Alveolar Hypoxia-Induced Pulmonary Inflammation: From Local Initiation to Secondary Promotion by Activated Systemic Inflammation

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Key Words

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Abstract

Pulmonary hypertension (PH) is a pathological condition with high mortality and morbidity. Hypoxic PH (HPH) is a common form of PH occurring mainly due to lung disease and/or hypoxia. Most causes of HPH are associated with persistent or intermittent alveolar hypoxia, including exposure to high altitude and chronic obstructive respiratory disease. Recent evidence suggests that inflammation is a critical step for HPH initiation and development. A detailed understanding of the initiation and progression of pulmonary inflammation would help in exploring potential clinical treatments for HPH. In this review, the mechanism for alveolar hypoxia-induced local lung inflammation and its progression are discussed as follows: (1) low alveolar PO₂ levels activate resident lung cells, mainly the alveolar macrophages, which initiate pulmonary inflammation; (2) systemic inflammation is induced by alveolar hypoxia through alveolar macrophage activation; (3) monocytes are recruited into the pulmonary circulation by alveolar hypoxia-induced macrophage activation, which then contributes to the progression of pulmo-

nary inflammation during the chronic phase of alveolar hypoxia, and (4) alveolar hypoxia-induced systemic inflammation contributes to the development of HPH. We hypothesize that a combination of alveolar hypoxia-induced local lung inflammation and the initiation of systemic inflammation ("second hit") is essential for HPH progression.

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Introduction

Pulmonary hypertension (PH) is defined as abnormally high blood pressure in the pulmonary arteries, which increases the workload of the right side of the heart and leads to cardiac hypertrophy over time; eventually, heart failure may result. There are 5 categories of PH classified by the World Health Organization (WHO) [1]. Group III consists of hypoxic PH (HPH) due to lung diseases and/or hypoxia, including obstructive airway diseases, pulmonary edema due to heart failure and acute lung injury, or physiological conditions, such as exposure to high altitude. Many of these pathological conditions are associated with persistent or intermittent alveolar hypoxia that occurs when alveolar epithelial cells (AEPs) are exposed to lower oxygen tensions [2, 3].

The basic mechanism for HPH is linked to sustained vascular constriction and remodeling resulting from a series of responses by pulmonary vascular cells to hypoxia. Recent reviews have addressed the potential cellular and molecular mechanisms that contribute to these processes [4–9]. In addition, accumulating evidence has shown that acute and chronic exposure of animals to even moderate alveolar hypoxia results in increased expression of lung inflammatory cytokines, chemokines, and adhesion molecules; it also leads to the accumulation of bloodstream monocytes within the lung interstitium and lung blood vessels [10–23].

Interestingly, with the exception of the pulmonary vascular system, no blood-born cell infiltration has been detected at other tissue vessels during the acute phase of alveolar hypoxia [24, 25]. Moreover, cell migration during pulmonary circulation is not initiated by low systemic PO_2 levels, but by the activation of alveolar macrophages (AMOs) [26–28]. HPH is usually characterized by mild to moderate pulmonary vascular remodeling that is thought to be reversible when oxygen deficiency is attenuated. However, there are severe PH cases in which pulmonary vascular remodeling is irreversible [1, 29]. The remarkable differences between the 2 situations are with respect to cell infiltration and cytokine secretion patterns in the pulmonary artery, especially in the adventitia. This suggests that inflammatory responses during pulmonary circulation are critical for HPH development. However, it is still unclear how alveolar hypoxia initiates and promotes the recruitment and arrest of cells from the bloodstream and into the pulmonary circulation.

The purpose of this review is to summarize published evidence that supports the following: (1) alveolar hypoxia evokes specific pulmonary responses; (2) low alveolar PO_2 levels activate resident lung cells, mainly AMOs, which then initiate pulmonary inflammation; (3) activation of AMOs results in alveolar hypoxia-induced systemic inflammation, also known as a systemic “second hit”, and (4) activation of AMOs also triggers the recruitment of circulatory monocytes to the pulmonary vasculature, which contributes to the progression of pulmonary inflammation during the chronic phase of alveolar hypoxia.

Alveolar Hypoxia Evokes Specific Pulmonary Responses

The lungs display specific responses to hypoxia compared to other systemic organs. First, the normal alveolar PO_2 level (110 mm Hg) is significantly higher compared

to that of other internal organs, in which oxygen tension ranges from 3 to 20 mm Hg for healthy patients [30]. Second, hypoxic responses are observed in the lungs at PO_2 values (50–60 mm Hg) that are higher than those of other organs, even under conditions of normal oxygenation [31]. Third, an acute onset of hypoxia leads to pulmonary arterial constriction, while it triggers vasodilatation in other organs [32]. Fourth, hypoxia alters the expression of specific genes in the lungs, which are not activated in other organs. RELM- α (resistin-like molecule- α), gremlin1, and CXCR7 (CXC chemokine receptor type 7) have been shown to be associated with HPH progression [33–39].

Recently, many studies have demonstrated that alveolar hypoxia results in an accumulation of monocytes/macrophages at the pulmonary vasculature. In a study by Wood et al. [40], alveolar hypoxia was produced in rats by having the animals breathe 10% O_2 , which induces increased leukocyte-endothelial interactions within minutes, followed by leukocyte emigration to the perivascular space within 2–4 h. A recent study by Burke et al. [25] showed an abundance of mononuclear cells at the pulmonary artery adventitia of infant rats and neonatal calves exposed to chronic hypoxic conditions. A time course (24 h to 4 weeks) analysis shows that monocytes appear early in the pulmonary artery adventitia and accumulate over the period of hypoxia exposure. Interestingly, the hypoxia-induced accumulation of monocytes/macrophages appears to be specific for the pulmonary artery, since no such recruitment is observed in systemic circulation, i.e., for the aorta. Nevertheless, a few T cells, but no B cells or neutrophils, can be observed at the pulmonary vessel wall at various time points. A study by Minamino et al. [41] demonstrates that mice exposed to 8–10% oxygen for 48 h to 5 days exhibit a marked induction of proinflammatory cytokines and chemokines, including monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-2, interleukin (IL)-1, IL-6, as well as increased numbers of macrophages and neutrophils, followed by pulmonary structural remodeling.

The effects of acute alveolar hypoxia on leukocyte recruitment and cytokine production have also been studied in humans. Patients with high-altitude pulmonary edema have a higher absolute number of cells in bronchoalveolar lavage fluid, especially neutrophils and macrophages, compared to healthy individuals [42, 43]. In addition, increased levels of TNF- α , IL-1, IL-6, and IL-8 have been reported, which quickly subside to normal levels upon recovery [44]. Ex vivo studies of human lung tissue isolated from patients with severe chronic pulmonary

arterial hypertension have revealed inflammatory cell infiltrates (macrophages and lymphocytes) in plexiform lesions, as well as increased expression of the chemokines RANTES and fractalkine [45–49].

Taken together, alveolar hypoxia induces leukocyte infiltration into the lungs and walls of pulmonary arteries, which are involved in vasoconstriction and vascular remodeling [4, 22]. Leukocytic invasion into other ischemic or hypoxic tissues has also been reported [50–57]. However, the triggers for these specific inflammatory responses of the pulmonary vascular system are still unidentified [58–61]. Recent progress on this issue will be discussed further.

Low Alveolar PO₂ Levels Activate Resident Lung Cells, Which Then Initiate Pulmonary Inflammation

Among the resident lung cells, AMOs and dendritic cells (DCs) are located in close proximity to the epithelial surface of the respiratory system and the capillaries where they can rapidly sense changes in oxygen tension. Besides these cells, vascular endothelial cells also play a key role in hypoxia-induced inflammation by secreting proinflammatory factors and mediating leukocyte activation. The immediate responses of these cells to low alveolar hypoxia result in increased secretion of inflammatory factors and cell migration in the pulmonary alveolus and pulmonary interstitium; these responses create an inflammatory microenvironment in the pulmonary circulation. The following experimental evidence supports the idea that resident lung cells initiate alveolar hypoxia-induced pulmonary inflammation.

Alveolar Macrophages

An AMO (or dust cell) is a type of macrophage found in the pulmonary alveolus, near the AEPC, but separated from the wall. The activity of AMOs is relatively high, since they are located at one of the major boundaries between the body and the outside world. AMOs are phagocytes that play a critical role in homeostasis, host defense, responses to foreign substances, and tissue remodeling. Activated AMOs release numerous secretory products and interact with other cells and molecules through the expression of several surface receptors. AMOs are also involved in the phagocytosis of apoptotic and necrotic cells that have undergone cell-death.

In vitro models have shown that reductions in alveolar PO₂ levels or oxygen concentration activate AMOs, which then results in the rapid release of mitogenic and angio-

genic factors, including H₂O₂, MCP-1 [62–64], IL-1, and IL-6 [17, 65, 66]. In addition, AMOs secrete inflammatory factors, such as 15d-PGJ₂ (15-deoxy- Δ (12, 14)-prostaglandin-J₂), in the bronchoalveolar lavage, which is followed by neutrophil recruitment [67]. It has also been shown that acute hypoxia induces enhanced NF- κ B activation by AMOs, which may result in the increased production and release of inflammatory cytokines such as TNF [68]. A study using an animal model showed that rats breathing 10% O₂ for periods ranging from 1 to 8 h exhibit increased albumin extravasation and pulmonary expression of HIF-1 α , NF- κ B, and proinflammatory cytokines. However, these changes are attenuated by the elimination of AMOs [10, 62, 68, 69].

Although AMOs and peritoneal macrophages originate from the same progenitor in the bone marrow, the different PO₂ levels in the environments in which they reside may determine their sensitivity to oxygen levels. Under normoxic conditions, AMOs are exposed to PO₂ levels of approximately 100 Torr, while systemic tissue macrophages reside in environments where PO₂ values range between 20 and 40 Torr. A study by Chao et al. [26] shows that lowering PO₂ levels from 100 to 70 Torr induces a transitory release of H₂O₂, a reflection of an AMO respiratory burst, into the supernatant of primary AMO cultures. Furthermore, the magnitude of H₂O₂ release is inversely related to the PO₂ level. In contrast, lowering PO₂ levels to 5 Torr did not induce a respiratory burst in peritoneal macrophages. To put these values into perspective, intact rats breathing 10% O₂ show cremaster microvascular PO₂ levels of approximately 6–8 Torr. AMOs have a high sensitivity to oxygen changes and lung location, thereby triggering pulmonary inflammation under alveolar hypoxia conditions. The discrepancies in hypoxia responses between AMOs and peritoneal macrophages highlight the different characteristics of these cell types. The different PO₂ levels of the environments in which they reside may determine their metabolic patterns and perhaps explain their different responses to hypoxia [70]. However, the mechanisms underlying this distinct activity of AMOs under low oxygen levels are still unclear.

Lung Dendritic Cells

Lung DCs play a fundamental role in sensing invading pathogens and in controlling tolerogenic responses in the respiratory tract. They are distributed along the airways and throughout the pulmonary interstitium in close proximity to the overlying AEPCs. At least 3 main subsets of lung DCs have been identified in mice: conventional DC (cDC), plasmacytoid DC (pDC), and IFN-producing

killer DC (IKDC). The cDC subset is the most prominent subset in the lungs [71, 72]. It has been shown that hypoxia inhibits the maturation and differentiation of monocyte-derived DCs, but promotes the production of proinflammatory cytokines [73]. However, the response of lung DCs to alveolar hypoxia and their role in the initiation of pulmonary inflammation remain unclear.

Alveolar Epithelial Cells

Another resident cell that reacts to low alveolar oxygen tension is the AEPC. The mammalian pulmonary alveolar epithelium is comprised of 2 types of cells, called AEPCII and AEPCI. AEPCII can transdifferentiate into AEPCI to repair damaged epithelium after lung injury or during fetal lung development. AEPCII grown in primary culture can be observed to undergo such transitions *in vitro*.

AEPCs provide a rich source of chemokines and modulate inflammatory cell migration. Recent studies propose that the chemokine CCL20 (MIP-3 α) may play an important role in DC recruitment. It has been shown that primary human AEPCII cells, but not human AMOs, produce CCL20. In addition, peripheral blood monocyte-derived CD1a⁺ DCs migrate in response to conditioned media from AEPCII cells but not those from AMOs. DC migration was significantly correlated with the amount of CCL20 detected in the media but not with any other chemokine measured [74]. A study by Beck-Schimmer et al. [69] detected the expression of adhesion molecules, ICAM-1 and VCAM-1, on AEPCs, and identified their biological function under hypoxic conditions. At 5% oxygen levels, ICAM-1 and VCAM-1 mRNA increased by 100%, peaking between 0.5 and 1 h, and further increasing between 2 and 4 h. Neutrophil adherence to hypoxia-induced AEPCs was enhanced by 115%, and was inhibited with an anti-ICAM-1 antibody. Adherence of AMOs to AEPCs increased by 118%, and was blocked with an anti-VCAM-1 antibody. These results suggest that adhesion of leukocytes and macrophages to lung epithelial cells might play an important role in pulmonary inflammation during hypoxic conditions.

Studies have shown that the interaction of AMOs and AEPCs results in the release of proinflammatory mediators in the lungs, which may then induce a systemic inflammatory response [75–78]. However, there is no direct evidence that an AMO-epithelial cell interaction is required for the initiation of alveolar hypoxia-induced pulmonary inflammation. An acute hypoxia-reoxygenation cell model has shown that activated AMOs enhance the proinflammatory response of AEPCs. AMO-produced TNF- α further induces AEPCs to produce key chemo-

kines that could then contribute to subsequent lung injury through neutrophil recruitment [78]. Another report suggested that interactions of AMOs with respiratory epithelium may play a key role in hyperoxia-induced lung inflammation [75]. In this study, pulmonary epithelial cells (A549) and monocyte/macrophage cells (THP-1) were cultured either singly, in contact coculture, or prevented from contact by a porous membrane, and then exposed to oxygen or room air. Neither cell line cultured alone produced detectable levels of IL-8 under hyperoxia conditions. Contact cocultures exposed to hyperoxia produced increased levels of IL-8, while in the noncontact coculture it was attenuated [76]. These results suggest that the interaction between AMOs and AEPCs contributes to the production of mediators that induce an inflammatory response [77]. Future studies should examine how the interaction of AMOs and AEPCs, the 2 oxygen-sensing biomolecular species in the lungs, leads to the initiation and development of alveolar hypoxia-induced pulmonary inflammation.

Vascular Endothelial Cells

Vascular endothelial cell activation is an important response to hypoxia in a variety of vascular beds. Vascular endothelial cells secrete a multitude of proinflammatory mediators and express adhesion molecules that promote leukocyte recruitment into hypoxic or injured areas. It has been shown that massive leukocytes that accumulate at the pulmonary artery originate from the bloodstream. However, it is still unknown whether endothelial cells initiate lung inflammation alone.

Pulmonary arterial endothelial cell responses to lung ischemia-reperfusion injury are not robust enough to drive the development of lung injury independently. The activation of AMOs enhances the proinflammatory response of endothelial cells to hypoxia [79]. A study by McCourtie et al. [79] provided evidence that the secretory products of AMOs amplify the response of cultured endothelial cells to lung hypoxia-reperfusion injury. The exposure of endothelial cells to activated AMOs media enhances chemokine secretion in response to hypoxia and reoxygenation. In the reciprocal experiment, activated endothelial cell media increased the production of MCP-1 from macrophages. AMOs propel the development of lung reperfusion injury by enhancing the production of proinflammatory chemokines from endothelial cells, which in turn imparts a degree of positive feedback on AMOs. This suggests that the activation of AMOs may induce vascular endothelial cells to regulate an inflammatory response in the lungs to alveolar hypoxia.

Alveolar hypoxia triggers specific hemodynamic effects during pulmonary circulation, which results in leukocyte recruitment to the lungs. Exposure to alveolar hypoxia leads to pulmonary arterial constriction within seconds and elevates pulmonary arterial pressure. Even a modest elevation of lung vascular pressure has been shown to activate pro-inflammatory responses in endothelial cells of the lung venular capillaries, including P-selectin upregulation [80], and increased production of reactive oxygen species (ROS) [81]. In addition, the pulmonary interstitium around the bronchus and vessels also hosts macrophages and DCs. These cells can be activated by alveolar hypoxia through the mechanical effects of vascular constriction and certain mediators secreted by vascular endothelial cells. Otherwise, hypoxia may exert different effects on chemokine and/or adhesion molecule expression in bronchial versus pulmonary arterial endothelial cells. Part of the bronchial circulatory system, such as the vasa vasorum, may also serve as a major portal for leukocyte entry into hypoxic lungs. However, these concepts are still unclear.

Taken together, experimental data suggest that alveolar hypoxia induces pulmonary inflammation at local sites through AMO activation and subsequent interactions with other resident lung cells. Consequently, those localized reactions create a proinflammatory microenvironment in the lungs, which then evokes a predominant chemotactic effect that allows a specific recruitment of inflammatory cells to the lungs (as shown in Fig. 1).

However, the main cell type that migrates to the pulmonary vessel wall originates from the bloodstream and the distribution is enlarged by the duration of hypoxia exposure [82, 83]. This suggests that alveolar hypoxia-induced local inflammatory responses of the lungs may trigger another series of processes that aggravate pulmonary inflammation, characterized as the progressed accumulation of bloodstream leukocytes in the lung, which induces acute lung injury during acute phases of hypoxia, or pulmonary vascular remodeling under chronic hypoxia conditions.

AMOs Are Required for Systemic Inflammation under Alveolar Hypoxia Conditions

As mentioned above, massive leukocytes that accumulate in the pulmonary artery originate from the bloodstream, which can be confirmed by CD45 and CD11b stains. This suggests that a widespread, systemic inflammatory response is involved in the pulmonary inflamma-

tion induced by alveolar hypoxia. There is mounting evidence that reduced alveolar PO₂ levels result in rapid, systemic inflammation characterized by ROS generation, mast cell degranulation, leukocyte-endothelial interactions, increased vascular permeability, and cell infiltration. Rats breathing 10% O₂ show a rapid inflammatory response in mesentery, skeletal muscle, and pial microcirculations within minutes of hypoxia onset [40, 84, 85]. However, mast cell degranulation and leukocyte recruitment occur only when the alveolar PO₂ level is reduced, independent of the systemic PO₂ level in skeletal muscle [84]. Cultured peritoneal mast cells did not show degranulation under low oxygen conditions. Immersion of mast cells in a supernatant of hypoxic AMOs, but not in that from hypoxic peritoneal macrophages, induces mast cell degranulation [26]. Interestingly, hypoxia induced a respiratory burst in alveolar, but not peritoneal, macrophages [26]. This suggests that AMO activation is required for alveolar hypoxia-induced systemic inflammation.

Systemic inflammation under alveolar hypoxia conditions is modulated by inflammatory mediators, which are released by AMOs. Studies by Chao et al. [26–28, 86] confirm that alveolar hypoxia-induced systemic inflammation requires AMO activation, independent of reduced systemic PO₂ levels or the activation of tissue macrophages. Another study found that plasma from alveolar hypoxic rats evokes an inflammatory response in tissues of normoxic rats, which suggests that a mediator released by hypoxic AMOs activates mast cells and triggers systemic inflammation [87]. Further research by the same group showed that systemic inflammation is initiated by circulating MCP-1, which is released from activated AMOs at low alveolar PO₂ levels. Circulating MCP-1 induces mast cell degranulation with renin release and activation of the local renin-angiotensin system (RAS), leading to microvascular leukocyte recruitment and increased vascular permeability [28].

A study using AMO-depleted, hypoxic rats provided direct evidence that AMO activation is required for the initiation of systemic inflammation. The supernatant of hypoxic AMO, but not of hypoxic systemic tissue macrophages, induced mast cell degranulation in vivo and in vitro [87]. Mast cells undergo degranulation when immersed in plasma from intact hypoxic rats, but not in plasma from AMO-depleted hypoxic rats [28]. Lastly, plasma MCP-1 concentration increases rapidly in rats breathing 10% O₂, but does not increase in AMO-depleted rats [87]. MCP-1 induces a concentration-dependent mast cell degranulation, and administration to normoxic

rats results in hypoxia-induced inflammation. Conversely, an MCP-1 receptor antagonist blocks alveolar hypoxia-induced systemic inflammation in intact rats. This is consistent with previous observations that AMO depletion prevents alveolar hypoxia-induced systemic inflammation, and that plasma from intact hypoxic rats, but not of AMO-depleted rats, elicits inflammation in normoxic tissues [28, 88].

In vitro experiments have shown that the permeability of single-layer endothelial cells and leukocyte-endothelial cell adhesion are induced under low oxygen conditions. This is not consistent with the above results showing that AMOs are necessary for alveolar hypoxia-induced systemic inflammation. However, different phases of alveolar hypoxia can explain this discrepancy. It was previously shown that alveolar hypoxia induces systemic inflammation within several minutes [27], although systemic PO₂ levels do not demonstrate obvious changes. With prolonged exposure to low alveolar PO₂ levels, systemic oxygen pressure decreases and exerts further effects on systemic inflammation. This suggests that AMOs trigger specific effects during the acute phase of hypoxia that are necessary for the initiation of systemic inflammation. However, other possibilities should be considered. Although most cell types are cultured under 21% oxygen conditions, it may not be appropriate to set 21% oxygen as a normoxic control. First, cells cultured in 21% oxygen conditions proliferate continuously and much faster than their physiological state in vivo. Second, oxygen pressure decreases in a gradient from alveoli to internal organs [30, 89]. It is impossible for blood to contain the same oxygen pressure as air. For example, it has been demonstrated that oxygen pressure in small arterioles is about 30–40 mm Hg under normoxic conditions [30]. According to Dalton's law, oxygen concentration is related to oxygen pressure; therefore, oxygen concentration at small arterioles is approximately 5%. If data from in vitro and in vivo experiments are compared, selection of oxygen levels characteristic of normoxic conditions is critical for prospective analyses. However, this issue is still under investigation.

Activated Systemic Inflammation Is Critical for the Progression of Pulmonary Inflammation and HPH Development

Inhibition of systemic inflammation prevents hypoxic pulmonary inflammation, and then attenuates HPH development. Dexamethasone treatment at any key step of

the inflammatory cascade results in significant inhibition of pulmonary inflammatory responses induced by alveolar hypoxia [90, 91]. A recent report has shown that lung-specific expression of heme oxygenase-1 (HO-1) may confer protection from HPH by effectively modifying the macrophage activation state during hypoxia. However, a brief, 2-day expression of high levels of HO-1 delayed, but did not prevent, the peak of hypoxic inflammation, and could not protect against HPH. In contrast, a 7-day expression of high levels of HO-1 during an entire period of hypoxic inflammation inhibited macrophage accumulation and activation, and prevented HPH development [92]. This suggests that alveolar hypoxia-induced systemic inflammation is crucial for the progression of pulmonary inflammation and HPH development in chronic hypoxia. The possible mechanisms are discussed below.

Activated Systemic Inflammation Prolongs the Accumulation of Monocytes in the Lungs by Promoting Cell Adhesion and Transendothelium Migration

Bloodstream leukocytes enter the pulmonary circulation and pulmonary vasculature system through the vascular endothelium. Interruption of leukocyte-endothelial cell interactions or protection against endothelium hyperpermeability attenuates hypoxia-induced inflammatory cell infiltration. Iptakalim is an adenosine triphosphate-sensitive potassium channel opener [93]. One study found that iptakalim prevented an increase in IL-1 β and a decrease in IL-10 in the peripheral blood and lung tissue, and alleviated inflammatory cell infiltration in rats with HPH [94]. Furthermore, iptakalim enhanced PECAM-1 and eNOS expression and prevented endothelial cell injury induced by hypoxic stimuli. Finally, increases in the mean pulmonary artery pressure, right ventricular systolic pressure, right ventricle to left ventricle plus septum ratio, and small pulmonary artery wall remodeling in HPH rats were attenuated. This suggests that suppression of pulmonary arterioles and systemic inflammatory responses and protection against endothelial damage are critical for the inhibition of HPH development.

Mast cells are known to increase in number at sites of inflammation, where they are believed to influence vascular permeability, cell accumulation, and tissue-specific responses [95]. Under hypoxic conditions, activated mast cells are involved in vascular hyperpermeability and leukocyte cell-endothelium adhesion. The activation of mast cells with compound 48/80 under normoxic conditions produced microvascular effects similar to those under hypoxic conditions. The blockade of mast cell degranula-

tion with cromolyn prevented or attenuated hypoxia-induced increases in ROS, leukocyte adherence/emigration, and vascular permeability [96].

An activated RAS mediates microvascular inflammation by increasing leukocyte-endothelial interactions and vascular hyperpermeability [86, 97]. It has been reported that MCP-1-induced MC degranulation is involved in RAS activation under alveolar hypoxia [86]. Western blots and immunocytochemistry demonstrated the presence of renin and angiotensin-converting enzyme in mast cells and their release upon degranulation. MCP-1-induced degranulation of mast cells incubated in plasma produced an increase in angiotensin II concentration, which was inhibited completely by the MCP-1 receptor antagonist RS-102895, the specific rat renin inhibitor WFML, or the angiotensin-converting enzyme inhibitor captopril. Captopril also inhibited angiotensin II generation by the incubation of mast cells in culture medium containing angiotensin I. The results show that peritoneal mast cells contain active renin, which activates the RAS upon degranulation.

Vascular endothelial growth factor (VEGF) has been implicated to contribute to tissue edema through its effect on vascular permeability. Hypoxia induces VEGF production by endothelial cells, which contributes to angiogenesis and vascular hyperpermeability. Recent research found that mast cells modulate VEGF levels under hypoxic conditions. A study revealed that the expression of VEGF, Flt-1, and Flk-1 increased significantly when myocardial microvascular endothelial cells were cocultured with mast cell granules or active mast cells [98]. It is interesting that mast cells are a source of VEGF secretion. Cobalt chloride (CoCl₂), a hypoxia-mimicking agent, promotes a sustained production of functional VEGF from mast cells and induces proliferation of endothelial cells in vitro [99].

Activated Systemic Inflammation Promotes Vascular Remodeling

Vascular remodeling is associated with collagenolysis and the activation of matrix metalloproteinases (MMPs). One of the possible sources of MMPs in hypoxic lung is mast cells. In order to define the role of lung mast cell collagenolytic activity in HPH, rats were treated with the inhibitor of mast cell degranulation disodium cromoglycate, DSCG, in an early or later phase of isobaric hypoxia. Lung hemodynamics, muscularization, and collagen metabolism in the walls of peripheral pulmonary vessels were measured. This study found that DSCG applied at an early phase of exposure to hypoxia inhibited muscu-

larization in peripheral pulmonary arteries and decreased the amount of collagen cleavage fragments in prealveolar vessels [100, 101]. This suggests that mast cell degranulation plays a role in the initiation of hypoxic pulmonary vascular remodeling.

It has also revealed that activated mast cells are participated in pulmonary artery remodeling [102, 103]. Exposure to acute hypoxia (barometric pressure of 263 mm Hg) for 8 h did not lead to increased numbers of mast cells in rat lungs. In contrast, adult rats maintained at a barometric pressure of 380 mm Hg for 35 days exhibit a proliferation of mast cells around pulmonary blood vessels and in alveolar septa. There is a correlation between perivascular lung mast cell density and medial thickness of muscular pulmonary arteries [104]. The MMP family of proteolytic enzymes is involved in remodeling of the extracellular matrix. Although proteolytic enzymes are produced by many cell types (mast cells, fibroblasts, vascular smooth muscle cells, and endothelial cells), mast cells are the most abundant source of proteolytic enzymes under normoxic and hypoxic conditions [105]. In order to verify that chronic hypoxia-induced vascular remodeling is initiated in the walls of prealveolar pulmonary arteries by MMPs released from activated mast cells, the distribution of mast cells and their expression of interstitial collagenase, MMP-13, in lung conduit, small muscular, and prealveolar arteries were examined in rats exposed to hypoxia for 4 and 20 days, as well as after a 7-day recovery from 20-day hypoxia (10% O₂). After 4 days, but not after 20 days of hypoxia, the number of mast cells increased significantly, and MMP-13 expression was found within prealveolar artery walls. In rats exposed to hypoxia for 20 days, MMP-13-positive mast cells accumulated within the walls of conduit arteries as well as subpleurally. In recovered rats, MMP-13-positive mast cells gather at prealveolar artery walls and in the walls of small muscular arteries. These mast cells also remained in the conduit part of the pulmonary vasculature [106]. This suggests that perivascular pulmonary mast cells contribute to vascular remodeling in rats by releasing interstitial collagenase.

ROS are important mediators of the proliferation response in pulmonary vascular cells. ROS increase the expression and/or activation of multiple factors involved in cellular growth, including PI3K/Akt, p38MAPK, c-Src, TGF- β 1, VEGF, PDGF, FGF-2, NFAT (nuclear factor of activated T cells), and PGC-1 α (peroxisome proliferator-activated receptor- γ coactivator 1 α). All these factors have been implicated in pulmonary vascular remodeling [107, 108]. ROS-dependent fluorescence is induced within minutes of hypoxia onset, and is observed in perivas-

Fig. 1. The initiation and progression of alveolar hypoxia-induced pulmonary inflammation. EC, endothelial cell; ET-1, endothelin-1; FBC, fibroblast cell; SMC, smooth muscle cell.

cular mast cells in endothelial layers of postcapillary venules, and at the sites of leukocyte-endothelial interactions [96, 109]. The magnitudes of ROS-dependent fluorescence intensity and leukocyte-endothelial interactions are inversely related to PO_2 values [110]. The antioxidants SOD/catalase and lipoic acid significantly inhibit ROS-dependent fluorescence intensity and increased leukocyte-endothelial interactions [96, 109].

Taken together, a secondary activation of systemic inflammation under alveolar hypoxia conditions is essential for the progression of pulmonary inflammation and HPH development, which is shown in Figure 1. However, studies in mast cell-deficient mice demonstrated that there were no obvious differences in the pulmonary re-

sponses of these mice and normal mice to alveolar hypoxia, since the right ventricular peak systolic pressure, the right ventricular hypertrophy index, and the pulmonary arterial structure had similar changes under chronic alveolar hypoxia conditions [111]. In this report, a mast cell-deficient mouse had less than 1% of the normal number of mast cells in the skin, stomach, cecum, and mesentery, and had no lung mast cells. Tissue mast cells are the main source of histamine, which is an important mediator for pulmonary responses to hypoxia. However, histamine content in mast cell-deficient and normal mouse lungs was similar. This implies that systemic inflammation alone is not sufficient to induce pulmonary inflammation and HPH under alveolar hypoxia.

A Combination of Alveolar Hypoxia-Induced Lung Local Inflammation and a Secondary Activation of Systemic Inflammation Controls Monocyte Infiltration in the Lungs

For the migration of blood-derived leukocytes into ischemic or hypoxic tissues, a series of events must occur. Leukocytes are activated and adhere to endothelial cells. Then, the transendothelium migrates and arrives at its destination by chemotactic effects. As discussed above, most of the inflammatory cells that accumulate in pulmonary circulation under alveolar hypoxia conditions are bloodstream mononuclear cell types. Their accumulation and retention is specific in pulmonary perivascular regions, while it is undetectable, or only minimally increased, at the adjoining lung parenchyma and systemic arteries (aorta and renal artery). These migrant cells are essential in the development of hypoxia-induced pulmonary vascular remodeling, which has been reviewed in detail previously [66, 83, 92]. Therefore, the mechanism for the specific recruitment and retention of these cells in the pulmonary vasculature under alveolar hypoxia conditions is necessary for the understanding of HPH initiation and development. Published evidence has been summarized that supports a combination of alveolar hypoxia-induced local lung inflammation and the initiation of systemic inflammation (“second hit”) that is necessary for the accumulation of bloodstream mononuclear cells in the lungs.

Alveolar Hypoxia-Induced Local Inflammation Evokes a Chemotactic Effect That Directs the Recruitment of Bloodstream Monocytes to the Lungs

It is well known that the primary step in leukocyte migration is the establishment of weak and transient adhesive interactions between leukocytes and vascular endothelial cells. However, the cues that regulate the site and route of monocyte migration along and through the endothelial cell barrier are critical since alveolar hypoxia-induced monocyte infiltration is specific to pulmonary circulation. This suggests that primary chemotactic signals exist in the lungs and determine the site of leukocyte migration into the lungs.

In order to identify potential mediators that are responsible for recruitment of circulating cells within the hypoxic pulmonary artery, mRNA expression of 44 different chemokine/chemokine receptor, cytokine, adhesion, growth, and differentiation genes in pulmonary arteries was examined by RT-PCR at several time points of hypoxic exposure (1, 7, and 28 days) in Wistar-Kyoto rats

[25]. Samples were obtained via laser capture microdissection in adjacent lung parenchyma and in systemic arteries. Consistent with other reports, this study also found that hypoxia induced the progressive accumulation of monocytes and DCs in the vessel wall with few T cells and no B cells or neutrophils. Upregulation of stromal cell-derived factor-1 (SDF-1), VEGF, GRO- α (growth-related oncogene protein- α), C5, ICAM-1, OPN (osteopontin), and TGF- β (transforming growth factor- β) preceded mononuclear cell influx. With time, a more complex pattern of gene expression developed with the persistent upregulation of adhesion molecules (ICAM-1, VCAM-1, and OPN) and monocyte/fibrocyte growth and differentiation factors (TGF- β , endothelin-1, and 5-lipoxygenase). On return to normoxia, expression of many genes (including SDF-1, MCP-1, C5, ICAM-1, and TGF- β) rapidly returned to control levels, changes that preceded the disappearance of monocytes and reversal of vascular remodeling. This suggests that sustained hypoxia leads to the development of a complex, pulmonary artery-specific, proinflammatory microenvironment capable of promoting the recruitment and retention of circulating monocytic cell populations that contribute to vascular remodeling.

The source of inflammatory factors in the lungs under alveolar hypoxia conditions is still under investigation. A local, activated lung cell, AMO, is a producer of inflammatory factors during the acute phase of alveolar hypoxia [1, 10, 62, 63, 68, 76, 78, 92, 112–116]. The distribution of leukocytes or migration of blood-derived leukocytes at the pulmonary artery is diminished remarkably after AMO clearance. AMOs actively secrete cytokines and chemokines, such as MCP-1, IL-6, and 5-HT, under hypoxic conditions. Among them, MCP-1 is indispensable for the development of alveolar hypoxia-induced lung local inflammation and systemic inflammation. If AMO-derived MCP-1 production decreases, the distribution of leukocytes in the lungs is reduced. In this regard, the pre-inflammatory condition is initiated by local cell activation, which may be related to a hyperactive lung's inflammatory response to alveolar hypoxia.

Activated Mast Cells Stimulate AMOs to Induce Monocyte Chemotactic Activity

Histamine is an important mediator for pulmonary responses to hypoxia [117–123]. Tissue mast cells are the main producer of histamine. AMOs secrete histamine-releasing factors, which induces histamine release by mast cells and basophils [124]. Furthermore, AMOs themselves are affected by histamine by the expression of

the histamine receptors H1 and H2 [125–127]. This suggests that activated systemic inflammation influences the function of AMOs through feedback.

Increasing evidence suggests that a continuous release of histamine from mast cells may modulate the functions of macrophages. Histamine induces exocytosis and IL-6 production from human lung macrophages through interaction with H1 receptors. Increases in histamine levels (10^9 – 10^6 M) in a concentration-dependent fashion result in the basal release of β -glucuronidase and IL-6 from human lung macrophages. Enhancement of β -glucuronidase release induced by histamine was evident after 30 min and peaked at 90 min, whereas that of IL-6 required 2–6 h of incubation. These effects were reproduced by an H1 agonist but not by an H2 agonist [127]. This suggests that histamine induces exocytosis and IL-6 production from human macrophages by activation of the H1 receptor, which contributes to the long-term sustainment of pulmonary inflammation.

More importantly, histamine stimulates AMOs to induce neutrophil and monocyte chemotactic activity [126]. In one study, bovine AMOs were cultured and supernatant fluids were evaluated for neutrophil chemotactic activity and monocyte chemotactic activity using a blind-well chamber technique. AMOs induced chemotactic activity in response to histamine in a dose- and time-dependent manner. Partial characterization and molecular sieve column chromatography revealed that low-molecular-weight lipid-soluble activity was predominant. Lipoxigenase inhibitors significantly blocked the induction of chemotactic activity. Leukotriene B4 receptor antagonists blocked the chemotactic activity. Immunoreactive leukotriene B4 significantly increased in supernatant fluids in response to histamine. The receptor responsible for the induction of chemotactic activity in response to histamine was the H2 receptor. These data demonstrate that AMOs release neutrophil and monocyte chemotactic activity in response to histamine and may thereby modulate inflammatory cell recruitment into the lungs.

Conclusion

Accumulated evidence supports the idea that continued recruitment of circulating monocytes into pulmonary circulation may play an important role in the development of PH in response to both acute and chronic alveolar hypoxia. However, the mechanisms responsible for leukocyte recruitment and migration into the pulmonary vasculature under alveolar hypoxia remain under investigation. In this review, we have discussed a series of studies that support the hypothesis that alveolar hypoxia induces a localized inflammatory response in the lung and a subsequent systemic inflammation (as a “second hit”), both of which are combined and essential to HPH development. As shown in Figure 1, alveolar hypoxia creates an inflammatory microenvironment in the lungs specifically, which triggers chemotactic effects on circulating monocytes to accumulate at the pulmonary vasculature. A secondary activation of systemic inflammation is necessary to trigger and allow monocyte migration into the lungs. A better understanding of the initiation and progression of alveolar hypoxia-induced pulmonary inflammation may lead to the identification of novel targets that could inhibit HPH development.

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Disclosure Statement

The authors have no conflicts of interest to report.

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