

Changes in Macrophages in Pulmonary Hypertension: A Focus on High-altitude Pulmonary Hypertension

ABSTRACT

High-altitude pulmonary hypertension (HAPH) is a condition characterized by elevated pulmonary arterial pressure exceeding normal physiological values, resulting from a combination of high-altitude low-pressure, hypoxic environments, genetic susceptibility, immune dysfunction, and neurogenic disturbances. This condition predominantly manifests as right heart failure, severely impacting quality of life and life expectancy. Macrophages, as one of the most prevalent innate immune cells, have been increasingly recognized for their crucial role in the pathogenesis of HAPH. The low-pressure and hypoxic environment, along with other etiological factors, lead to metabolic abnormalities in tissue cells and the microenvironment. This results in increased secretion of chemokines, cytokines, and growth factors in the microenvironment, which promote the proliferation of tissue-resident macrophages and the differentiation of monocytes recruited from the blood into macrophages. This exacerbates the inflammatory cascade, further promoting cell proliferation, tissue repair, and inhibition of apoptosis. These processes contribute to the migration and proliferation of pulmonary arterial smooth muscle cells, endothelial cells, and fibroblasts, leading to vascular remodeling and ultimately the development of pulmonary arterial hypertension. This review examines the role of macrophage-mediated immune responses in high-altitude pulmonary arterial hypertension, with a focus on hypoxia as a key feature.

Keywords: High-altitude pulmonary hypertension, hypoxia-inducible factors, macrophages, pulmonary hypertension, transforming growth factor-beta

INTRODUCTION

High-altitude pulmonary hypertension (HAPH) is a condition affecting adults or children residing at altitudes above 2500 meters. It is caused by a combination of factors including decreased oxygen content due to higher altitudes, increased blood viscosity, enhanced sympathetic nervous activity, and genetic predispositions. These factors contribute to pathological and physiological responses such as proliferation of small pulmonary vessels, pulmonary vasoconstriction, and endothelial cell (EC) damage, leading to elevated PAP. Clinically, this condition is characterized by symptoms such as dyspnea, cough, cyanosis, sleep disturbances, irritability, and right heart failure. The condition is typically defined by a mean PAP (mPAP) ≥ 30 mmHg or a systolic PAP ≥ 50 mmHg measured at the residence location.¹

According to the 2022 European Society of Cardiology and European Respiratory Society guidelines for the diagnosis and treatment of pulmonary hypertension (PH), PH is classified into 5 groups: Group 1—Pulmonary arterial hypertension; Group 2—PH due to left heart disease; Group 3—PH due to lung diseases and/or hypoxia; Group 4—PH due to chronic thromboembolic disease; and Group 5—PH with unclear and/or multifactorial etiology.² This group has revised the hemodynamic criteria for PH, defining it as a mPAP > 20 mm Hg at rest. Pulmonary hypertension associated with lung diseases and/or hypoxia includes PH caused by chronic lung diseases such as chronic obstructive pulmonary disease (COPD), interstitial lung disease, and bronchiectasis. It also encompasses PH resulting from chronic

REVIEW

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or intermittent hypoxia induced by high-altitude environments, chronic lung diseases, or sleep-disordered breathing.

Both chronic lung diseases and high-altitude hypoxic environments contribute to the development of PH through mechanisms involving hypoxia, inflammation, and pulmonary vascular remodeling, highlighting significant similarities and crosstalk between the 2 conditions. At the 7th World Symposium on HAPH, PH associated with lung diseases and/or hypoxia was a key topic of discussion.³ Chronic lung diseases, characterized by pathological changes such as alveolar destruction, impaired airway function, pulmonary fibrosis, and extracellular matrix (ECM) remodeling, lead to persistent ventilation/perfusion mismatches and hypoxemia.⁴ Prolonged hypoxia activates signaling pathways such as the hypoxia-inducible factors (HIFs) pathway, disrupting inflammatory response balance, promoting immune cell infiltration (e.g., macrophages), increasing cytokine secretion, and driving abnormal proliferation of pulmonary vascular smooth muscle cells, ECs, and fibroblasts, which results in vascular wall thickening, fibrosis, and pulmonary vascular remodeling.⁵ High-altitude hypoxia further exacerbates these processes and is a critical factor in the initiation and progression of PH, particularly in patients with chronic lung diseases. These patients already experience varying degrees of hypoxemia due to impaired lung function and ventilation/perfusion mismatch; high-altitude hypoxia further reduces arterial oxygen partial pressure, exacerbates hypoxic pulmonary vasoconstriction (HPV), and increases pulmonary vascular resistance (PVR).^{6,7} Additionally, high-altitude hypoxia enhances inflammatory responses and oxidative stress, activating pathological functions of immune cells like macrophages and driving pulmonary vascular fibrosis.⁸ The interplay between high-altitude hypoxia and chronic lung diseases significantly increases the risk and severity of group 3 PH, with patients exhibiting poorer tolerance to hypoxia in high-altitude environments, faster disease progression, and more severe symptoms. Understanding the characteristics

of chronic lung diseases and high-altitude environments is crucial to mitigating the cumulative damage to the pulmonary vascular system caused by these overlapping factors.

In addition to PH caused by lung diseases, group 3 PH also includes hypoxia-associated PH. Among these, HAPH is a significant subtype of hypoxia-associated PH, with its pathogenesis primarily linked to prolonged exposure to hypoxic conditions in high-altitude environments. Hypoxia is one of the most distinctive and extensively studied factors contributing to HAPH. Hypoxia-induced cellular responses regulate various cellular changes, including signal transduction, transcription, translation, post-translational modifications, and alterations in metabolite patterns.⁹ As altitude increases, atmospheric pressure decreases, leading to a reduction in the partial pressure of oxygen in the atmosphere. According to the oxygen-hemoglobin dissociation curve, the oxygen content in arterial blood significantly decreases at altitudes above 2500 meters (Figure 1).¹⁰ The pulmonary and systemic circulatory systems respond differently to hypoxia; the pulmonary vascular system exhibits vasoconstriction, while the systemic circulation shows vasodilation.¹¹ The marked reduction in oxygen content increases the body's demand

HIGHLIGHTS

- **Key Role of Macrophages in High-Altitude Pulmonary Hypertension (HAPH):** Research reveals that macrophages play a critical role in the development of HAPH through hypoxia-induced inflammatory responses and vascular remodeling.
- **Hypoxia-Induced Hypoxia-Inducible Factors (HIFs) Signaling Pathway Regulation:** In hypoxic environments, the stabilization of HIFs (HIF-1 α and HIF-2 α) promotes the proliferation of pulmonary artery smooth muscle and endothelial cells, further driving vascular remodeling.
- **Macrophage Polarization and Pulmonary Vascular Remodeling:** The dynamic polarization of M1 and M2 macrophages contributes to the pro-inflammatory and anti-inflammatory phases of HAPH, offering potential intervention targets for disease progression and treatment.

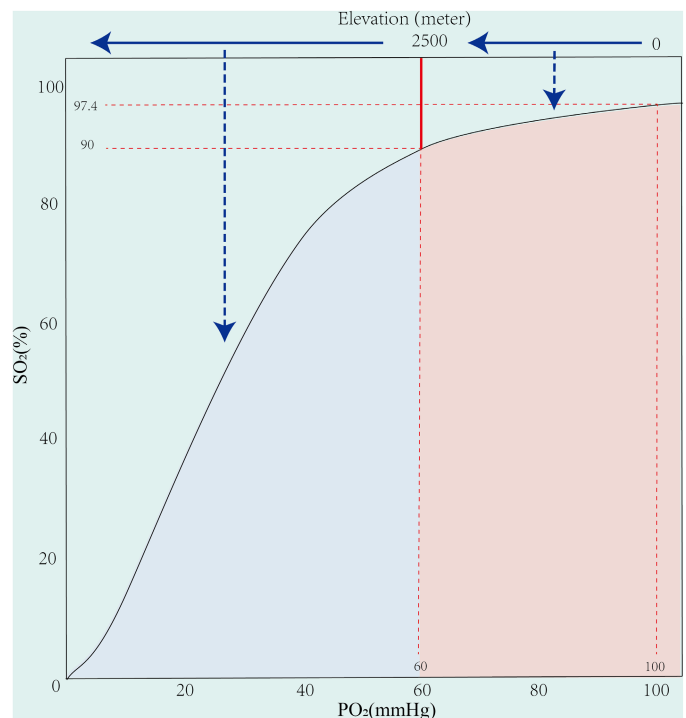


Figure 1. The decrease in PO_2 is accompanied by a decrease in SaO_2 . When PO_2 decreases from 100 mm Hg to 60 mm Hg, based on the characteristic oxygen dissociation curve of hemoglobin (Hb), the decline is relatively gradual, maintaining SaO_2 above 90%, ensuring sufficient oxygen content within the body. When PO_2 falls below 60 mm Hg, the oxygen dissociation curve becomes steeper, with SaO_2 dropping below 90%, indicating a state of hypoxia in the body. At an altitude of approximately 8000 feet, where the air is thin due to high altitude, the relative decrease in oxygen leads to a decrease in SaO_2 to below 90%.

for oxygen. Under hypoxic conditions, pulmonary tissue responds with HPV, constricting pulmonary arterial vessels and redistributing blood flow to better-ventilated areas of the lung, thereby improving the ventilation/perfusion ratio and gas exchange. Prolonged and persistent hypoxia maintains HPV, activating various molecular pathways in the local tissue microenvironment. This leads to abnormal apoptosis and proliferation of pulmonary vascular smooth muscle cells, vascular wall fibrosis, and abnormal apoptosis and proliferation of pulmonary arterial ECs (PA-ECs), resulting in pulmonary vascular remodeling and the onset of HAPH.^{12,13} Therefore, exploring the mechanisms and related signaling pathways of HAPH, with hypoxia as a primary etiological factor combined with other pathogenic elements, is crucial. This research aims to provide a theoretical basis for preventive and therapeutic measures for individuals who are new to or living at high altitudes.

HYPOXIA-INDUCIBLE FACTORS AND HIGH-ALTITUDE PULMONARY HYPERTENSION

One of the primary characteristics of HAPH is exposure to low-pressure, low-oxygen environments at high altitudes, leading to insufficient oxygen supply to cells and tissues. Both acute and chronic or intermittent hypoxia can trigger a series of cellular responses and biological processes at the molecular, cellular, and tissue levels. The pathological and physiological changes caused by hypoxia can lead to pulmonary vascular remodeling and contribute to the development of PH, with these processes beginning within the first few hours of hypoxic exposure.¹⁴

Hypoxia-inducible factors play a crucial role in regulating and adapting to hypoxic environments. Cellular responses to hypoxia involve 3 transcription factors known as HIF-1 α , HIF-2 α , and HIF-3 α . These transcription factors dimerize with constitutively expressed β -subunits to form HIF-1, HIF-2, and HIF-3. Among the 3 HIF subtypes present in mammals, HIF-1 and HIF-2 are well-studied, while research on HIF-3 is limited and its role remains unclear.¹⁵ Typically, cells respond to hypoxia by stabilizing HIFs.¹⁶

Hypoxia-inducible factor activity is mainly regulated by α -subunit expression. Under normoxic conditions, HIF-1 α and HIF-2 α are hydroxylated by prolyl hydroxylase domain (PHD) proteins and subsequently ubiquitinated by von Hippel-Lindau (VHL) protein, leading to rapid degradation. During hypoxia, PHD activity decreases significantly, resulting in the stabilization and accumulation of HIF-1 α /2 α . These stabilized HIFs then dimerize with HIF- β and translocate to the nucleus to exert physiological effects.¹⁷ Activated HIFs regulate the transcription of multiple genes and modulate cellular responses to hypoxia by inducing or inhibiting a wide range of genes involved in regulating vascular tone, cell metabolism, proliferation, survival, and autophagy.¹⁸

The sustained low-pressure and hypoxic environment at high altitudes are key factor in the development of HAPH. Exposure to high-altitude conditions results in a reduction in oxygen partial pressure, which activates the HIF signaling pathway, triggering EC injury and smooth muscle cell

proliferation in the pulmonary vasculature, ultimately leading to vascular wall thickening and remodeling. Studies using rats with HAPH induced by a simulated altitude of 5000 meters show that average mPAP, right ventricular systolic pressure (RVSP), PVR, and right ventricular hypertrophy gradually alleviate as the altitude decreases.¹⁹ The aforementioned studies indicate that PH at high altitudes is reversible, and early intervention may effectively improve patients' symptoms. Research conducted on earthquake relief in Yushu, China, indicates that residents from low-altitude areas who rapidly ascended from 1500 meters to 3700 meters showed a positive correlation between average PAP, serum HIF-1 α , and vascular endothelial growth factor (VEGF). However, these parameters normalized within 15 days of returning to lower altitudes.²⁰ Daily intermittent, non-continuous, short-term reoxygenation partially prevented HAPH induced by 5000 meters hypoxia in rats.²¹ This further confirms the critical role of HIFs in the development of HAPH and highlights the importance of correcting HIF-related signaling pathways, such as through oxygen therapy, as a significant approach to treating HAPH.

In PH, pulmonary arterial remodeling occurs in the 3-layered structure of the vessel wall, including the intima, media, and adventitia. This remodeling primarily involves pathological changes in PA-ECs, pulmonary arterial smooth muscle cells (PA-SMCs), pulmonary arterial fibroblasts (PA-Fibs), and the ECM.²² Existing research confirms that HIF regulation of PH is complex and multifaceted, with abnormalities in HIF signaling present in PA-ECs, PA-SMCs, and PA-Fibs (Figure 2).

THE ROLE OF HYPOXIA-INDUCIBLE FACTOR IN PULMONARY ARTERIAL ENDOTHELIAL CELLS

Endothelial cells (ECs) play a critical role in maintaining normal cardiovascular function. Endothelial cells exhibit different phenotypes, including apoptotic and proliferative forms, which contribute differently to the development of PH. In the early stages of PH, the presence of pathogenic factors damages and induces apoptosis in the cells within the pulmonary vascular wall. Endothelial cells that survive apoptosis undergo epigenetic reprogramming, entering an abnormal proliferation phase. Proliferative ECs gradually emerge with the progression of PH and become the predominant phenotype in later stages.²³

For instance, Yamaji-Kegan et al²⁴ induced PH in mice by injecting hypoxia-induced mitotic factor (HIMF, also known as FIZZ1 or RELM α). On the seventh day, they observed a significant increase in EC apoptosis in the pulmonary vasculature of the mice.²⁴ Early EC apoptosis can directly lead to the loss of integrity in small distal pulmonary arteries, indirectly contributing to the formation of complex and obstructive arterial lesions.²⁵ Endothelial dysfunction results in a deficiency of endothelial-derived vasodilators, such as prostacyclin and nitric oxide (NO), and an increase in endothelial-derived vasoconstrictors, such as endothelin, which promotes the development of PH.^{25,26} As the disease progresses, abnormal EC proliferation in PH becomes predominant, driving increased vascular resistance and exacerbating the condition through the promotion of intimal proliferation,

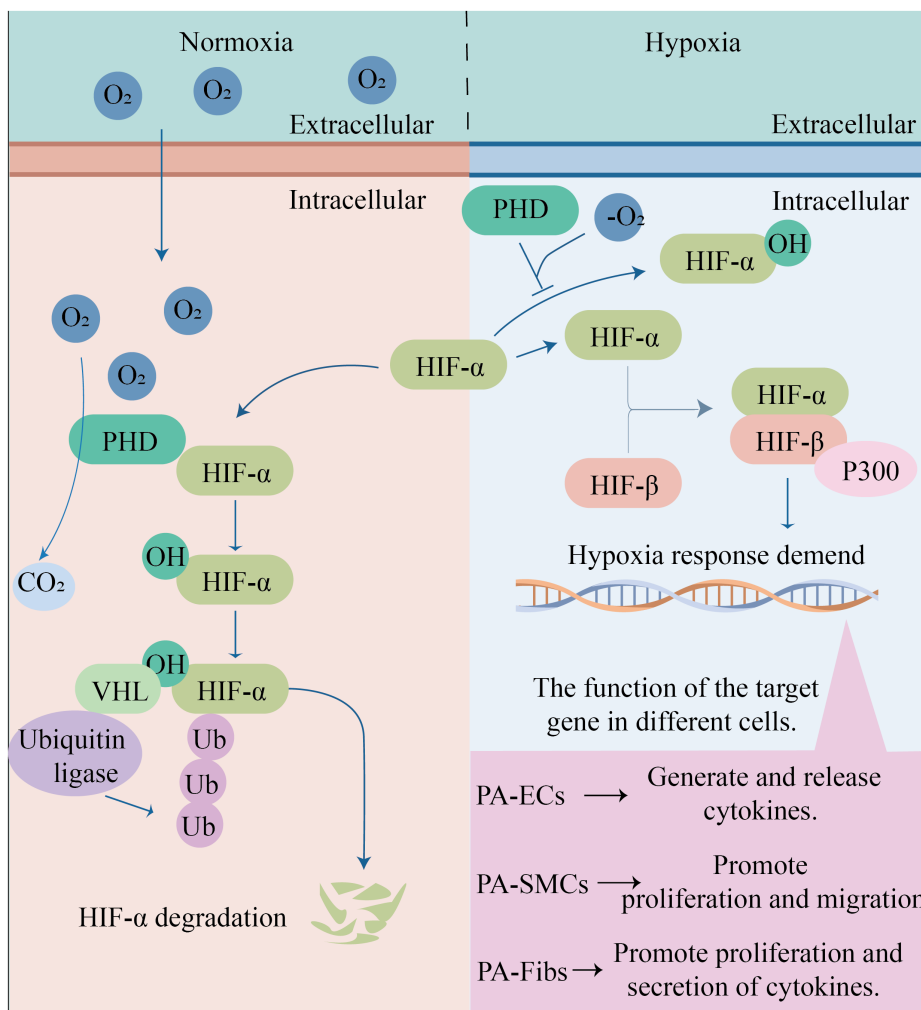


Figure 2. Under normoxic conditions, the HIF- α subunit within the cell undergoes hydroxylation by PHD, followed by further ubiquitination by VHL, leading to degradation. In hypoxia, decreased PHD activity inhibits hydroxylation of the HIF- α subunit, resulting in its intracellular accumulation. Upon binding with the HIF- β subunit, stable HIF complexes are formed, which modulate gene expression to exert biological effects. In hypoxic lung tissue, stable HIF regulates the production and release of cytokines by PA-ECs; HIF in PA-SMCs and PA-Fibs regulates cell proliferation and migration, among other biological effects.

obstructive arterial remodeling, and the formation of tuft-like lesions.²⁵

Under hypoxic conditions, HIF plays a crucial role in the development of PH by promoting the production and release of cytokines from PA-ECs. In PH mouse models, knocking down the gene encoding PHD2 (*Egln1*) leads to decreased PHD2 expression in PA-ECs at obstructive vascular lesions, thereby increasing the stability of HIF-2 α and promoting PH formation, accompanied by significant proliferation of ECs in pulmonary vascular tissues.²⁷ Further research has confirmed that genetic deletion of HIF-2 α in PA-ECs and pharmacological inhibitors of HIF-2 α can suppress the formation of PH.²⁸⁻³⁰

THE ROLE OF HYPOXIA-INDUCIBLE FACTORS IN PULMONARY ARTERIAL SMOOTH MUSCLE CELLS

In PH, vascular remodeling is primarily driven by interactions among molecules and cells in the intima, media, and adventitia of the pulmonary arterial wall, as well as the surrounding vascular space. Pulmonary arterial smooth muscle

cells, which are a major component of the media, contribute to pulmonary vascular remodeling through proliferation, hypertrophy, and the secretion of chemokines and cytokines, as well as through the promotion of ECM production and degradation.³¹

During the progression of PH, various factors can induce senescence (not apoptosis) in PA-SMCs. Senescent PA-SMCs can promote their own proliferation by increasing the expression of paracrine cytokines such as interleukin (IL)-6.³² Enhancing PA-SMC apoptosis through pharmacological intervention in rats has been shown to mitigate induced PH.³³

Hypoxia-inducible factor subunits are widely expressed in various tissues and cells, including PA-SMCs, which are among the cells with high expression of HIF subunits. In the PA-SMCs of PH patients, the stability and activity of HIF subunits are increased, promoting pulmonary artery narrowing and thickening of the pulmonary arterial wall through

a series of signaling pathways. Correcting HIF signaling can improve the pathological progression of PH caused by PA-SMC proliferation. Studies have shown that primary cultures of rat PA-SMCs exposed to 4% O₂ for 60 hours exhibit increased expression of the hypoxia-sensitive HIF-1 α subunit, HIF target genes, and HIF-1 α mRNA, while the mRNA and protein expression of PHD2, which is responsible for degrading HIF-2 α , is reduced.³⁴ The increased expression of HIF-1 α under hypoxic conditions significantly enhances PA-SMC proliferation, while downregulation of HIF-related subunits can inhibit PA-SMC proliferation and migration.^{35,36} Research by Deng Jun³⁶ and colleagues has demonstrated that intervention with the drug Rutaecarpine, which reduces the protein and mRNA expression of HIF-1 α , promotes PA-SMC apoptosis and reverses the formation of PH. Therefore, modulation and inhibition of HIF-related signaling pathways may hold potential therapeutic value in the treatment of PH.

THE ROLE OF HYPOXIA-INDUCIBLE FACTORS IN PULMONARY FIBROBLASTS

In HAPH and COPD, PA-Fibs are activated, exhibiting excessive proliferation, increased migration, and enhanced inflammatory activity.^{37,38} Pulmonary arterial fibroblasts, as one of the primary cells responsible for secreting ECM, are found in conjunction with ECM in the pulmonary adventitia. Research has demonstrated that in both animal models and humans with PH, PH fibroblasts (PH-Fibs) are among the cell types with the highest and most significantly increased levels of cytokines, chemokines, and growth factors.³⁹

Under hypoxic conditions, the expression of HIF and its related subunits is elevated in PH, and these factors contribute to the activation of PH-Fibs, thereby facilitating the development of PH. In PH-Fibs, the expression of HIF target genes, such as *CA9*, *GLUT1*, and *NDRG1*, is significantly increased.³⁹ Primary fibroblasts exposed to 1% O₂ become activated, with elevated levels of HIF-1 α , α -smooth muscle actin (α -SMA) protein, and mRNA expression, which are blocked upon silencing HIF-1 α .⁴⁰ Under hypoxic conditions, the knockout of HIF-1 α can increase the expression of downstream miR-29a-3p in PH-Fibs through the HIF-1 α /SMAD3-related pathway, leading to reduced proliferation of PH-Fibs.³⁷

THE ROLE OF IMMUNE RESPONSES IN PULMONARY ARTERIAL HYPERTENSION

The immune system plays a crucial role in maintaining tissue homeostasis and responding to infections and injuries. Imbalances between immune response defects or enhancements can lead to disease development. Under normal physiological conditions, immune cells adapt their functional characteristics according to changes in their environment, ranging from adequate oxygen supply in the circulatory system to severely hypoxic pathological sites.⁹ However, under pathological conditions such as inflammation, injury, infection, ischemia, and cancer, severe hypoxia alters immune cells, leading to dysregulation of immune responses and ultimately resulting in tissue damage, cancer progression, and autoimmune diseases.⁹

It is well-established that during immune responses to damage or pathogen infiltration, early recruitment of immune cells is mediated by chemokines and inflammatory factors produced by the local microenvironment. These cells perform functions such as phagocytosis and pathogen clearance, releasing pro-inflammatory cytokines like interferon- γ (IFN- γ), IL-1 β , and IL-6 during the initial stages of inflammation. In later stages of tissue repair, immune cells primarily secrete anti-inflammatory cytokines such as arginase 1 (Arg1), transforming growth factor- β (TGF- β), and IL-10, which stimulate cell proliferation, matrix synthesis, angiogenesis, and immune regulation, thereby promoting tissue repair and reconstruction.

Immune dysfunction plays a key role in the development of various diseases, including cancer, asthma, and autoimmune disorders. Similarly, in PH, the imbalance between pro-inflammatory and anti-inflammatory immune responses has been increasingly documented. During the early stages of PH, PA-Fibs exhibit a pro-inflammatory phenotype, with increased expression of inflammatory mediators driving the recruitment of innate immune cells, and elevated levels of inflammatory mediators in the pulmonary circulation.⁴¹ The accumulation of various immune cells such as macrophages, neutrophils, dendritic cells, mast cells, T lymphocytes, and B lymphocytes around the pulmonary vasculature in PH patients has been observed.⁴¹ Additionally, inflammatory infiltration around the pulmonary vessels typically occurs prior to pulmonary vascular remodeling, indicating that maladaptive immune responses play a critical role in this remodeling process.⁴²

Beyond the role of pro-inflammatory immune responses in PH, anti-inflammatory immune responses also significantly impact the progression of PH. Anti-inflammatory immune cells, such as M2 macrophages and regulatory T cells, contribute to PH development by secreting anti-inflammatory cytokines, suppressing pro-inflammatory immune responses, promoting cell proliferation, inhibiting apoptosis, and facilitating ECM secretion and fibrosis, all of which contribute to pulmonary vascular remodeling and PH formation.⁴³⁻⁴⁵

INTERACTION BETWEEN HYPOXIA AND IMMUNE RESPONSE

The objective presence of hypoxia adds complexity to the pathophysiology of HAPH. Hypoxia-related signaling, particularly involving HIFs, plays a critical role in intercellular communication. Beyond direct interactions between hypoxia and PA-ECs, PA-SMCs, and PH-Fibs that facilitate pulmonary vascular remodeling, existing research confirms that crosstalk between perivascular immune cells and hypoxia is also crucial in the development of PH.

Hypoxia-induced tissue damage and the inflammatory environment resulting from changes in microenvironmental metabolism can recruit immune cells to infiltrate affected areas. Immune cells migrate from well-oxygenated vascular systems to hypoxic, inflamed regions, where there is a sharp increase in the energy demands for metabolism and involvement in pathological processes, such as inflammatory

cytokines, enzymes, and inflammatory mediators.⁴⁶ Concurrently, under hypoxic conditions, inhibition of mitochondrial oxidative phosphorylation and the electron transport chain further increase reactive oxygen species (ROS) production in macrophages, thereby activating and stabilizing HIF-1 α .⁴⁷ Hypoxia-inducible factors serves as a key regulatory factor in adapting to these conditions, influencing the migration, antigen presentation, cytokine and antimicrobial peptide production, phagocytosis, and metabolic reprogramming of various adaptive and innate immune cells.⁴⁸

In diseases characterized by choroidal neovascularization, such as age-related macular degeneration, neutrophils infiltrating around blood vessels can activate Toll-like receptor 4 to promote HIF-1 α expression. The HIF-1 α , in turn, regulates the expression of matrix metalloproteinase 9 (MMP9) and IL-1 β , further driving inflammation and vascular formation.⁴⁹

In PH, HIF stabilization promotes the formation of PH by enhancing the migration and differentiation of immune cells. In hypoxic PH, stabilization of HIF-1 α can activate downstream adenosine pathways.⁵⁰ Adenosine, a signaling nucleoside produced under cell damage and stress conditions, activates signaling pathways by binding to specific adenosine receptors. Notably, the adenosine axis can promote M2 macrophage polarization. The M2 macrophages possess significant anti-inflammatory and repair capabilities. In PH, the accumulation of M2 macrophages around the pulmonary arteries plays a crucial role in promoting pulmonary vascular remodeling. In HAPH, the stabilizing effects of hypoxia-induced HIF on tissue cells through cytokine secretion and immune cell recruitment contribute to vascular remodeling.⁵¹ Furthermore, HIF enhances immune cell survival by providing necessary energy through inhibiting apoptosis pathways and modulating glycolysis pathways. Early studies indicated that HIF-1 α could enhance the survival of hypoxia-induced centriolar cells through NF- κ B signaling.⁵² Research by Sormendi et al⁵³ found that constitutive loss of PHD-2, leading to activation of HIF-2 α , increased neutrophil migration in highly confined environments. Other studies have confirmed that centriolar cells, through myeloperoxidase-catalyzed ROS production and secretion of proteolytic enzymes, contribute to adverse remodeling of pulmonary arteries and surrounding tissues through abnormal protein degradation.^{54,55} Arginase 1, a cytokine secreted by M2 macrophages, decreases in expression upon specific knockdown of HIF-2 α in PA-ECs, mitigating PH formation. This further confirms that HIF genes and their subunits regulate immune responses and contribute to the pathological progression of PH.²⁹

THE ROLE OF MACROPHAGES IN PULMONARY ARTERIAL HYPERTENSION

Macrophages are innate immune cells present in nearly all tissues and organs. In the immune response, macrophages generally originate from 2 sources. One type, the tissue-resident macrophages, derives from embryonic progenitor cells that develop into self-maintaining populations through local proliferation during embryogenesis.⁵⁶ These macrophages

participate in inflammation and immune responses primarily by phagocytosing pathogens, necrotic cells, cellular debris, presenting antigens, and releasing cytokines. Additionally, macrophages contribute to tissue regeneration and repair through the release of growth factors, MMPs, and anti-inflammatory cytokines.⁵⁷ These precursor cells enter specific tissues, mature, and localize there to form tissue-resident macrophages, such as Kupffer cells in the liver, microglia in the central nervous system, interstitial macrophages, and alveolar macrophages in the lungs.^{58,59} In adulthood, tissue-resident macrophages maintain and renew their populations through self-proliferation and the recruitment of monocyte precursors from the bloodstream to address various physiological and pathological states. Beyond tissue-resident macrophages, when the body is subjected to infection, damage, or inflammatory stimuli, immune cells, damaged cells, and surrounding tissues release cytokines, chemokines, and transcription factors, leading to the recruitment of monocytes from the blood to the inflammatory sites, where they further differentiate into macrophages and participate in the immune response.⁵⁹

In lung tissue, there are 2 types of macrophages: alveolar macrophages and interstitial macrophages. Alveolar macrophages, originating from the fetal liver, colonize the lungs during embryonic development and maintain their population through self-renewal. They clear microorganisms and other foreign substances entering the alveoli and serve as the first line of immune defense in the lungs, playing a crucial role in pathogen phagocytosis and antigen presentation.⁶⁰ On the other hand, interstitial macrophages, which exist between blood monocytes and alveolar macrophage phenotypes, have origins from both embryonic yolk sac residency and blood monocyte recruitment. They play a vital role in regulating the local microenvironment, the secretion of cytokines, and immune responses to maintain lung stability and immune balance.^{61,62} In both human and animal models of PH, inflammation in the lung and surrounding pulmonary vasculature is considered a hallmark of PH development.⁶³ Macrophage infiltration into the perivascular tissues of the lungs has been confirmed by numerous studies. Research on macrophage-related immune responses in high-altitude areas and their association with PH is limited, with most studies providing theoretical foundations for HAPH through hypoxia and simulated high-altitude animal models. Hypoxia-induced HIF-1 α knockout PH mice (MycHIF1KO) exhibit reduced RVSP, a decreased ratio of right ventricular to left ventricular plus interventricular septum, and reduced macrophage infiltration, validating that HIF-1 α in macrophages contributes to the progression of pulmonary vascular remodeling and PH induced by chronic hypoxia.⁶⁴ Moreover, some researchers believe that during the pathological process of PH, interstitial macrophages mainly transition to an anti-inflammatory phenotype, while alveolar macrophages retain a pro-inflammatory phenotype.⁶⁵ Studies by Valérie Amsellem and colleagues found significant increases in CX3CR1, CCR2, and their corresponding ligands CX3CL1 and CCL2 in the lung tissue of hypoxia-induced PH mice, accompanied by a rapid increase in bone marrow monocytes at

different time points of hypoxia exposure, further indicating that both tissue-resident macrophages and recruited blood monocytes are involved in PH development.⁶⁶

Macrophages respond to infections, pathological damage, and other stimuli through a polarization process that results in specific functional phenotypes and immune functions. Based on their functions and activation states, macrophages are primarily classified into two subtypes: classical M1 macrophages and alternatively activated M2 macrophages.⁶⁷ The M1 macrophages are primarily pro-inflammatory. When the body is in states of infection, damage, or autoimmune diseases, pathogens and inflammatory cytokines in the local microenvironment, such as bacterial lipopolysaccharides, tumor necrosis factor- α (TNF- α), and granulocyte-macrophage colony-stimulating factor (GM-CSF), activate macrophages to polarize into the M1 phenotype. The M1 macrophages produce inflammatory cytokines like IL-1 β and TNF- α , inhibiting surrounding cell proliferation, damaging adjacent tissues, and eliminating pathogens and abnormal immune responses.^{67,68} The M2 macrophages, on the other hand, have anti-inflammatory roles and can be further subdivided into M2a, M2b, M2c, and M2d subtypes based on their responses to various cytokines. Research has extensively studied the M2a, M2b, and M2c subtypes.⁶⁹ The M2a macrophages polarize in response to IL-4 and IL-13 and play significant roles in tissue repair, remodeling, and healing through the secretion of cytokines and chemokines such as Arg-1, IL-10, TGF- β , and CCL5.^{70,71} The M2b macrophages are stimulated by TLRs and immune complexes and are important in anti-inflammatory responses and tumor progression.⁷¹ M2c

macrophages, polarized in response to IL-10 and TGF- β , secrete cytokines like IL-10, TGF- β , and VEGF, contributing to tissue remodeling, repair, inflammation resolution, and immune regulation(Figure 3).^{70,71}

Macrophages ensure appropriate inflammatory and repair responses during the immune response process through polarization. They play a crucial role in controlling infections, limiting lesion spread, and reconstructing damaged tissues. Although research on macrophage polarization in HAPH is limited, studies on other types of PH and hypoxic PH, which share many similarities with HAPH, indicate that in PH animal models and patients with PH, the predominant macrophage polarization phenotype in the perivascular regions is M2. Intermittent hypoxia-induced mice show an increase in M2 macrophages.⁷² Additionally, transgenic male mice depleted of CD68 (M0 macrophages) exhibit reduced expression of iNOS (M1 macrophages) and increased expression of CD206+ (M2 macrophages) during hypoxia-induced PH formation.⁷³ These studies provide a theoretical basis for understanding macrophage polarization and the mechanisms of inflammatory factor secretion in HAPH. Interestingly, in an experiment where PH was induced in rats by colchicine, macrophage polarization exhibited dynamic changes over time. Initially, M1 macrophage polarization predominates, while later, M2 macrophage polarization becomes more prevalent. It is hypothesized that M1 macrophages participate in the initial inflammatory phase by accelerating EC apoptosis, while M2 macrophages dominate during the inflammation repair phase and subsequent abnormal tissue remodeling by promoting the proliferation of smooth muscle cells and ECs.⁷⁴

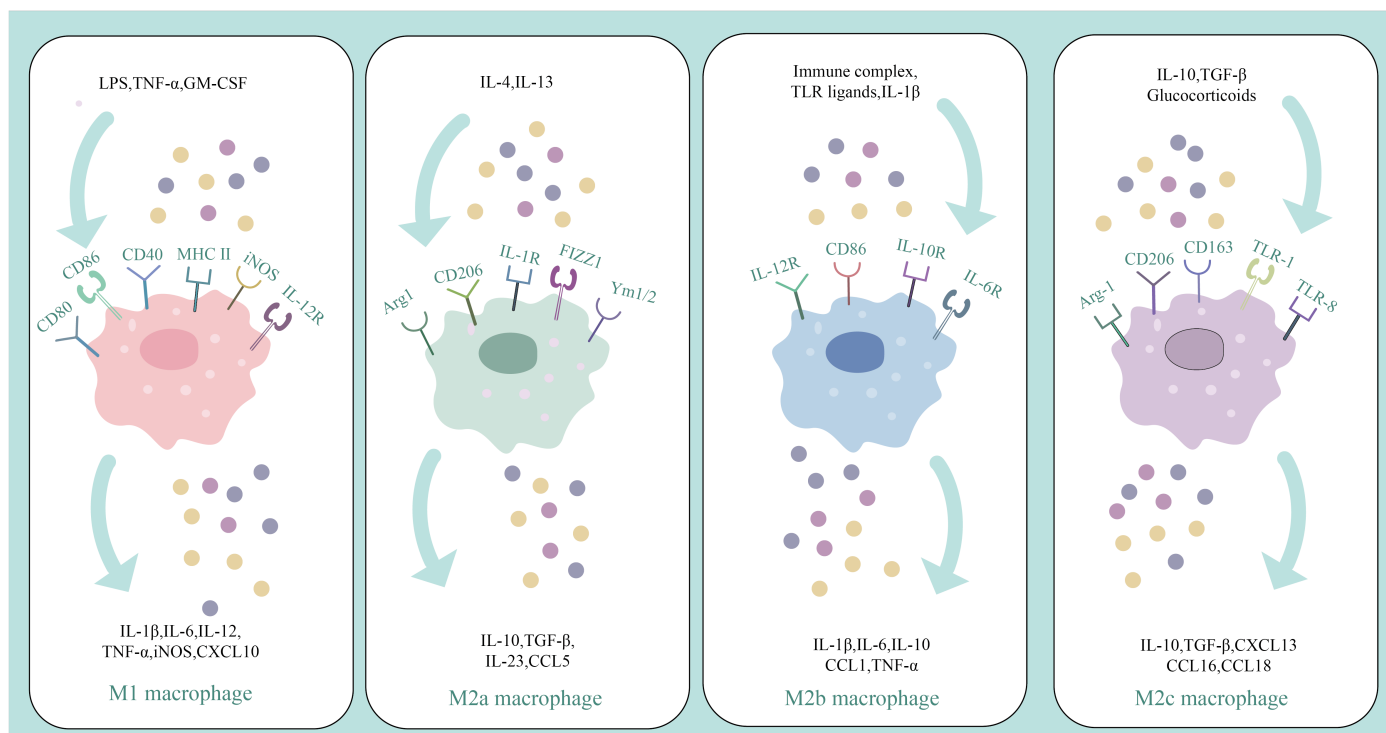


Figure 3. Macrophage subtypes polarize differently in response to stimuli from diverse local microenvironments, each exhibiting unique surface molecular markers and playing pivotal roles in both pro-inflammatory and anti-inflammatory responses by secreting distinct cytokines.

THE ROLE OF MACROPHAGE-RELATED CYTOKINES IN PULMONARY ARTERIAL HYPERTENSION

The M2 macrophages exert their biological functions through anti-inflammatory responses, foreign body clearance, and tissue repair and regeneration. These functions are primarily driven by signaling molecules such as chemokines, cytokines, and growth factors. Therefore, exploring the molecular mechanisms of M2 macrophage recruitment and polarization, as well as their biological functions, provides a theoretical basis for treating PH. Previous research in fields such as breast cancer,⁷⁵ ankylosing spondylitis,⁷⁶ pulmonary fibrosis,⁷⁷ and tissue repair⁷⁸ has shown that IL-4 and IL-13 promote macrophage polarization towards the M2 phenotype mainly through the phosphorylation of downstream STAT6, which is a primary pathway for macrophage alternative activation. However, studies investigating IL-4 and STAT6 in macrophage polarization in PH are limited, and results vary depending on the type of PH. In vitro studies have demonstrated that IL-4 stimulation of human PA-ECs enhances the expression of CXCL-8 mRNA and protein, promoting neutrophil recruitment to the lung tissue, indicating that IL-4 aids in immune cell recruitment.⁷⁹ Murine models of PH induced by *Schistosoma mansoni* and asthma, which exhibit elevated IL-4 and IL-13 levels, suggest a potential association with Type II hypersensitivity.^{80,81} Conversely, IL-4 expression in PH patients shows no significant change, and some studies have reported decreased IL-4 levels in idiopathic pulmonary arterial hypertension.^{82,83} These discrepancies may relate to differences in the etiologies and triggers of PH as well as variations between in vivo and in vitro experiments. Further exploration of macrophage subtypes and their functions could provide a more comprehensive explanation of whether M2 macrophages exhibit a bias in PH.

Chemokines are small molecular proteins with a conserved secondary structure, consisting of a flexible N-terminus, three anti-parallel β -folds, and a C-terminal α -helix.⁸⁴ They play significant roles in regulating macrophage migration and polarization. Chemokines are categorized into 4 families based on their N-terminal cysteine residues: CXC (α -chemokines), CC (β -chemokines), XC (γ -chemokines), and CX3C (δ -chemokines).⁸⁵ Dysregulation of chemokines and their receptors in HAPH has been increasingly studied, showing that M2 macrophage polarization is predominantly observed. Acute exposure to 3400 meters altitude leads to elevated levels of macrophage inflammatory protein-1 α (MIP-1 α), monocyte chemoattractant protein-1 (MCP-1), and IL-8 in the blood, with MIP-1 α and MCP-1 further recruiting monocytes.⁸⁶ In hypoxia-induced PH mouse models, the CCL2-CCR2, CCL5-CCR5, and CX3CL1/CX3CR1 pathways stimulate M2 macrophage increase and PA-SMCs proliferation, promoting PH formation; inhibition of these pathways can prevent or reverse PH.⁸⁷ The CX3CR1 knockout mice and those treated with the CX3CR1 inhibitor F1 show reduced M2 macrophages, lower RVSP, reduced right ventricle-to-left ventricle plus septum (RV/LV+S) ratios, and alleviated pulmonary vascular remodeling, validating CX3CR1's role in macrophage polarization and recruitment.⁸⁶

Transforming growth factor- β , produced by tissue and immune cells, plays a critical role in tissue repair and healing. There is a close relationship between M2 macrophages and TGF- β in pulmonary tissue. The M2 macrophages secrete large amounts of TGF- β , contributing to pathological responses, while TGF- β drives macrophages towards the M2 phenotype.^{88,89} The TGF- β regulates various cellular processes including proliferation, phenotype remodeling, migration, metabolism, and immune responses, impacting embryonic development, tissue homeostasis, and damage repair.⁹⁰ Additionally, TGF- β serves as an effective signal for fibroblast, connective tissue, and epithelial cell production and remodeling of ECM.⁹⁰ The TGF- β superfamily, including TGF- β 1, TGF- β 2, and TGF- β 3, influences immune response, cell proliferation, differentiation, and ECM synthesis. Transforming growth factor- β , in its dimeric form, binds to type I and type II receptors, resulting in phosphorylation of the type I receptor by the type II receptor and activation of intracellular kinase domains. This process transmits signals via classical SMAD pathways and non-classical SMAD pathways.⁹¹ Enhanced expression of TGF- β 1 in lung tissue is associated with the severity of PH.⁹² Classical SMAD pathway studies indicate overactive SMAD2/3 signaling in PA-ECs and PA-SMCs, and decreased SMAD1/5/8 signaling, contributing to pulmonary vascular remodeling.^{91,93} The TGF- β promotes PA-SMC proliferation and differentiation, inhibits NO synthesis and release by PA-ECs, induces endothelial-mesenchymal transition (EndMT), and stimulates PA-Fib proliferation and ECM secretion. In vitro stimulation of rat PA-SMCs with TGF- β increases SMAD2/3 expression and PA-SMC proliferation.⁹⁴ Transforming growth factor- β exposure leads to mitochondrial dysfunction in PA-ECs, decreased ATP levels, and reduced interaction between hsp90/eNOS, resulting in diminished NO release.⁹⁵ The TGF- β 1-treated human umbilical vein ECs (HUVECs) show a transition from polygonal cobblestone shapes to more spindle-shaped, fibroblast-like morphology, with increased α -SMA expression, indicating EndMT.⁹⁶ The EndMT is a significant contributor to endothelial dysfunction, a hallmark of PH, and adversely affects vascular homeostasis.⁹⁷ In hypoxia-induced PH mice, TGF- β levels are significantly elevated, with increased α -SMA levels in PA-Fibs, confirming TGF- β 's role in PA-Fib activation.⁹⁸ Under hypoxic conditions, cultured PA-Fibs exhibit increased proliferation, migration, and metabolism, with elevated cytokine and collagen levels. Inhibition of TGF- β with SB-431542 suppresses PA-Fib activation, reducing cytokine and collagen production.⁹⁹ TGF- β also promotes PH by acting on immune cells. In macrophages, TGF- β activates the transcription factor SNAIL through SMAD2/3 and PI3K/Akt pathways, characterized by reduced pro-inflammatory cytokines and increased anti-inflammatory cytokine IL-10, inducing M2 polarization.¹⁰⁰

Cytokines are secreted proteins that regulate cell proliferation, death, activation, or inhibition through autocrine, paracrine, and endocrine signaling.¹⁰¹ They play crucial roles in intercellular communication and cell function regulation. Numerous studies have confirmed that cytokines drive M2 macrophage polarization, enhancing the anti-inflammatory

response and contributing to pulmonary arterial remodeling and PH progression. In hypoxia-induced PH mice, the IL-6/IL-21 signaling axis promotes PH through M2 macrophage polarization. Blocking IL-6 with the monoclonal antibody MR16-1 significantly reduces downstream IL-21 expression, and IL-21 treatment of alveolar macrophages from bronchoalveolar lavage fluid upregulates M2-related gene mRNA levels (such as Fizz1, Arg1, and CXCL12).¹⁰² Additionally, anti-inflammatory cytokines like IL-10 and IL-13 are elevated in PH patients and animal models.^{82,103} The IL-13 overexpression induces pulmonary vascular remodeling and spontaneous PH in wild-type mice, with increased production of anti-inflammatory cytokines Arg1, Arg2, and NOS3.¹⁰³

Arginine metabolism plays a critical role in the development and progression of PH. Arginine can be hydrolyzed by arginase to produce ornithine or oxidized by NOS to produce NO.¹⁰⁴ The NO is a vasodilator that counteracts vascular remodeling in PH by dilating blood vessels and inhibiting cell proliferation. Under pathological hypoxia, excessive arginase activity hydrolyzes arginine, leading to excessive ornithine production and insufficient NO production.^{105,106} In HAPH, chronic hypoxia stabilizes HIF-2 α , increasing downstream Arg1 and Arg2 expression. Arg1 and Arg2 decrease NO levels in the blood, promoting pulmonary vascular remodeling. Knockdown of HIF-2 α in ECs reduces Arg1 expression and mitigates hypoxia-induced PH.²⁹ Studies on rats exposed to 5000 meters altitude show that arginine supplementation significantly decreases PAP and alleviates PH symptoms.¹⁰⁷ High-altitude broiler chickens benefit from arginine supplementation to reduce HAPH incidence.¹⁰⁸ The use of macitentan, which reduces Arg1 and Arg2 expression, significantly relieves symptoms related to HAPH, including mPAP and right ventricular hypertrophy.¹⁰⁴

PROSPECT

In recent years, with the improvement in living standards and advancements in science and technology, there has been a notable increase in the number of people traveling to high-altitude areas for work, exploration, and tourism. The various challenges posed by acute and chronic mountain sickness have become increasingly apparent, significantly impeding these activities. Research into acute and chronic mountain sickness has also become more thorough and comprehensive. With a deeper understanding of the mechanisms underlying HAPH, and by drawing parallels with the pathophysiological mechanisms of other types of PH, we can more precisely develop prevention and treatment strategies for HAPH.

Given the critical role of macrophages in HAPH, designing specific drugs and employing gene technology to regulate abnormal macrophage polarization, mitigate inflammatory responses, and prevent pulmonary vascular remodeling represents significant breakthrough directions. Additionally, beyond the primary factors of high altitude, low pressure, and hypoxia, genetic susceptibility, immune abnormalities, and neurological dysfunctions also play crucial pathogenic roles. Conducting further genetic research and developing personalized treatment strategies through molecular

biology and genomics can enhance our understanding of individual patient differences, offering more possibilities for personalized treatments.

Currently, to address the impact of high-altitude hypoxia on HAPH, advanced oxygen therapy technologies, bloodletting, and climate adjustment methods can be introduced to alleviate or prevent the onset of HAPH in high-altitude regions.

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