Respiratory Physiology & Neurobiology 178 (2011) 439–448

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Review

Alveolar macrophages initiate the systemic microvascular inflammatory response to alveolar hypoxia☆,☆☆

Jie Chao[a,1], John G. Wood[b], Norberto C. Gonzalez[a,*]

[a] Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, USA
[b] Department of Surgery, University of Kansas Medical Center, Kansas City, KS 66160, USA

ARTICLE INFO

Article history:
Accepted 7 March 2011

Keywords:
Alveolar hypoxia
Alveolar macrophages
Systemic inflammation
Mast cells
MCP-1/CCL2

ABSTRACT

Alveolar hypoxia occurs as a result of a decrease in the environmental P_{O_2}, as in altitude, or in clinical conditions associated with a global or regional decrease in alveolar ventilation. Systemic effects, in most of which an inflammatory component has been identified, frequently accompany both acute and chronic forms of alveolar hypoxia. Experimentally, it has been shown that acute exposure to environmental hypoxia causes a widespread systemic inflammatory response in rats and mice.

Recent research has demonstrated that alveolar macrophages, in addition to their well known intrapulmonary functions, have systemic, extrapulmonary effects when activated, and indirect evidence suggest these cells may play a role in the systemic consequences of alveolar hypoxia.

This article reviews studies showing that the systemic inflammation of acute alveolar hypoxia observed in rats is not initiated by the low systemic tissue P_{O_2}, but rather by a chemokine, Monocyte Chemoattractant Protein-1 (MCP-1, or CCL2) released by alveolar macrophages stimulated by hypoxia and transported by the circulation. Circulating MCP-1, in turn, activates perivascular mast cells to initiate the microvascular inflammatory cascade.

The research reviewed here highlights the extrapulmonary effects of alveolar macrophages and provides a possible mechanism for some of the systemic effects of alveolar hypoxia.

1. Introduction

Alveolar hypoxia may occur as a result of reduced inspired P_{O_2} as in altitude, or in acute or chronic pulmonary diseases that lead to hypoventilation and the resulting fall in alveolar O_2 levels. In addition to its pulmonary effects, such as hypoxic pulmonary hypertension and high altitude pulmonary edema, alveolar hypoxia is frequently associated with systemic effects. These include the illnesses of high altitude: acute mountain sickness and high altitude cerebral edema (HACE) (Basnyat and Murdoch, 2003; Beidleman et al., 2006; Hartmann et al., 2000), the multiple organ failure secondary to atelectasis (Kisala et al., 1993), acute lung injury (Ciesla et al., 2006; Puneet et al., 2005; St John et al., 1993), and pulmonary contusion (Perl et al., 2005), and the systemic inflammation of pneumonia (Fernandez-Serrano et al., 2003; Yende et al., 2008). Chronic alveolar hypoxia is also frequently associated with systemic effects, such as the cachexia and muscle wasting of chronic obstructive pulmonary disease (Eickhoff et al., 2008; Koechlin et al., 2005; Wouters, 2005; Wust and Degens, 2007), the insufficient hemopoietic response in pulmonary fibrosis (Tsantes et al., 2003), and the cardiovascular and metabolic dysfunctions in sleep apnea (Jelic et al., 2008; Morgan, 2007; Punjabi and Beamer, 2007). Most of the conditions listed above show an inflammatory component; while it is possible that systemic inflammation does not play a causal role in every one of them, it is reasonable to assume that inflammation influences their development and outcome.

In addition to these overall deleterious effects, systemic inflammation may influence the adaptive responses that accompany exposure to alveolar hypoxia. The central role played by the changes in gene expression induced by Hypoxia-Inducible Factor (HIF) in the adaptation of intact organisms to hypoxia continues to be a fertile area of research. It is now clear that in addition to reduced tissue P_{O_2} inflammatory agents and reactive O_2 species (ROS) are capable of promoting the stabilization of HIF and thereby influence the expression of genes that control O_2 delivery and uptake; in this respect, numerous links between hypoxia and inflammation have been demonstrated (Bonello et al., 2007; Dehne

☆ This paper is part of a special issue entitled "Inflammation and Cardio-Respiratory Control", guest-edited by Frank L. Powell and Yu Ru Kou.

☆☆ Supported by National Institutes of Health USA Grant HL 39443 (NCG).

* Corresponding author at: Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160, USA. Tel.: +1 913 588 7028; fax: +1 913 588 7430.
E-mail address: ngonzale@kumc.edu (N.C. Gonzalez).
[1] Dr Chao was a pre-doctoral Fellow of the American Heart Association. His current address is Department of Physiology, University of Nebraska Medical Center, Omaha, NE, USA.

1569-9048/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.resp.2011.03.008

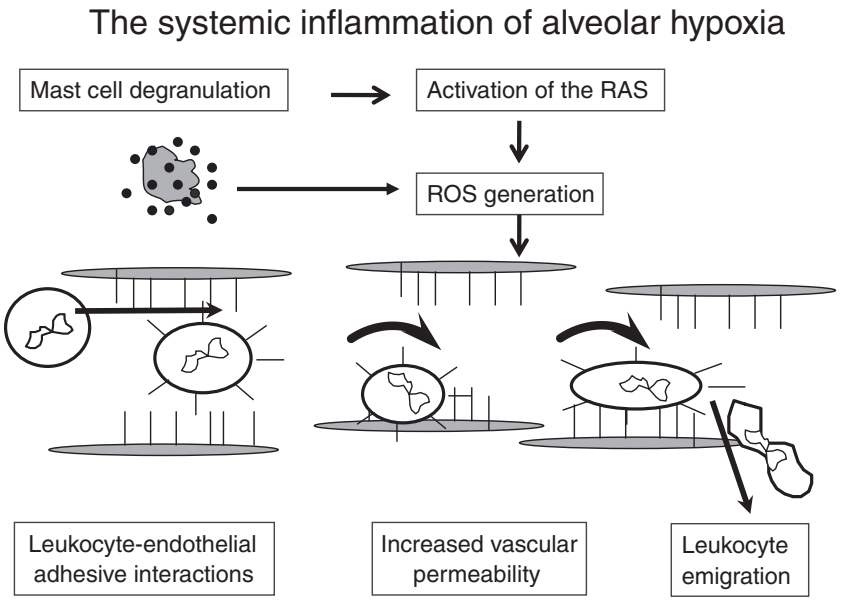

Fig. 1. Schematic representation of the inflammatory events taking place in a post-capillary venule after the onset of alveolar hypoxia.

and Brune, 2009; Dosek et al., 2007; Fitzpatrick et al., 2011; Richard et al., 2000; Taylor, 2008).

Recent research has shown that in addition to its well-known intrapulmonary effects, activation of alveolar macrophages has systemic, extrapulmonary consequences. For instance, the respiratory burst elicited by phagocytosis of particulate matter is accompanied by the release of alveolar macrophage (AMO) cytokines into the circulation. These cytokines act on the bone marrow to mobilize leukocytes and platelets and initiate systemic tissue inflammation (Ishii et al., 2005; Nurkiewicz et al., 2006; van Eeden et al., 2001). Another example is the possible "overspill" of agents generated by pulmonary cells, including alveolar macrophages, as a contributor to the systemic effects of chronic obstructive pulmonary disease (Sinden and Stockley, 2010).

Taken together, these observations show that investigation of the links between alveolar macrophages, alveolar hypoxia, and systemic inflammation could provide insights into the pathogenesis of the systemic effects present in conditions associated with alveolar hypoxia, and increase the understanding of the mechanisms of adaptation of entire organisms to hypoxia.

The objective of this article is to review studies on a novel subject, the systemic inflammatory response to acute alveolar hypoxia. Specifically, this review will discuss the evidence supporting a role of an AMO-borne chemokine, Monocyte Chemoattractant Protein-1 (MCP-1) also known as Chemokine (C–C motif) ligand 2 (CCL2), as the agent that initiates the systemic inflammation of hypoxia.

2. The systemic inflammation of alveolar hypoxia

Acute alveolar hypoxia results in a widespread systemic inflammatory response that develops within minutes of reduction of the inspired P_{O_2} of rats and mice. The inflammation has been observed directly in mesentery and skeletal muscle (Dix et al., 2003; Wood et al., 1999b); in addition, markers of inflammation, including expression of inflammatory mediators and increased vascular permeability have been documented in brain (Himadri et al., 2010; Kalpana et al., 2008) myocardium (Fitzpatrick et al., 2011) and lung (Beck-Schimmer et al., 2001; Fitzpatrick et al., 2011; Madjdpour et al., 2003) of hypoxic rats and mice. In particular, a role of AMO in the inflammatory response of the lung to hypoxia has been documented (Leeper-Woodford and Detmer, 1999; Madjdpour et al., 2003; VanOtteren et al., 1995; Zee et al., 2006). Indices of systemic inflammation have also been reported in humans exposed to altitude (Beidleman et al., 2006; Hartmann et al., 2000). The inflammation is characterized by generation of ROS (Wood et al., 1999a), reduced microvascular levels of nitric oxide (NO) (Steiner et al., 2002) degranulation of perivascular mast cells (MCs), (Steiner et al., 2003), increased leukocyte–endothelial adhesive interactions (Wood et al., 1999b), leukocyte emigration, and increased vascular permeability (Wood et al., 2000) (Fig. 1). ROS-dependent fluorescence signals are observed within MCs, in the endothelial layer of post-capillary venules, and at sites of leukocyte adherence to the post-capillary venular endothelium (Steiner et al., 2003). The ROS signals, as well as all the inflammatory markers, are attenuated by antioxidants and by exogenous NO donors (Wood et al., 1999b). Both the NO precursor L-arginine and NO donors are equally effective in attenuating the inflammation (Steiner et al., 2002). This suggests that the reduction of NO levels in hypoxia is not due to a decreased rate of NO synthesis induced by the low levels of O_2 substrate, but rather to NO depletion as a result of interaction with the elevated ROS.

MCs play a key role in the inflammation: MC degranulation is observed within 2 min of reduction in inspired P_{O_2} (Fig. 2); stabilization of MCs with sodium cromoglycate (Cromolyn) blocks the

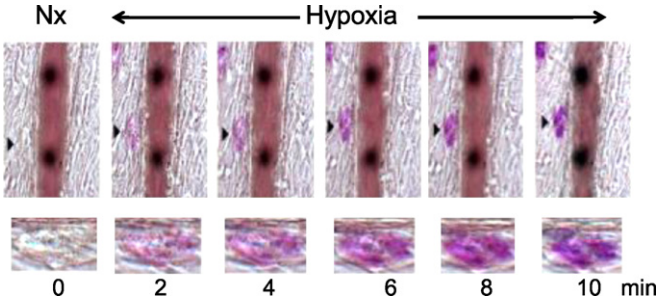

Fig. 2. Time course of MC degranulation, evidenced by the uptake of Ruthenium Red after the onset of alveolar hypoxia. Microphotographs of a mesenteric post capillary venule were obtained every 2 min. The large black dots are used to align the optical Doppler velocimeter used to measure red blood cell velocity. The bottom row is a magnification of the MC shown immediately to the left of the venule. Adapted from Steiner et al. (2003).

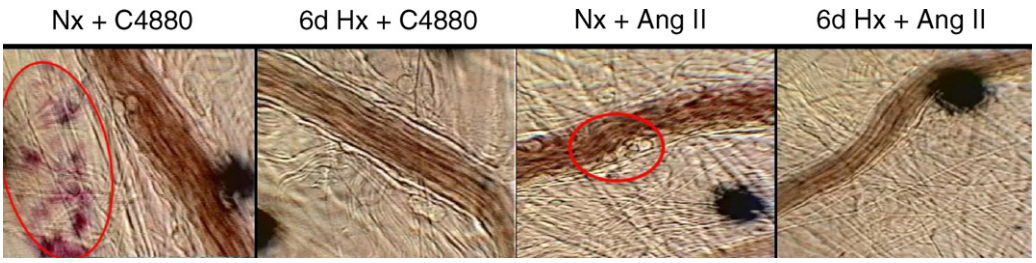

Fig. 3. Effects of topical administration of the MC secretagogue C4880 and of Angiotensin II (Ang II) on the mesenteric microcirculation of normoxic, non acclimatized rats (Nx) and of rats exposed to hypobaric hypoxia (PB = 380 Torr) for 6 days (6d Hx). The red ovals highlight degranulated MCs (Nx + C4880) and adherent leukocytes (Nx + Ang II).

MC degranulation and the inflammatory response (Steiner et al., 2003). Degranulation of MCs activates the local renin–angiotensin system (RAS) with generation of Angiotensin II (Ang II), a potent mediator of leukocyte recruitment and vascular permeability (Gonzalez et al., 2007a). Ang II is generated downstream of MCs, possibly as a result of liberation of renin contained in MCs (Chao et al., 2009a). Blockade of Ang II receptors, inhibitors of the angiotensin-converting enzyme (ACE) (Gonzalez et al., 2007a), as well as apocynin, an inhibitor of NADPH-oxidase assembly (Chao et al., 2009b) attenuate the systemic inflammatory response to hypoxia in intact rats. The inflammation is accompanied by initial arteriolar vasoconstriction that eventually subsides and is replaced by vasodilation. The initial vasoconstriction is blocked by Cromolyn and by inhibitors of the RAS system (Gonzalez et al., 2007a). While it is clear that Ang II contributes substantially to the microvascular inflammation of hypoxia, the participation of other MC-borne mediators in the inflammation cannot be ruled out.

After several days of exposure to hypoxia, the inflammation resolves spontaneously; furthermore, the microcirculation becomes resistant to more severe hypoxia (Wood et al., 1999b) and to exogenous inflammatory mediators. Fig. 3 compares the responses of post-capillary venules of the mesentery microcirculation to topical application of the general MC secretagogue C4880, and of Ang II on the mesentery of normoxic, non-acclimatized rats, and of rats exposed to hypobaric hypoxia (barometric pressure = 380 Torr) for 6 days. C4880 induces the expected degranulation of MCs and the accompanying increase in leukocyte endothelial adherence in the normoxic non-acclimatized rats, and fails to elicit a response in the acclimatized rats. Ang II, which acts downstream of MCs, also fail to induce increased leukocyte endothelial adherence. These results indicate that the process of acclimatization of the microcirculation takes place at the level of the MCs and also at the leukocyte–endothelial interface. Indirect evidence suggests that the increased resistance of the acclimatized microcirculation to inflammatory mediators may be in part the result of increased microvascular NO levels resulting from inducible NOs (iNOs) expression, since iNOs inhibitors restore the inflammation in acclimatized rats (Wood et al., 1999a). While it is clear that further research on the mechanism of the acclimatization of the microcirculation to prolonged hypoxia is necessary, the phenomenon itself is interesting, and the role of the acute inflammation on this process is a fertile area of future research.

3. The systemic inflammation of alveolar hypoxia is not initiated by the low systemic P_{O_2}

Considerable evidence indicates that the systemic inflammation of alveolar hypoxia is not initiated by the resulting fall in local tissue P_{O_2}; rather, it appears that the inflammatory cascade is triggered by activation of MCs by a circulating agent released by hypoxic AMO. The cascade illustrated in Fig. 4 best describes our current understanding of this phenomenon: AMO activated by alveolar hypoxia release MCP-1 into the circulation. MCP-1 induces degranulation of perivascular MCs, which leads to the activation of the local RAS and generation of Ang II. The activation of the RAS, in turn, promotes leukocyte–endothelial adhesive interactions and increased vascular permeability, in part as a result of NADPH-oxidase assembly. The following experimental evidence supports this sequence of events.

3.1. *The systemic inflammation develops only when alveolar P_{O_2} is reduced, independent of the systemic tissue P_{O_2}*

The first line of evidence suggesting that the systemic tissue P_{O_2} does not initiate the inflammation was the finding that inflammation in the cremaster muscle could be dissociated from the cremaster microvascular P_{O_2} (Dix et al., 2003; Shah et al., 2003). In experiments in intact rats, microvascular P_{O_2} of the cremaster muscle was altered independently of the systemic arterial P_{O_2} by equilibrating the cremaster with a given gas mixture while the animal breathed gas of a different composition. Arterial blood P_{O_2} and cremaster microvascular P_{O_2} were determined simultaneously, the latter being estimated using a phosphorescence quenching method. Reduction of alveolar P_{O_2} always produced mast cell degranula-

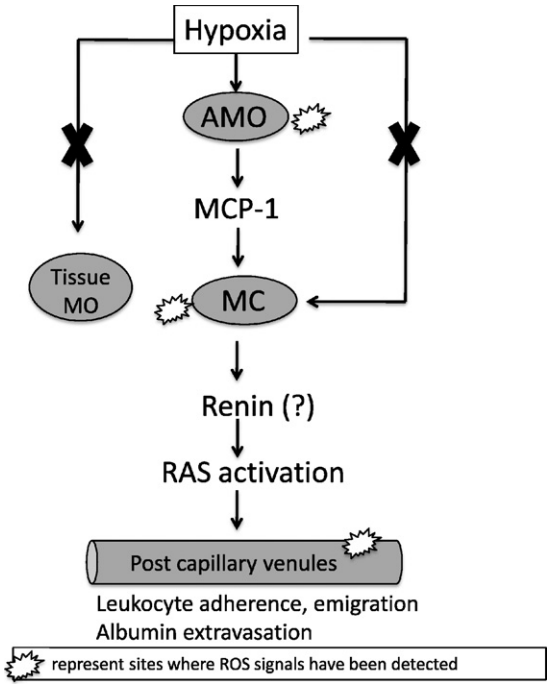

Fig. 4. Schematic representation of the inflammatory cascade initiated by alveolar hypoxia: AMO, alveolar macrophages, MCP-1: Monocyte Chemoattractant Protein-1 (also known as CCL2), tissue MO: systemic tissue macrophages, MC: mast cells; RAS: local renin–angiotensin system. ROS: reactive O_2 species.

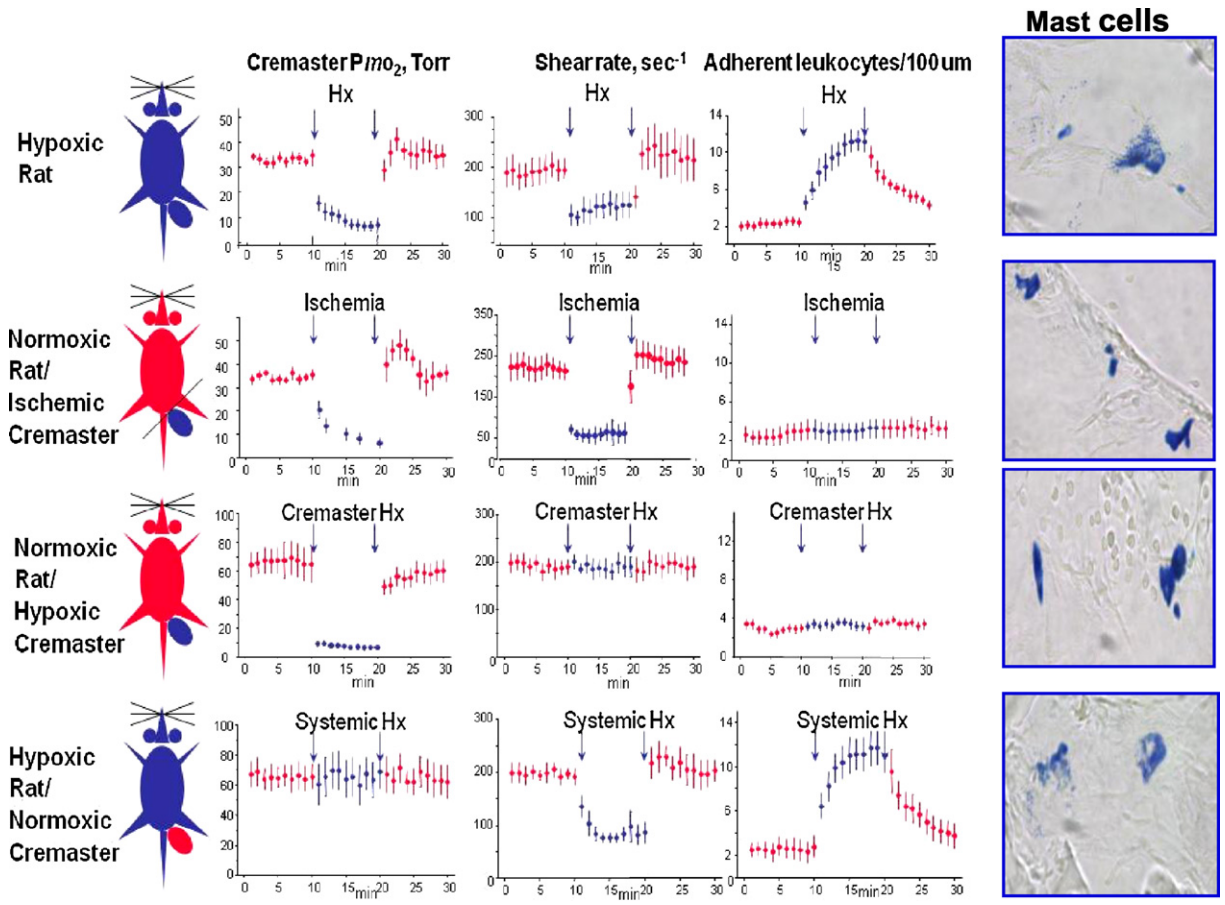

Fig. 5. Effects of independent changes in alveolar and cremaster muscle P_{O_2} (Pmo_2) in intact rats. Alveolar P_{O_2} was altered by changing the inspired P_{O_2}. Cremaster muscle P_{O_2} (estimated using a phosphorescence-quenching method) was altered by equilibrating the cremaster with either 95% N_2, 5% CO_2 (cremaster hypoxia), or 10% O_2, 85% N_2, 5% CO_2 (Cremaster normoxia). Cremaster ischemia was induced by mechanical compression of the cremaster pedicle. Cremaster mast cells were identified using Toluidine Blue staining.
Adapted from Dix et al. (2003) and Shah et al. (2003).

tion and increased leukocyte–endothelial adherence, independent of the value of cremaster microvascular P_{O_2} (Fig. 5, rows 1 and 4). On the other hand, reduction of cremaster microvascular P_{O_2} either by reduction of cremaster blood flow or by equilibration of the cremaster with a hypoxic gas mixture, did not elicit expression of inflammatory markers as long as alveolar P_{O_2} was maintained within normoxic levels (Fig. 5, rows 2 and 3, respectively.)

3.2. Plasma from hypoxic rats elicits inflammation in normoxic tissue

Although there are alternative explanations, the dissociation between tissue P_{O_2} and inflammatory response illustrated in Fig. 5 is compatible with the hypothesis that a circulating mediator released from a distant site initiates the inflammation. If this were correct, it would be expected that plasma from hypoxic rats, containing the mediator, would trigger inflammation in normoxic tissues. This was shown to be the case: plasma obtained from conscious rats at 5 min of breathing 10% O_2 produced MC degranulation, leukocyte–endothelial adherence (Orth et al., 2005) and increased vascular permeability (Gonzalez et al., 2007a) when applied to the cremaster muscle of normoxic rats. Normoxic rat plasma was ineffective, showing that the inflammatory effect is specific for hypoxic plasma. The agent responsible for the inflammation does not generate in blood cells, since plasma separated after *in vitro* equilibration of blood with hypoxic gas mixtures did not produce inflammation. Furthermore, plasma obtained from hypoxic rats in which the systemic inflammation was prevented by MC stabilization with Cromolyn did produce inflammation in normoxic cremaster. This indicates that the agent responsible for the inflammation does not originate in activated leukocytes or mast cells released into the plasma of the donor rat (Orth et al., 2005).

3.3. Alveolar macrophages are necessary for the systemic inflammation of alveolar hypoxia

Since the systemic inflammation develops only when alveolar P_{O_2} is reduced, independent of the local tissue P_{O_2} the lungs were considered a likely source of the inflammatory mediator contained in plasma of hypoxic rats. AMO, located in the surface of the alveoli is exposed to alveolar P_{O_2} and synthesize and release numerous agents that could contribute to an inflammatory response. As indicated above, evidence of extrapulmonary roles of AMO has been documented. Three independent lines of evidence confirmed AMO as the source of the circulating mediator of inflammation (Gonzalez et al., 2007b): first, alveolar hypoxia failed to elicit systemic inflammation in rats in which AMO was depleted by the intra-tracheal administration of clodronate-containing liposomes. AMO depletion prevented MC degranulation, leukocyte endothelial adherence and increased extravasation of albumin induced by alveolar hypoxia in cremaster (Fig. 6) and mesentery (Chao et al., 2009b). AMO-deficient animals did develop inflammation in response to the MC secretagogue C4880 and to Ang II, indicating that AMO depletion does not influence the ability of these animals to mount an inflam-

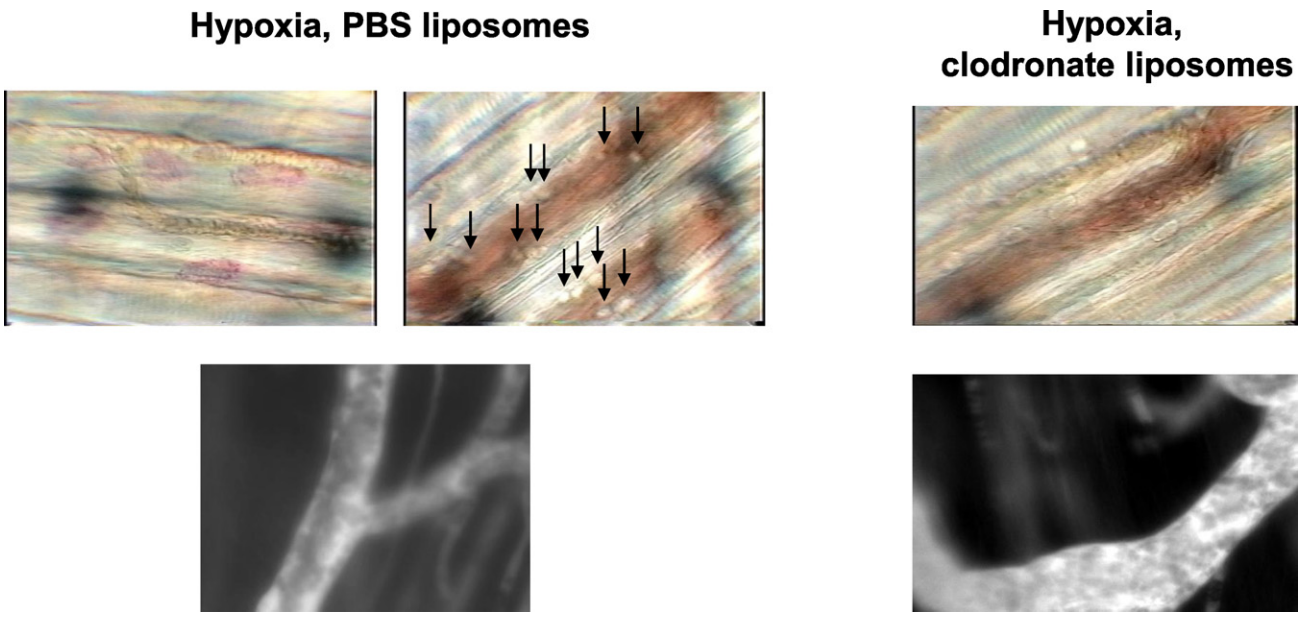

Fig. 6. Representative microphotographs of the cremaster microcirculation showing the effects of alveolar hypoxia (10% O_2 breathing) in rats with normal AMO count (Hypoxia, PBS liposomes) and in AMO-depleted rats (Hypoxia, clodronate liposomes). The top two bright field images of the PBS liposomes rat show the expected effect of hypoxia on MC degranulation (left photograph), and on leukocyte endothelial adherence (Right). The bottom photograph shows the increased extravasation of fluorescence-labeled albumin (FITC). The photographs of the clodronate liposomes rat show no MC degranulation or leukocyte–endothelial adherence, and minimal FITC albumin extravasation during hypoxia.
Adapted from Gonzalez et al. (2007b).

matory response (Gonzalez et al., 2007b). Second, plasma obtained from hypoxic, AMO-depleted rats failed to induce inflammation in normoxic tissues in contrast to plasma obtained from hypoxic rats treated with PBS-containing liposomes. Third, supernatant of primary cultures of AMO exposed to hypoxia, but not of normoxic AMO, also induced an inflammatory response in normoxic cremaster and mesentery (Gonzalez et al., 2007b; Chao et al., 2009b).

4. An AMO-borne circulating mediator initiates the systemic inflammation

The data presented so far supports the hypothesis that a mediator released into the circulation by hypoxic AMO, rather than the local tissue hypoxia, initiates the inflammation. This hypothesis is supported by the following findings.

4.1. Alveolar macrophages, but not resident tissue macrophages, are directly activated by low P_{O_2}

Primary cultures of AMO and of peritoneal macrophages were exposed to hypoxia for 30 min (Chao et al., 2009b). The different P_{O_2} environments to which alveolar and systemic macrophages are exposed *in vivo* were taken into account. Under normoxic conditions, AMO are exposed to P_{O_2} of approximately 100 Torr, while systemic tissue macrophages reside in environments where to P_{O_2} values range between 20 and 40 Torr. Fig. 7A shows that lowering P_{O_2} from 100 to 70 Torr induces a transitory release of H_2O_2 into the supernatant of primary AMO cultures. This is a relatively mild level of alveolar hypoxia; the magnitude of the release of H_2O_2, a reflection of the AMO respiratory burst, is inversely related to the P_{O_2} (Chao et al., 2009b). In contrast, lowering P_{O_2} to ~5 Torr did not induce a respiratory burst in peritoneal macrophages. To put this value into perspective, intact rats breathing 10% O_2 show a cremaster microvascular P_{O_2} of approximately 6–8 Torr (Fig. 5). The discrepancy in the response to hypoxia between AMO and peritoneal macrophages highlights the different characteristics of these cell types. Although both are originated from the same progenitor in the bone marrow, the different P_{O_2} of the environments in which they reside may determine their different metabolic patterns (Simon et al., 1977) and perhaps explains their different responses to hypoxia.

4.2. MCs are not directly activated by hypoxia, but degranulate in contact with hypoxic AMO supernatant, but not with supernatant hypoxic peritoneal macrophages

Fig. 7B shows that isolated peritoneal MCs do not degranulate when medium P_{O_2} is reduced to approximately 5 Torr for 30 min, indicating that, at least within the time frame of the development of the systemic inflammation, hypoxia has no direct effect on MC degranulation. On the other hand, MCs underwent degranulation when immersed in supernatant of hypoxic AMO but not of hypoxic peritoneal macrophages. This indicates that the agent responsible for MC activation during hypoxia is released by AMO, but not by peritoneal macrophages, and is consistent with the different effects of hypoxia in the activation of AMO and peritoneal macrophages. The lack of a direct effect of hypoxia on MCs and on systemic tissue macrophages is in agreement with the *in vivo* findings represented in Fig. 5, in which selective cremaster hypoxia failed to induce cremaster MC degranulation and inflammation as long as alveolar P_{O_2} remained within normoxic levels. In this respect, it should be noted that the similarity of responses of MCs in skeletal muscle and peritoneum are consistent with the notion of a widespread inflammation initiated by a circulating mediator.

5. MCP-1 triggers the systemic inflammation of hypoxia

The results described above provided useful clues to identify the agent that initiates the systemic inflammation of hypoxia. Given the rapid onset of the inflammation, it is reasonable to assume that the mediator is a mast cell secretagogue either stored or rapidly synthesized by AMO. The first step of the process of identification of the AMO-borne mediator of the systemic inflammation was to determine the concentration of various MC secretagogues in the

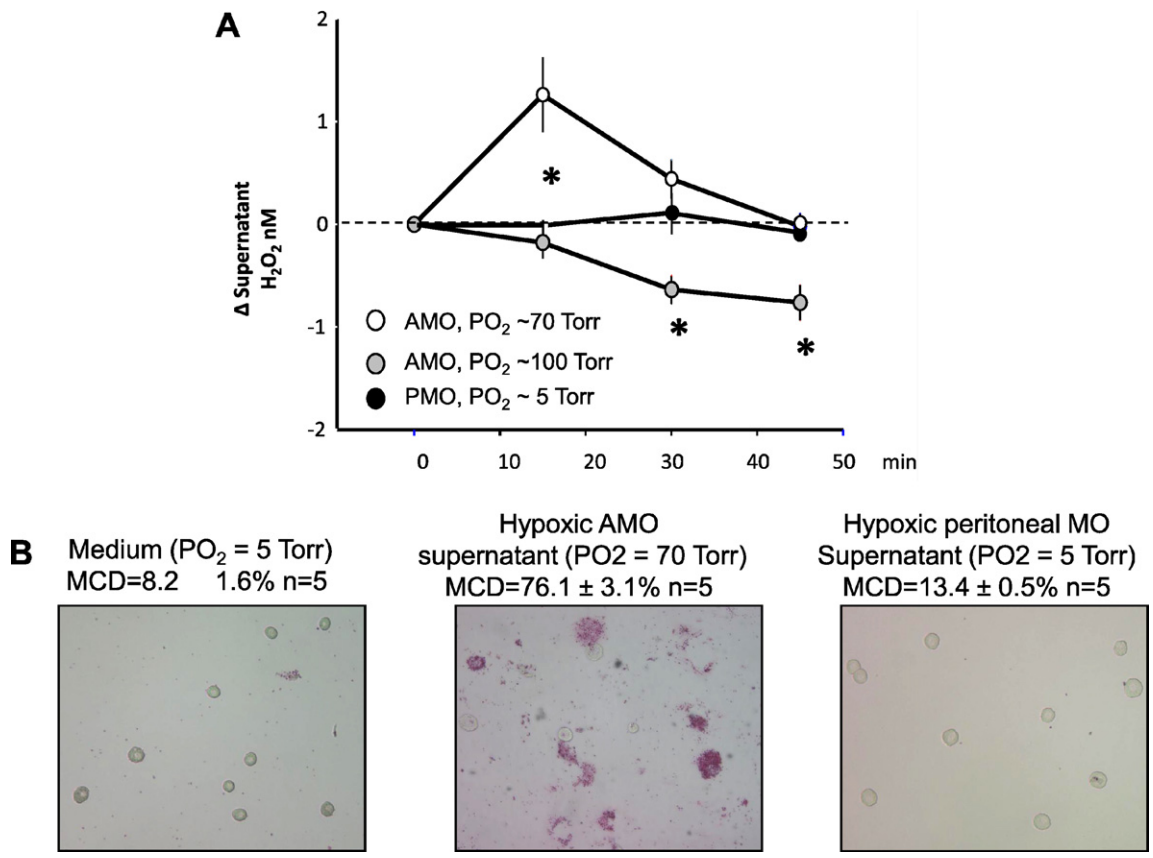

Fig. 7. (A) Changes in the macrophage supernatant concentration of H_2O_2 as a function of time in hypoxia or normoxia. Adapted from Chao et al. (2009b). (B) Representative microphotographs of MCs immersed in culture medium (left) supernatant of AMO equilibrated with P_{O_2} 70 Torr (center) and supernatant of peritoneal macrophages equilibrated with P_{O_2} 5 Torr. MC degranulation is evidenced by uptake of Ruthenium Red. Adapted from Chao et al. (2009b).

supernatant of primary cultures of AMO exposed to hypoxia for 30 min. Of 12 different agents, only MCP-1 was released into the supernatant of AMO exposed to hypoxia within this time frame (Fig. 8).

MCP-1 fits the criteria for a putative mediator of the inflammation of alveolar hypoxia: MCP-1 induces chemotaxis of alveolar macrophages, mast cells and human T-lymphocytes (de Boer et al., 2000), and is released from AMO *in vitro* in response to hypoxia (Fig. 8) and hypoxia-reoxygenation (Krishnadasan et al., 2003; Zhao et al., 2006). Hypoxia induces MCP-1 release from AMO but not from peritoneal MCs or peritoneal macrophages (Chao et al., 2009b). However, in order to demonstrate that MCP-1 is the AMO-borne circulating agent that initiates the systemic inflammation of hypoxia, several criteria must be met: first, hypoxia must induce an increase in plasma MCP-1 concentration with a time course compatible with the development of the inflammation, and of a magnitude sufficient to initiate the inflammatory response; and AMO must be demonstrated as a source of this increase in plasma MCP-1. Second, the inflammatory response to hypoxia must be linked to MCP-1, in other words, MCP-1 must replicate the inflammation of hypoxia in normoxic animals, and antagonism of the effects of MCP-1 must abrogate the inflammation in hypoxic animals. The data reviewed below (Chao et al., 2010) confirms the hypothesis that AMO-borne MCP-1 initiates the systemic inflammation of hypoxia.

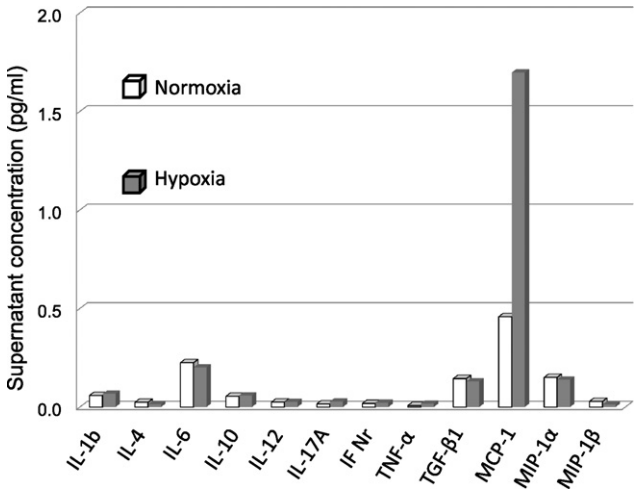

Fig. 8. Cytokine and chemokine concentration in the supernatant of primary AMO cultures during normoxia and after 30 min of equilibration with P_{O_2} 70 Torr.

5.1. Plasma MCP-1 concentration increases in intact rats during hypoxia

Conscious intact rats breathing 10% O_2 showed a rapid and sustained increase in plasma MCP-1 concentration which was elevated already at 5 min of hypoxia (Fig. 9, black triangles). The major source of the initial increase of MCP-1 is AMO, since AMO depletion by tracheal instillation of clodronate liposomes completely abolished the increase in MCP-1 observed at 5 and 30 min of hypoxia (Fig. 9, gray circles). At 1 h of 10% O_2 breathing, however, the plasma concentration of MCP-1 in the AMO-depleted rats had increased to approximately 40% of the value observed in the intact rats at the same time of hypoxia. The source of the late increase in plasma MCP-1 in these rats is likely to be cells other than AMO, rather than the small number of AMO remaining after treatment with clo-

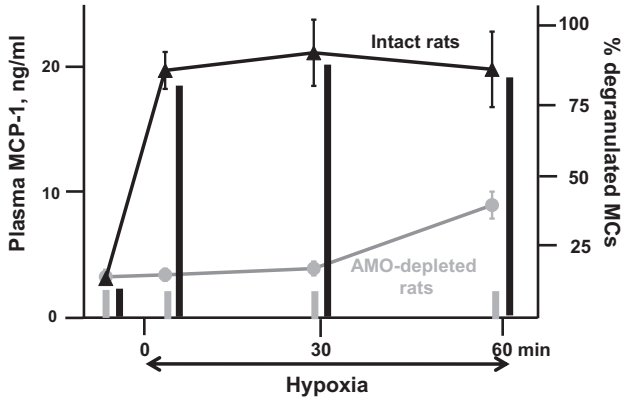

Fig. 9. The left vertical axis represents the plasma concentration of MCP-1 in conscious intact rats (black triangles) and in AMO-depleted rats (gray circles) during normoxia and hypoxia (10% O_2 breathing). The left axis represents the percentage of degranulated MCs immersed in the plasma of the samples obtained in both groups of rats. Black vertical bars: intact rats; gray vertical bars, AMO-depleted rats.
Adapted from Chao et al. (2010).

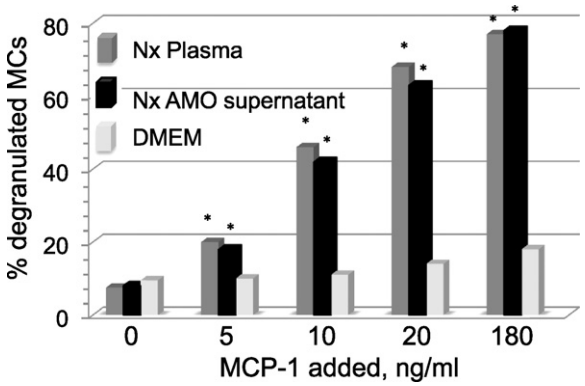

Fig. 10. MCP-1 concentration-dependence of Mc degranulation: the percentage of degranulated MCs observed after immersion in normoxic rats plasma (NX plasma), supernatant of primary AMO cultures equilibrated in normoxia (Nx AMO supernatant) and serum-free DMEM cell culture medium (DMEM) are plotted against the concentrations of MCP-1 added.
Adapted from Chao et al. (2010).

dronate liposomes, which is less than 5% of the number of AMO recovered with broncho-alveolar lavage from intact rats (Gonzalez et al., 2007b). In addition to the disproportionate levels of plasma MCP-1 in relation to the number of AMO, MCP-1 released from the remaining AMO should follow a time course similar to that observed in the intact rats. Whether the stimulus for the late increase in MCP-1 is the low tissue P_{O_2} or some other factor associated with hypoxia, it is clear that MCP-1 from these alternative sources does not play a role in the rapid onset of inflammation, although it may contribute to the inflammatory process at later stages of hypoxia.

5.2. The plasma concentration of MCP-1 reached during hypoxia is sufficient to elicit MC degranulation

To determine whether the plasma MCP-1 concentration reached during hypoxia was sufficient to induce MC degranulation and thus initiate the inflammation, peritoneal MCs were immersed in plasma of the samples obtained in the intact and in the AMO-depleted rats before and during 10% O_2 breathing. Immersion of MCs in plasma obtained during normoxia in either group produced minimal degranulation; in contrast, approximately 80% of the MCs immersed in the plasma obtained from the intact hypoxic rats underwent degranulation (Fig. 9, black vertical bars). On the other hand, the percentage of degranulated MCs immersed in plasma of hypoxic, AMO-depleted rats was not significantly different from that observed in MCs suspended in normoxic plasma (Fig. 9, gray vertical bars). This was the case even in the MCs immersed in plasma obtained at 60 min of hypoxia in the AMO-depleted rats, in which MCP-1 concentration was higher than in normoxia (Chao et al., 2010).

5.3. MCP-1 produces a concentration-dependent increase in the number of degranulated MCs

To determine whether the degranulation of MCs immersed in the plasma of intact hypoxic rats was due to the elevated MCP-1 concentration, or to some other factor related to hypoxia or AMO, the dependence of MC degranulation on the MCP-1 concentration was studied. MCP-1 added to either normoxic rat plasma or normoxic AMO supernatant produced a similar concentration-dependent increase in the number of degranulated MCs (Fig. 10). The data obtained in MCs immersed in plasma from intact hypoxic rats fits this relationship (compare Figs. 9 and 10). On the other hand, the low level of MC degranulation observed in MC immersed in plasma of normoxic intact rats, or in plasma of hypoxic, AMO-depleted rats, is in agreement with their lower levels of MCP-1. The only exception to the MCP-1 concentration-dependence of MC degranulation was the sample obtained at 60 min of hypoxia in the AMO-depleted rat: in this case, degranulation of MC immersed in this sample was low, in spite of a MCP-1 concentration that would have produced substantial MC degranulation when added to normal plasma or to AMO supernatant.

MCP-1 added to serum-free DMEM culture medium, on the other hand, did not produce MC degranulation, even at the highest concentrations. This finding may explain earlier results (Fureder et al., 1995) showing that MCP-1 concentrations even higher than those observed in hypoxic rat plasma failed to activate *in vitro* cultures of MCs. This lack of effect of MCP-1 *in vitro*, in turn, contradicts the documented effectiveness of MCP-1 to elicit MC degranulation *in vivo* (Wan et al., 2003). A possible explanation of this apparent discrepancy is that MCP-1 requires a co-factor to activate MC at biologically relevant concentrations. This co-factor would be present in plasma from normoxic intact rats and in the supernatant of normoxic AMO, and absent in standard culture media. Direct evidence of the existence of a co-factor for MCP-1 was provided by experiments where normoxic plasma and AMO supernatant were filtered through different pore sizes: 30, 50, and 100 kDa. MCP-1 and peritoneal MCs were added to both the filtrate and the retained fractions of each sample; the reasoning being that if a co-factor for MCP-1 is needed, MC degranulation would occur only in the fraction where the co-factor is present. Similar results were obtained in plasma and in AMO supernatant: MCP-1 induced MC degranulation only in the retained fraction of the 30 kDa filter and in the filtered fraction of the 100 kDa filter. No MC degranulation was observed either in the retained or the filtered fraction of the 50 kDa filter (Chao et al., 2010). These results provide persuasive evidence of the existence of the co-factor and suggest that it is composed of two fractions: one with a size between 30 and 50 kDa, and another between 50 and 100 kDa. Both fractions would be retained by the 30 kDa filter, and pass through the 100 kDa filter. In the 50 kDa filter both fractions would be separated, and MCP-1 would be unable to act.

While the nature of the MCP-1 co-factor is unknown, the results suggest that it is constitutively expressed in AMO and secreted into plasma. In addition to its presence in the supernatant of normoxic AMO, evidence for an AMO origin of this agent is that the MCP-1 concentration-dependence of MC degranulation is essentially the same in plasma and AMO supernatant, and that AMO appear to

be necessary for MCP-1 to induce MC degranulation *in vivo*: MC immersed in plasma of AMO-depleted rats exposed to 60 min of hypoxia underwent minimal degranulation, in site of the presence of MCP-1 in a concentration that could induce substantial MC degranulation. Another example that AMO presence is required for MCP-1 to activate MCs *in vivo* will be provided below.

5.4. MCP-1 administration to normoxic animals replicates the systemic inflammation of hypoxia

Another line of evidence of the key role of MCP-1 in the inflammation of hypoxia is that topical administration of MCP-1 to the mesentery of intact normoxic rats produces perivascular MC degranulation and increased leukocyte–endothelial adhesive interactions (Chao et al., 2010). A complementary finding is that RS-102895, a selective antagonist of CCR2b, the putative MCP-1 receptor expressed in MCs, blocks the MC degranulation and leukocyte endothelial adherence that accompanies acute alveolar hypoxia. Interestingly, topical application of MCP-1 dissolved in DMEM does not produce inflammation in the mesentery of normoxic AMO-depleted rats. On the other hand, these animals do show an inflammatory response to the application of MCP-1 dissolved in plasma obtained from intact rats with normal AMO count (Chao et al., 2010). These results provide additional evidence that AMO are necessary for the expression of the MCP-1 co-factor in biological fluids, by implying that its absence in the mesenteric environment of the AMO-depleted rats prevents MC degranulation in response to MCP-1 with DMEM as a vehicle. When the co-factor is provided in the plasma of intact rats, MCP-1 elicits the normal inflammatory response in these AMO-depleted rats.

In summary, the evidence reviewed above shows that MCP-1 fulfills the criteria to be considered the mediator of the systemic inflammation of hypoxia: (a) AMO-borne MCP-1 plasma concentration of intact animals increases during hypoxia with a time course compatible to the development of the systemic inflammation; (b) the plasma concentration reached during hypoxia is sufficient to activate MCs; (c) the extent of MC degranulation is dependent on the MCP-1 concentration; (d) administration of MCP-1 to normoxic animals replicates the inflammation of hypoxia and (e) the inflammation of hypoxia is attenuated by MCP-1 receptor antagonism. In addition, the results indicate that, in order for biologically relevant plasma concentrations of MCP-1 to effectively initiate the systemic inflammation of hypoxia, the presence of a co-factor constitutively expressed in AMO is necessary.

The studies summarized here highlight the extrapulmonary functions of AMO. The results show that AMO are capable of initiating the inflammation by providing, in addition to MCP-1, the co-factor necessary for MCP-1 to act at biologically relevant concentrations. Although the nature of the co-factor is still unknown, the evidence supports a substance constitutively expressed in AMO and released to the body fluids. The results provide additional support to previous observations of important extrapulmonary effects of AMO.

While the data presented provide persuasive evidence supporting the cascade represented in Fig. 4, several issues remain unclear and should be the subject of further research. In addition to the nature of the co-factor for MCP-1, relevant questions include the mechanisms of activation of the RAS by MCs, and a related issue, namely whether Ang II is the only inflammatory agent through which MCs produce microvascular inflammation.

6. Role of the AMO-initiated inflammation in the overall response to hypoxia

The data reviewed above indicate that reduction of inspired P_{O_2} in intact animals results in a rapid systemic inflammatory response initiated by activation of AMO. It should be kept in mind, however, that exposure of intact organisms to reduced environmental O_2 levels is a complex stimulus that elicits multiple responses. AMO-independent phenomena with a different time course, chiefly functional responses initiated by changes in gene expression initiated by the low P_{O_2} are likely to modify the initial response at later times in the course of hypoxia. Examples of AMO-independent inflammatory responses to hypoxia are the *in vitro* increased leukocyte–endothelial interactions observed under hypoxic conditions (Michiels et al., 2000; Montoya et al., 1997), and the release of inflammatory mediators in response to local hypoxia of adipose tissue (Trayhurn et al., 2008). Certainly, *in vivo* conditions of local hypoxia due to reduced convective O_2 delivery to the tissues are well known to elicit an inflammatory response, which is naturally not mediated by AMO activation.

Within this context, it must be also recognized that the AMO-dependent inflammation described here was observed in conditions similar to those observed in altitude, where hypoxia is accompanied by hypocapnia. In contrast, hypoxia in the clinical setting is usually accompanied by elevated CO_2 levels. Recent evidence indicates that CO_2 may suppress genes involved in the regulation of inflammation and innate immunity, in part as a result of modification of NF-κB activity (Taylor and Cummins, 2011). Accordingly, it is possible that the accompanying levels of CO_2 may in part modulate the inflammatory response to alveolar hypoxia.

Clearly the most relevant question in this area is the role of the AMO-induced inflammation on the overall response of intact organisms to alveolar hypoxia. At this moment, two main possibilities are visualized: on one hand, the inflammation may provide the pathophysiological basis for diseases of acute alveolar hypoxia, such as the illnesses of high altitude or the systemic effects of clinical conditions presenting alveolar hypoxia described above. Certainly, the number of conditions associated presenting alveolar hypoxia in association with systemic inflammation makes this a strong possibility.

Alternatively, the inflammation may help set in motion the processes that eventually lead to a state of microvascular "acclimatization" of the immune system in which either exogenous inflammatory agents (Fig. 3) or more severe hypoxia (Wood et al., 1999b) do not elicit microvascular inflammation. Whether this adaptive response includes the processes that are traditionally considered components of the acclimatization to hypoxia, i.e. phenomena that influence the supply of O_2 to the tissues, should also be considered. The extensive links between hypoxia and inflammation, and the documented effects of ROS and inflammatory mediators to promote HIF-1 stabilization suggest that an inflammatory response could be involved in the development of the acclimatization process; this process would include an increased tolerance to inflammatory stimuli as well as improved O_2 supply to the tissues. One example of a possible role of inflammation on the mechanisms of O_2 transport that contribute to the adaptation to hypoxia is the inflammatory nature of the increased peripheral chemoreceptor O_2 sensitivity that develops during the first few days of hypoxia (Liu et al., 2009; Powell and Fu, 2008), a phenomenon thought to underlie the ventilatory acclimatization to hypoxia.

There is no intrinsic contradiction between the apparently opposite alternatives of inflammation having deleterious effects and also contributing to hypoxia adaptation. One scenario is that the adverse effects of inflammation are most commonly overcome, but that the presence of some specific factors may lead to adverse consequences. For instance, while most individuals that ascend to altitude acclimatize successfully and do not develop altitude illnesses after acclimatization, the presence of risk factors, such as rate of ascent, altitude reached, and severity of exertion, may determine that a small fraction becomes ill.

The study of these alternative possibilities as the functional role of the systemic inflammation of hypoxia is an interesting and possibly fruitful avenue for future research.

References

Basnyat, B., Murdoch, D.R., 2003. High-altitude illness. Lancet 361, 1967–1974.

Beck-Schimmer, B., Schimmer, R.C., Madjdpour, C., Bonvini, J.M., Pasch, T., Ward, P.A., 2001. Hypoxia mediates increased neutrophil and macrophage adhesiveness to alveolar epithelial cells. Am. J. Respir. Cell Mol. Biol. 25 (6), 780–787.

Beidleman, B.A., Muza, S.R., Fulco, C.S., Cymerman, A., Staab, J.E., Sawka, M.N., Lewis, S.F., Skrinar, G.S., 2006. White blood cell and hormonal responses to 4300 m altitude before and after intermittent altitude exposure. Clin. Sci. (Lond.) 111, 163–169.

Bonello, S., Zahringer, C., BelAiba, R.S., Djordjevic, T., 2007. Reactive O_2 species activate the HIF-1α promoter via a functional NF-κB site. Arterioscler. Thromb. Vasc. Biol. 27, 755–761.

Chao, J., Wood, J.G., Blanco, V.G., Gonzalez, N.C., 2009a. Renin released from activated mast cells mediates the systemic inflammation of alveolar hypoxia. FASEB J. 23, 762.5.

Chao, J., Wood, J.G., Blanco, V.G., Gonzalez, N.C., 2009b. The systemic inflammation of alveolar hypoxia is initiated by an alveolar macrophage-borne mediator(s). Am. J. Respir. Cell Mol. Biol. 41, 576–582.

Chao, J., Donham, P., van Rooijen, N., Wood, J.G., Gonzalez, N.C., 2010. MCP-1/CCL2 released from alveolar macrophages mediates the systemic inflammation of alveolar hypoxia. Am. J. Respir. Cell Mol. Biol. 2 (September) (Epub ahead of print).

Ciesla, D.J., Moore, E.E., Johnson, J.L., Cothren, C.C., Banerjee, A., Burch, J.M., Sauaia, A., 2006. Decreased progression of postinjury lung dysfunction to the acute respiratory distress syndrome and multiple organ failure. Surgery 140, 640–647.

de Boer, W.I., Sont, J.K., van Schadewijk, A., Stolk, J., van Krieken, J.H., Hiemstra, P.S., 2000. Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD. J. Pathol. 190, 619–626.

Dehne, N., Brune, B., 2009. HIF-1 in the inflammatory microenvironment. Exp. Cell. Res. 315, 1791–1797.

Dix, R., Orth, T., Allen, J., Wood, J.G., Gonzalez, N.C., 2003. Activation of mast cells by systemic hypoxia, but not by local hypoxia, mediates increased leukocyte–endothelial adherence in cremaster venules. J. Appl. Physiol. 95, 2495–2502.

Dosek, A., Ohno, H., Taylor, A.W., Radak, Z., 2007. High altitude and oxidative stress. Resp. Physiol. Neurobiol. 158, 128–131.

Eickhoff, P., Valipour, A., Kiss, D., Schreder, M., Cekici, L., Geyer, K., Kohansal, R., Burghuber, O.C., 2008. Determinants of systemic vascular function in patients with stable chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 178, 1211–1218.

Fernandez-Serrano, S., Dorca, J., Coromines, M., Carratala, J., Gudiol, F., Manresa, F., 2003. Molecular inflammatory responses measured in blood of patients with severe community-acquired pneumonia. Clin. Diagn. Lab. Immunol. 10, 813–820.

Fitzpatrick, S.F., Tambulawa, M.M., Brunning, U., et al., 2011. An intact canonical NF-kB pathway is required for inflammatory gene expression in response to hypoxia. J. Immunol. 186, 1091–1096.

Fureder, W., Agis, H., Semper, H., Keil, F., Maier, U., Muller, M.R., Czerwenka, K., Hofler, H., Lechner, K., Valent, P., 1995. Differential response of human basophils and mast cells to recombinant chemokines. Ann. Hematol. 70, 251–258.

Gonzalez, N.C., Allen, J., Schmidt, E.J., Casillan, A.J., Orth, T., Wood, J.G., 2007a. Role of the renin–angiotensin system in the systemic microvascular inflammation of alveolar hypoxia. Am. J. Physiol. Heart Circ. Physiol. 292, H2285–H2294.

Gonzalez, N.C., Allen, J., Blanco, V.G., Schmidt, E.J., van Rooijen, N., Wood, J.G., 2007b. Alveolar macrophages are necessary for the systemic inflammation of acute alveolar hypoxia. J. Appl. Physiol. 103, 1386–1394.

Hartmann, G., Tschop, M., Fischer, R., Bidlingmaier, C., Riepl, R., Tschop, K., Hautmann, H., Endres, S., Toepfer, M., 2000. High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. Cytokine 12, 246–252.

Himadri, P., Kumari, M., Chitharanjan, M., Dhananjay, S., 2010. Role of oxidative stress and inflammation inhypoxia-induced cerebral edema: a molecular approach. High Alt. Med. Biol. 11, 231–244.

Ishii, H., Hayashi, S., Hogg, J.C., Fujii, T., Goto, Y., Sakamoto, N., Mukae, H., Vincent, R., van Eeden, S.F., 2005. Alveolar macrophage–epithelial cell interaction following exposure to atmospheric particles induces the release of mediators involved in monocyte mobilization and recruitment. Respir. Res. 6, 87.

Jelic, S., Padeletti, M., Kawut, S.M., Higgins, C., Canfield, S.M., Onat, D., Colombo, P.C., Basner, R.C., Factor, P., LeJemtel, T.H., 2008. Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea. Circulation 117, 2270–2278.

Kalpana, S., Dhananjay, S., Anju, B., Ram, E., 2008. Cobalt chloride attenuates hypobaric hypoxia induced vascular leakage in rat brain: molecular mechanisms of action of cobalt chloride. Toxicol. Appl. Pharmacol. 231, 354–363.

Kisala, J.M., Ayala, A., Stephan, R.N., Chaudry, I.H., 1993. A model of pulmonary atelectasis in rats: activation of alveolar macrophage and cytokine release. Am. J. Physiol. 26, R610–614.

Koechlin, C., Maltais, F., Saey, D., Michaud, A., LeBlanc, P., Hayot, M., Prefaut, C., 2005. Hypoxaemia enhances peripheral muscle oxidative stress in chronic obstructive pulmonary disease. Thorax 60, 834–841.

Krishnadasan, B., Naidu, B.V., Byrne, K., Fraga, C., Verrier, E.D., Mulligan, M.S., 2003. The role of inflammatory cytokines in lung ischemia–reperfusion injury. J. Thorac. Cardiovasc. Surg. 203, 261–272.

Leeper-Woodford, S.K., Detmer, K., 1999. Acute hypoxia increases alveolar macrophage tumor necrosis factor activity and alters NF-kappaB expression. Am. J. Physiol. 276, L909–916.

Liu, X., He, L., Stensaas, L., Dinger, B., Fidone, S., 2009. Adaptation to chronic hypoxia involves immune cell invasion and increased expression of inflammatory cytokines in rat carotid body. Am. J. Physiol. Lung Cell. Mol. Physiol. 296, L158–L166.

Madjdpour, C., Jewell, U.R., Kneller, S., Ziegler, U., Schwendener, R., Booy, C., Klausli, L., Pasch, T., Schimmer, R.C., Beck-Schimmer, B., 2003. Decreased alveolar oxygen induces lung inflammation. Am. J. Physiol. Lung Cell. Mol. Physiol. 284, L360–L367.

Michiels, C., Arnoud, T., Remacle, J., 2000. Endothelial cell responses to hypoxia: initiation of a cascade of cellular interactions. Biochim. Biophys. Acta 1497, 1–10.

Montoya, M.C., Luscinskas, F.W., del Pozo, M.A., Aragones, J., de Landazuri, M.O., 1997. Reduced intracellular metabolism promotes firm adhesion of polymorphonuclear leukocytes to vascular endothelium under flow conditions. Eur. J. Immunol. 27, 1942–1951.

Morgan, B.J., 2007. Vascular consequences of intermittent hypoxia. Adv. Exp. Med. Biol. 618, 69–84.

Nurkiewicz, T.R., Porter, D.W., Barger, M., Millecchia, L., Rao, K.M., Marvar, P.J., Hubbs, A.F., Castranova, V., Boegehold, M.A., 2006. Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure. Environ. Health Perspect. 114, 412–419.

Orth, T., Allen, J.A., Wood, J.G., Gonzalez, N.C., 2005. Plasma from conscious hypoxic rats stimulates leukocyte–endothelial interactions in normoxic cremaster venules. J. Appl. Physiol. 99, 290–297.

Perl, M., Gebhard, F., Bruckner, U.B., Ayala, A., Braumuller, S., Buttner, C., Kinzl, L., Knoferl, M.W., 2005. Pulmonary contusion causes impairment of macrophage and lymphocyte immune functions and increases mortality associated with a subsequent septic challenge. Crit. Care Med. 33, 1351–1358.

Powell, F.L., Fu, Z., 2008. HIF-1 and ventilatory acclimatization to chronic hypoxia 2008. Respir. Physiol. Neurobiol. 164, 282–287.

Puneet, P., Moochhala, S., Bhatia, M., 2005. Chemokines in acute respiratory distress syndrome. Am. J. Physiol. Lung Cell. Mol. Physiol. 288, L3–L15.

Punjabi, N.M., Beamer, B.A., 2007. C-reactive protein is associated with sleep disordered breathing independent of adiposity. Sleep 30, 29–34.

Richard, D.E., Berra, E., Pouyssegur, J., 2000. Non-hypoxic pathway mediates the induction of hypoxia inducible factor 1alpha in vascular smooth muscle cells. J. Biol. Chem. 275, 26765–26771.

Shah, S., Allen, J., Wood, J.G., Gonzalez, N.C., 2003. Dissociation between skeletal muscle microvascular P_{O_2} and hypoxia-induced microvascular inflammation. J. Appl. Physiol. 94, 2323–2329.

Simon, L.M., Robin, E.D., Phillips, J.R., Acevedo, J., Axline, S.G., Theodore, J., 1977. Enzymatic basis for bioenergetic differences of alveolar versus peritoneal macrophages and enzyme regulation by molecular O_2. J. Clin. Invest. 59, 443–448.

Sinden, N.A., Stockley, R.A., 2010. Systemic inflammation and comorbidity in COPD: a result of "overspill" of inflammatory mediators from the lungs? Rev. Evid. Thorax 65, 930–936.

St John, R.C., Mizer, L.A., Kindt, G.C., Weisbrode, S.E., Moore, S.A., Dorinsky, P.M., 1993. Acid aspiration-induced acute lung injury causes leukocyte-dependent systemic organ injury. J. Appl. Physiol. 74, 1994–2003.

Steiner, D.R., Gonzalez, N.C., Wood, J.G., 2002. Interaction between reactive oxygen species and nitric oxide in the microvascular response to systemic hypoxia. J. Appl. Physiol. 93, 1411–1418.

Steiner, D.R., Gonzalez, N.C., Wood, J.G., 2003. Mast cells mediate the microvascular inflammatory response to systemic hypoxia. J. Appl. Physiol. 94, 325–334.

Taylor, C.T., 2008. Interdependent roles for hypoxia inducible factor and nuclear factor-κB in hypoxic inflammation. J. Physiol. (Lond.) 586, 4055–4059.

Taylor, C.T., Cummins, E.P., 2011. Regulation of gene expression by carbon dioxide. J. Physiol. 589, 797–803.

Trayhurn, P., Wang, B., Wood, I.S., 2008. Hypoxia in adipose tissue: a basis for the dysregulayion of tissue function in obesity? Br. J. Nutr. 100, 227–235.

Tsantes, A., Tassiopoulos, S., Papadhimitriou, S.I., Bonovas, S., Kavalierou, L., Vaiopoulos, G., Meletis, I., 2003. Suboptimal erythropoietic response to hypoxemia in idiopathic pulmonary fibrosis. Chest 124, 548–553.

van Eeden, S.F., Tan, W.C., Suwa, T., Mukae, H., Terashima, T., Fujii, T., Qui, D., Vincent, R., Hogg, J.C., 2001. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). Am. J. Respir. Crit. Care Med. 164 (5), 826–830.

VanOtteren, G.M., Standiford, T.J., Kunkel, S.L., Danforth, J.M., Strieter, R.M., 1995. Alterations of ambient oxygen tension modulate the expression of tumor necrosis factor and macrophage inflammatory protein-1 alpha from murine alveolar macrophages. Am. J. Respir. Cell Mol. Biol. 13 (4), 399–409.

Wan, M.X., Wang, Y., Liu, Q., Schramm, R., Thorlacius, H., 2003. CC chemokines induce p-selectin-dependent neutrophil rolling and recruitment in vivo: intermediary role of mast cells. Br. J. Pharmacol. 138, 698–706.

Wood, J.G., Johnson, J.S., Mattioli, L.F., Gonzalez, N.C., 1999a. Systemic hypoxia promotes leukocyte–endothelial adherence via reactive oxidant generation. J. Appl. Physiol. 87, 1734–1740.

Wood, J.G., Mattioli, L.F., Gonzalez, N.C., 1999b. Hypoxia causes leukocyte adherence to mesenteric venules in non acclimatized, but not in acclimatized, rats. J. Appl. Physiol. 87 (3), 873–881.

Wood, J.G., Johnson, J.S., Mattioli, L.F., Gonzalez, N.C., 2000. Systemic hypoxia increases leukocyte emigration and vascular permeability in conscious rats. J. Appl. Physiol. 89, 1561–1568.

Wouters, E.F., 2005. Local and systemic inflammation in chronic obstructive pulmonary disease. Proc. Am. Thorac. Soc. 2, 26–33.

Wust, R.C., Degens, H., 2007. Factors contributing to muscle wasting and dysfunction in COPD patients. Int. J. Chron. Obstruct. Pulmon. Dis. 2, 289–300.

Yende, S., D'Angelo, G., Kellum, J.A., Weissfeld, L., Fine, J., Welch, R.D., Kong, L., Carter, M., Angus, D.C., 2008. Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. Am. J. Respir. Crit. Care Med. 177, 1242–1247.

Zee, E.D., Schomberg, S., Carpenter, T.C., 2006. Hypoxia upregulates lung microvascular neurokinin-1 receptor expression. Am. J. Physiol. Lung Cell. Mol. Physiol. 291, L102–L110.

Zhao, M., Fernandez, L.G., Doctor, A., Sharma, A.K., Zarbock, A., Tribble, C.G., Kron, I.L., Laubach, V.E., 2006. Alveolar macrophage activation is a key initiation signal for acute lung ischemia–reperfusion injury. Am. J. Physiol. Lung Cell. Mol. Physiol. 291, L1018–L1026.