD86, and MHC class II, enhancing their activation capacity toward effector T cells. This intense pro-inflammatory activation coincides with a substantial 40–50% reduction in the local population of immunosuppressive $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells (Tregs). The hyperoxia-driven depletion of Tregs, coupled with the macrophage-mediated release of HMGB1, effectively removes critical anti-inflammatory brakes, thereby sustaining the immune-mediated secondary lung injury.[87,88]

Emerging Pharmacological and Nanomedicine Strategies Targeting Macrophages

Therapeutic interventions specifically targeting macrophage plasticity are rapidly emerging as promising strategies for HALI. Pharmacologically, the selective Caspase-1 inhibitor VX-765 has demonstrated robust efficacy in preclinical BPD models. By explicitly blocking macrophage pyroptosis, VX-765 prevents the cleavage of Gasdermin D and the subsequent massive extracellular release of mature IL-1β, thereby shielding the alveolar epithelium and preserving alveolarization without the broad immunosuppressive side effects of systemic corticosteroids. Concurrently, endogenous pro-resolving lipid mediators, such as Lipoxin A4, have been shown to actively drive macrophage polarization toward a reparative M2 phenotype, suppressing pyroptosis via the modulation of exosomal microRNA transfer.[79] Beyond small molecules, advanced nanoplatforms capitalize on the intrinsic phagocytic capacity of macrophages. Engineered cerium-based tannic acid nanozymes have been deployed to efficiently scavenge local ROS within the

highly oxidative alveolar niche, actively catalyzing the reprogramming of macrophages toward tissue-repairing M2 profiles. Similarly, ROS-responsive carbon dots (RCMNs) and aerosolized core-shell liposomal nanoplatforms delivering an mRNA-based CRISPR/Cas9 system—designed to precisely knock out hexokinase 2 (HK2) and halt pathogenic macrophage glycolysis—represent cutting-edge methodologies to therapeutically re-educate the immune microenvironment in situ.[22]

Conclusion and Perspectives

In summary, accumulating evidence indicates that macrophages in hyperoxia-induced lung injury are not merely sources of inflammatory mediators, but function as critical intermediaries linking oxidative stress to tissue injury and repair. Hyperoxic exposure disrupts macrophage homeostasis and promotes maladaptive phenotypic shifts through coordinated alterations in mitochondrial function, cellular metabolism, epigenetic regulation, and inflammatory signaling pathways. Dysregulation of antioxidant defense systems such as the Nrf2/KEAP1 axis, aberrant activation of the NLRP3 inflammasome, non-hypoxic stabilization of HIF-1α, and persistent endoplasmic reticulum stress together form an interconnected regulatory network that sustains inflammatory amplification. In parallel, altered macrophage communication with alveolar epithelial cells, endothelial cells, and fibroblasts via extracellular vesicles and metabolic intermediates contributes to impaired alveolarization, microvascular abnormalities, and fibrotic remodeling.

Future studies focusing on the translational relevance of macrophage-targeted interventions may provide novel opportunities for modulating hyperoxia-driven inflammation. Emerging strategies, including macrophage-derived or macrophage-modulating extracellular vesicle–based approaches, warrant further investigation. In addition, advances in single-cell and spatial transcriptomic technologies are expected to refine our understanding of macrophage heterogeneity within distinct pulmonary microanatomical niches under hyperoxic stress, thereby facilitating more precise therapeutic targeting.

Importantly, improving our understanding of macrophage phenotypic plasticity—particularly inflammatory cell death programs such as pyroptosis, immunometabolic reprogramming, and non-coding RNA–mediated regulatory networks—may deepen insights into the pathogenesis of bronchopulmonary dysplasia and acute respiratory distress syndrome. Such knowledge could ultimately inform the development of anti-inflammatory strategies aimed at restoring immune balance and promoting lung repair in patients with hyperoxic lung injury.

Despite rapid conceptual and technological advancements, translating macrophage-targeted therapies to the clinical management of HALI and BPD faces formidable challenges and prominent knowledge gaps. The foremost critical hurdle is the intrinsic phenotypic instability of macrophages in vivo; therapeutically administered or locally re-programmed "reparative" macrophages are highly susceptible to being powerfully "re-educated" by the hostile, hyperoxic alveolar milieu. Consequently, these therapeutic cells can rapidly undergo phenotypic reversion back into pathogenic, pro-inflammatory, or pro-fibrotic states, negating clinical efficacy and potentially exacerbating injury. Additionally, pharmacological targeting is severely hindered by stubborn pharmacokinetic barriers, including the unintended systemic "lung trapping" of cellular therapeutics, as well as the premature intracellular degradation or uncontrolled leakage of nanoparticle payloads before they can successfully navigate to specific sub-alveolar disease niches. Finally, the profound surface receptor heterogeneity among macrophage subsets makes it exceedingly difficult to design targeting ligands that exclusively neutralize pathogenic subpopulations (eg, profibrotic interstitial subsets) without simultaneously inciting disastrous off-target immunotoxicities in healthy, tissue-resident populations systemic-wide. Future breakthroughs will mandate the sophisticated integration of spatial transcriptomic diagnostics with precisely tunable, bio-responsive smart nanodelivery platforms capable of overcoming this extraordinary microenvironmental plasticity.

Data Sharing Statement

Data sharing is not applicable to this article as no data were created or analysed in this study.

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Author Contributions

WF: Writing – original draft, Writing – review and editing, Conceptualization, Visualization. YWY: Writing–original draft, Writing – review and editing, Visualization. TYM: Writing – original draft, Writing – review and editing, Visualization. YLL: Writing – review and editing, Supervision, Conceptualization. All authors have agreed on the final version of the article for publication, have agreed on the journal to which the paper is submitted, and have agreed to be accountable for the work published.

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