ACUTE (MINUTES TO HOURS) DECREASES in inspired oxygen concentrations cause vasoconstriction of pulmonary arteries, a process that has been termed “hypoxic pulmonary vasoconstriction.” Extended (days to weeks) exposure to low oxygen concentrations, either persistent or intermittent, results in the development of chronic pulmonary hypertension and pulmonary vascular remodeling, which is often characterized by medial and adventitial thickening due to increases in cell size and number, as well as increased extracellular matrix protein accumulation. Recent reviews have addressed many of the potential cellular and molecular mechanisms that contribute to hypoxic vasoconstriction and remodeling (1, 2, 27, 40, 59). However, little attention has been given to the possibility that pulmonary inflammation and/or a noninflammatory accumulation of circulating monocytes/macrophages might contribute to vasoconstriction and remodeling. Yet there is accumulating evidence to support the idea that both acute and chronic exposure of animals to even moderate hypoxia result in the increased expression of lung inflammatory cytokines, chemokines, and adhesion molecules, as well as the accumulation of leukocytes both within the lungs and the lung blood vessels themselves. The purpose of this brief review is to 1) provide evidence that hypoxia induces recruitment of circulating leukocytes to the lung vasculature, 2) review potential mechanisms through which the hypoxia-induced recruitment of leukocytes might occur, and 3) discuss the potential contribution of these cells to changes in lung vessel function, structure, and the pulmonary hypertensive process.

EVIDENCE FOR HYPOXIA-INDUCED LEUKOCYTE RECRUITMENT TO TISSUES INCLUDING THE LUNG

Hypoxia and leukocytes in the systemic circulation. Leukocytic invasion into ischemic or hypoxic tissues is now well documented. For instance, macrophages represent an important component of the leukocytic infiltrate in a majority of malig-
nant tumors, in some instances comprising up to 50% of the tumor cell mass. These tumor-associated macrophages are thought to originate from peripheral blood monocytes that are recruited into the tumor from the circulation (rather than from resident macrophages present in tissue before the tumor developed). There is good evidence that these macrophages are recruited to the tumor environment specifically via hypoxia-regulated signaling pathways (30, 36, 53). Macrophages are believed to contribute to tumor angiogenesis through the secretion of a variety of proangiogenic factors and to support tumor growth by secreting a number of factors important for proliferation, invasion, and metastasis of tumor and stromal cells (Table 1). Increased numbers of macrophages have also been observed in ischemic areas of dermal wounds, atherosclerotic plaques, synovium of joints with rheumatoid arthritis, and eyes with proliferative retinopathy (7, 9, 14, 22). Wood and colleagues (16, 57) demonstrated in rats that systemic hypoxia induces increased leukocyte-leukocytic interactions within minutes, followed by leukocyte emigration to the perivascular space within 2–4 h. This response is observed in several (mesenteric, remaster, and pial) microcirculation beds, suggesting a generalized response to hypoxia (16, 57). Thus the nascent theme that hypoxia-mediated recruitment of leukocytes is an important contributor to various systemic disease processes raises the question as to whether hypoxia causes leukocytic invasion into the lung.

Acute hypoxia and leukocytes in the lung. Several recent studies document the enhanced expression of inflammatory mediators and increased numbers of macrophages and neutrophils in the lungs of mice and rats exposed to acute hypoxia. Minamino et al. (35) demonstrated that mice exposed to 8–10% oxygen for 48 h to 5 days exhibited a marked induction of proinflammatory cytokines and chemokines (monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-2, interleukin (IL)-1β, IL-6) in association with increased numbers of macrophages and neutrophils, both processes preceding the development of pulmonary structural remodeling. Interestingly, in this model, the authors found no evidence for induction of tumor necrosis factor (TNF)-α, a cytokine whose increased expression is observed in the setting of lung inflammation induced by lipopolysaccharide, raising the possibility that hypoxia induces a network of cytokines and chemokines that is distinct from other inflammatory subpathways (35, 60). Madjdpour et al. (33) reported similar, but not identical, findings in rats acutely exposed to hypoxia. These investigators found that exposure to 10% oxygen for as little as 60 min induced a marked increase in the number of alveolar macrophages within the lung. In addition, they reported the brief and transient (between 4 and 6 h) appearance of neutrophils in the acutely hypoxic lung. The increase in macrophage and neutrophil numbers in the lung was associated with an increase in albumin extravasation, possibly indicating the development of a mild vascular injury in response to acute hypoxia, a finding similar to the acute changes in permeability induced by hypoxia in the mesenteric circulation (33, 16, 57). In this model, acute hypoxia led to increased expression of inflammatory mediators including MCP-1, MIP-1β, ICAM-1, as well as TNF-α. Others also reported increased expression of TNF-α in the hypoxic rat lung (56), raising the possibility that there are species-specific differences in the acute (hours to days) response of the lung to hypoxic exposure.

The effect of acute hypoxia on leukocyte recruitment and cytokine production has also been studied in humans. In patients with high-altitude pulmonary edema (HAPE), increases in the absolute number of cells in bronchoalveolar lavage fluid (BALF), especially neutrophils and macrophages, compared with healthy individuals have been observed (31, 48). In addition, increased levels of TNF-α, IL-1β, IL-6, and IL-8 have been noted, levels that quickly return to normal on recovery (19, 31, 48). Whether the inflammatory cells directly contribute to the hypoxia-induced edema formation or are a secondary or late phenomenon is currently unclear (5).

Chronic hypoxia and leukocytes in the lung. Chronic hypoxic exposure, especially in the young, is characterized by the development of striking fibroproliferative changes in the adventitia of both large and small pulmonary arteries (52). These changes have long been assumed to be due to increased proliferation and extracellular matrix protein production by resident pulmonary artery adventitial fibroblasts. However, the cell-restricted nature of this concept has recently been challenged by observations in the systemic circulation showing that circulating mononuclear cells with fibroblast-like properties (termed “fibrocytes”) are recruited to sites of tissue injury and participate in the repair process (6, 39, 34, 58, 47). In the lung, Phillips et al. (38) recently showed that circulating mononuclear fibrocytes are the major contributors to lung fibrosis after bleomycin-induced injury. Furthermore, Hashimoto et al. (20) demonstrated the bone marrow origin of fibroblast precursors that contribute to pulmonary fibrosis. With regard to the pulmonary circulation, we recently presented data demonstrating the robust appearance of mononuclear cells in the pulmonary artery adventitia of both infant rats and neonatal calves exposed to chronic hypoxia (15). The time course (24 h–4 wk) analysis showed the early appearance of monocytes in the pulmonary artery adventitia and their continued accumulation over time of hypoxic exposure. We established a nonresident (circulating) origin for these cells. Importantly, the hypoxia-induced accumulation of macrophages appeared to be specific for the pulmonary circulation, because no macrophage recruitment was noted in the systemic circulation (aorta). Furthermore, no neutrophils were identified in the pulmonary perivascular areas at the time points analyzed.

Table 1. Mitogenic and angiogenic factors known to be produced by macrophages/fibrocytes

<table>
<thead>
<tr>
<th>Factor</th>
<th>MCP-1</th>
<th>MIP-1α</th>
<th>MIP-2</th>
<th>MPP-9, -19</th>
<th>PDGF-A, B</th>
<th>TIMP-1</th>
<th>TGF-α</th>
<th>TGF-β</th>
<th>uPA</th>
<th>VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>bFGF, basic fibroblast growth factor</td>
<td>EGF, endothelial growth factor</td>
<td>IGF, insulin-like growth factor</td>
<td>IL, interleukin</td>
<td>MCP, monocyte chemoattractant protein</td>
<td>MMP, matrix metalloproteinases</td>
<td>TGF, transforming growth factor</td>
<td>VEGF, vascular endothelial growth factor</td>
<td>GM-CSF, granulocyte macrophage-colony stimulating factor</td>
<td>M-CSF, macrophage-colony stimulating factor</td>
<td>PDGF, platelet-derived growth factor</td>
</tr>
</tbody>
</table>

bFGF, basic fibroblast growth factor; EGF, endothelial growth factor; IGF, insulin-like growth factor; IL, interleukin; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinases; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; GM-CSF, granulocyte macrophage-colony stimulating factor; M-CSF, macrophage-colony stimulating factor; PDGF, platelet-derived growth factor; TIMP, tissue inhibitor of metalloprotease; uPA, urokinase type plasminogen activator.
Ex vivo studies in human lung tissue have revealed inflammatory cell infiltrates (macrophages and lymphocytes) in the areas of plexiform lesions in severe chronic pulmonary arterial hypertension, as well as an increased expression of the chemokines, RANTES and fractalkine (13, 45). Collectively, these findings in both animal models and humans provide strong support for the idea that hypoxic exposure induces leukocyte infiltration into the lung and wall of pulmonary arteries where they can have both acute and long-lasting effects on vascular function and structure.

RECRUITMENT OF MONOCYTES INTO HYPOXIC TISSUES: POTENTIAL MECHANISMS

A variety of factors are involved in the recruitment of monocytes from the bloodstream to the specific tissue sites. Factors include chemokines, cytokines, and mitogenic peptides (30, 36). Chemokines are known to induce recruitment of monocytes from the bloodstream into tissues. Chemokines are divided into four subclasses (CXC, CC, C, and CX3C), and their specific effects on target cells (monocytes/macrophages) are mediated via members of a family of seven transmembrane G protein-coupled receptors (3, 55). Several CC chemokines, in particular CCL2 (MCP-1) and CCL5 (RANTES), specifically attract and activate monocytes, and these molecules have been most heavily implicated in monocyte recruitment in tumors and other inflammatory disease processes (36). CCL2 and CCL5 are expressed by tumor cells, fibroblasts, and endothelial cells, and their expression has been shown to positively correlate with the number of macrophages, at least in a variety of tumor settings. Furthermore, both CCL2 and CCL5 have been found to stimulate monocyctic cell lines, blood monocytes, and tissue macrophages to secrete matrix metalloproteinases, including MMP-9, MMP-19, and μ-PA (42). These proteases degrade basement membranes and extracellular matrix components and appear to aid leukocyte migration into tissues. They have also been implicated in the proteolytic remodeling of the extracellular matrix and in modulating local growth factor availability, which may be important in supporting adventitial fibroblast and/or smooth muscle cell (SMC) activation (27, 40). Importantly, increases in CCL2 (MCP-1) expression have been found in the lung and blood vessels of all animal models of hypoxic exposure described above (33, 35, 60).

Other chemokines may also be involved in monocyte recruitment to the hypoxic vessel wall. Monocytes and macrophages respond to the chemotactic effects of CXCL12 [stromal cell-derived factor-1 (SDF-1)] via the expression of the CXCR4 receptor. In fact, many tissues, including the lung, may constitutively express/secrete CXCL12. However, hypoxia has been shown to significantly upregulate expression of CXCL12/SDF-1 in resident fibroblasts and endothelial cells, and circulating monocytes have been shown to increase their expression of CXCR4 under hypoxic conditions (8, 34, 46).

A number of cytokines have also been implicated in the recruitment of leukocytes into tumors and other hypoxic tissues. Perhaps the most important is the heparin-binding glycoprotein vascular endothelial growth factor (VEGF), whose expression is known to be upregulated under hypoxic conditions. VEGF-A has been clearly shown to be chemotactic for monocytes and macrophages via activation of the VEGFR-1 (Flt-1) receptor by these cells (4, 45). A positive correlation between elevated VEGF expression and the number of infiltrating macrophages has been demonstrated in breast tumors (32). We demonstrated upregulated VEGF-A expression in the pulmonary artery adventitia of chronically hypoxic animals in association with increased neovascularization and monocyte accumulation (12, 15). Thus VEGF, produced locally by a number of different cell types in response to hypoxia, can attract monocytes into the local environment.

Endothelins and TGF-β family members may also participate in the recruitment of monocytes to the pulmonary circulation. ETs-1–3 are small vasoactive and mitogenic peptides that are secreted by a wide variety of cell types, including fibroblasts, in response to hypoxia. Like chemokines, endothelins mediate their effects on monocytes by binding to seven transmembrane G protein-coupled receptors (ET-RA and ET-RB) (17). ET-1 is known to be chemoattractant for monocytes through a mechanism involving binding to the ET-RA on these cells. ET-2 is a chemoattractant for macrophages by binding the ET-RB (which is not expressed on monocytes so they do not migrate toward ET-2) (10, 17). Numerous investigators have documented increased concentrations of ET-1 in the lung and pulmonary arteries of hypoxic animals (27). TGF-β family members are also known to be regulated by hypoxia and to be chemoattractant for monocytes (44). We have preliminary evidence suggesting upregulation of active TGF-β1 in the pulmonary artery adventitia of hypoxic animals, making it another candidate for inducing monocyte recruitment to the hypoxic vessel wall.

Within the pulmonary circulation, one must consider that hypoxia may not be the only stimulus driving the expression of molecules that induce leukocyte recruitment. Exposure to hypoxia elevates pulmonary arterial pressure. Even a modest elevation of lung vascular pressure has been shown to activate proinflammatory responses (such as P-selectin upregulation) in endothelial cells of the lung venular capillaries (28). This response appears to be coupled through reactive oxygen species generated by mitochondria (23). Thus both hypoxia and its attendant hemodynamic effects could participate in leukocyte recruitment.

Because it is clear that the factors involved in leukocyte recruitment may be tissue specific and vary in their sensitivity to hypoxic regulation, a systematic evaluation of the effects of hypoxia, in the presence and absence of hemodynamic changes, on pulmonary vascular cell production of chemokines and cytokines is needed. In addition, the possibility that hypoxia exerts different effects on chemokine and/or adhesion molecule expression in bronchial vs. pulmonary artery endothelial cells also needs to be considered because the bronchial circulation (vasa vasorum) may serve as a major portal of monocyte entry into the hypoxic lung.

POTENTIAL CONTRIBUTION OF LEUKOCYTES TO ABNORMALITIES IN LUNG VASCULAR TONE AND TO STRUCTURAL REMODELING

Leukocytes and vascular tone. Recruitment of leukocytes into the lung airspaces or the vessel wall itself can have significant impact on the function and structure of lung blood vessels. Activated macrophages and/or neutrophils can release a variety of factors including reactive oxygen species (ROS) and proteolytic enzymes capable of directly causing vasocon-
striction and increases in vascular permeability (41, 57). It has also been postulated that activated adventitial fibroblasts, through the production of ROS, can act as a “sink” for nitric oxide (NO) produced by the endothelium (41). Thus, under certain circumstances, less NO may be available to the medial SMC, leading indirectly to vasoconstriction. It is possible that activated monocytes/macrophages, accumulating on the outside of the vessel wall, could also, through release of ROS, contribute to abnormalities in tone. Further support for the idea that hypoxia-induced changes in the lung leukocyte recruitment may alter vascular tone comes from observations that TNF-α−/− mice are completely protected against hypoxia-induced increases in pulmonary artery pressure (50).

Leukocytes and vascular structural remodeling. Monocytes and circulating mononuclear fibrocytes recruited to the pulmonary vessel wall could contribute to the changes in SMC and fibroblast proliferative status and phenotype observed in response to chronic hypoxic exposure (52) through the release of numerous growth factors (Table 1) in a manner similar to their reported effects on stromal cell proliferation and differentiation in tumors (30, 36). It has been reported that hypoxic pulmonary artery adventitial fibroblasts secrete factors, regulated by HIF-1α expression, capable of stimulating vascular SMC proliferation (43). Therefore, the possibility that leukocytes recruited to the hypoxic pulmonary vessel wall in large numbers (15) secrete factors, which trigger the growth and/or migration of resident fibroblasts and SMCs, needs to be considered.

Marked production and accumulation of extracellular matrix proteins, especially type I collagen, is observed in hypoxia-induced vascular remodeling (27, 40, 52). The cells responsible for the matrix protein production have long been assumed to be resident fibroblasts or SMC. The possibility that nonresident cells may contribute to increased matrix protein accumulation within various tissues was raised at least 150 years ago, beginning with the studies of Paget in the 1850s (37). A decade ago, Bucala and colleagues (34, 39, 58) described the subpopulation of circulating leukocytes, which they termed “fibrocytes” that can be recruited from the circulation into wounds and can produce collagen. Since then, many studies have supported the idea that at least some collagen-producing cells in wounds and various injured tissues are derived from circulating mononuclear fibrocytes. The support for the idea that circulating rather than resident cells are critical in collagen production in lung fibrotic responses, comes from observations by Phan’s group (20), demonstrating that, in the majority of collagen-producing cells, bleomycin-induced pulmonary fibrosis are the bone marrow-derived fibroblast precursors. Phillips et al. (38) most recently demonstrated that bleomycin-induced pulmonary fibrosis, in fact, is mediated by circulating mononuclear fibrocytes that accumulate within the lung. Our preliminary studies in chronically hypoxic calves and rats demonstrate that collagen-producing cells in the pulmonary artery adventitia express leukocyte/macrophage markers (CD11b, CD14, CD45, CD68/ED1) (15).

The accumulation of myofibroblasts [α-smooth muscle (SM)-actin expressing fibroblasts] in the pulmonary artery adventitia of chronically hypoxic animals is well documented, and their presence is speculated to contribute to high pulmonary vascular resistance (49). It has even been suggested that the adventitial myofibroblasts can migrate and incorporate into the media, assume at least some SMC-like characteristics, and contribute to medial thickening (29, 51). Hypoxia can directly induce resident pulmonary artery adventitial fibroblasts to express α-SM-actin (49). In addition, it has been shown that

![Fig. 1. MCP, monocyte chemoattractant protein; SDF, stromal cell-derived factor; VEGF, vascular endothelial growth factor; ET, endothelin; TGF, transforming growth factor; PA, pulmonary artery; SMC, smooth muscle cell; ETR, ET receptor; VEGFR, VEGF receptor; ETR, ET receptor.](https://jap.physiology.org/)

J Appl Physiol • VOL 98 • FEBRUARY 2005 • www.jap.org
circulating fibrocytes can be induced to express α-SM-actin by factors including TGF-β (34, 39, 58). Thus it is possible that newly appearing myofibroblasts in the adventitia and media of chronically hypoxic animals are derived from nonresident cells. Support for this possibility comes from studies of the bronchial tissue of asthmatic lungs where the appearance of myofibroblasts was shown to be due, at least in part, to the recruitment of circulating fibrocytes (47). Our recent findings in both calves and rats also suggest that chronic hypoxic exposure induces the appearance of cells expressing a macrophage marker CD68/ED1 and α-SM-actin in the pulmonary artery adventitia. Thus circulating mononuclear cells/fibrocytes may contribute to the accumulation of collagen-producing cells and myofibroblasts observed in hypoxic forms of pulmonary hypertension.

It is also well known that tissue macrophages and fibrocytes are potent producers of pro-angiogenic factors (Table 1) and thus are important contributors to new blood vessel formation (18, 34, 36, 39). This concept is now well accepted in tumor biology, where macrophages have been shown to play critical roles in tumor angiogenesis (30, 34, 36). The appearance of monocytes in the adventitia of hypoxic animals thus may help explain our previously published finding of marked neovascularization in the adventitia of chronically hypoxic animals (12). One hypothesis is that mononuclear cells/fibrocytes are recruited to the arterial adventitia and stimulate neovascularization, which in turn acts as a conduit for continued delivery of circulating precursor cells. Thus a feedforward loop develops whereby circulating monocyctic cells are continuously delivered to the adventitial wall to participate in the remodeling process (Fig. 1).

Monocyte inactivation studies. The pathological role of circulating monocytes in vascular injury and repair has been highlighted by observations that demonstrate that transient inactivation of monocytes and macrophages by chemical inhibitors (clodronate-encapsulated liposomes, gadolinium chloride, and Mac-1 inhibitor NPC15669) ameliorates vascular remodeling in a number of settings (54, 26). Experiments using clodronate-encapsulated liposomes in balloon carotid injury models and vein grafts have demonstrated that inactivation or reduction in the number of circulating monocytes reduces significantly vascular remodeling (11, 21, 54). In addition, other studies demonstrated that monocyte depletion by clodronate liposomes decreases pathological neovascularization in a model of proliferative retinopathy (25). This is consistent with the hypothesis raised above that monocytes are critical participants in a feedforward loop of neovascularization and remodeling in the hypoxic pulmonary artery.

Studies based on other experimental approaches have also supported the idea that leukocytes are important players in various models of pulmonary hypertension. Kourembanas and colleagues (35, 60) demonstrated that transgenic mice overexpressing hemoxygenase-1 (HO-1) are protected from the development of pulmonary inflammation, as well as pulmonary hypertension and vessel wall hypertrophy induced by hypoxia. Significantly, the hypoxic induction of proinflammatory cytokines and chemokines was suppressed in HO-1 transgenic mice. Ikeda et al. (24) demonstrated that a dominant negative inhibitor of CCL2/MCP-1 chemokine significantly reduced the progression of monocrotaline-induced pulmonary hypertension as evaluated by right ventricular systolic pressure and hypertrophy, medial hypertrophy of pulmonary arteries, and mononuclear cell infiltration into the lung (24). Furthermore, gadolinium chloride injections prevented right ventricular hypertrophy and smooth muscle hyperplasia around pulmonary vessels in a neonatal rat model of hypoxia-induced (60% O2) pulmonary hypertension (26).

CONCLUSION

Experimental data are rapidly accumulating in support of the idea that circulating monocytes and/or mononuclear fibrocytes may be recruited to the pulmonary circulation in response to both acute and chronic hypoxia and that these cells play an important role in the pulmonary hypertensive process. Early leukocyte recruitment could participate in increasing permeability of the pulmonary and/or bronchial circulation. Early changes in permeability may be an early critical step in the remodeling process. Continued recruitment of monocytes and mononuclear fibrocytes might contribute directly (via differentiation into collagen producing cells and/or myofibroblasts) to structural changes. These cells could also induce phenotypic changes in resident SMC and fibroblasts. They could also stimulate angiogenesis of the vasa vasorum as suggested in atherosclerosis, which, in turn, would provide a conduit for further delivery of leukocytes and progenitor cells. If indeed nonresident cells play a significant role in the vascular remodeling process, new therapeutic options for the treatment of pulmonary hypertension are raised. Understanding the mechanisms of cell recruitment and the pathways used to differentiate into mesenchymal cells will be critical first steps in unraveling the role of these cells in the hypoxic pulmonary hypertensive process.

ACKNOWLEDGMENTS

The authors gratefully acknowledge J. A. Brunetti and D. L. Burke for excellent technical assistance, Dr. I. F. McMurtry for valuable discussion, and M. McGowan and S. E. Hofmeister for help in preparation of this manuscript.

GRANTS

The project was supported by National Heart, Lung, and Blood Institute Grants SCOR 5 P50-HL-57144-08, PPG 5 PO1-HL-14985-32.

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Invited Review

HYPOXIA, LEUKOCYTES, AND THE PULMONARY CIRCULATION


