Alveolar Hypoxia-Induced Systemic Inflammation: What Low PO_2 Does and Does Not Do

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Abstract Reduction of alveolar PO_2 (alveolar hypoxia, AH) may occur in pulmonary diseases such as chronic obstructive pulmonary disease (COPD), or in healthy individuals ascending to altitude. Altitude illnesses may develop in non-acclimatized persons who ascend rapidly. The mechanisms underlying these illnesses are not well understood, and systemic inflammation has been suggested as a possible contributor. Similarly, there is evidence of systemic inflammation in the systemic alterations present in COPD patients, although its role as a causative factor is not clear.

We have observed that AH, induced by breathing 10% O_2 produces a rapid (minutes) and widespread micro vascular inflammation in rats and mice. This inflammation has been observed directly in the mesenteric, skeletal muscle, and pial microcirculations. The inflammation is characterized by mast cell degranulation, generation of reactive O_2 species, reduced nitric oxide levels, increased leukocyte-endothelial adherence in post-capillary venules, and extravasation of albumin. Activated mast cells stimulate the renin-angiotensin system (RAS) which leads to the inflammatory response via activation of NADPH oxidase. If the animals remain in hypoxia for several days, the inflammation resolves and exposure to lower PO_2 does not elicit further inflammation, suggesting that the vascular endothelium has "acclimatized" to hypoxia.

Recent experiments in cremaster microcirculation suggest that the initial trigger of the inflammation is not the reduced tissue PO_2, but rather an intermediary re-leased by alveolar macrophages into the circulation. The putative intermediary ac-tivates mast cells, which, in turn, stimulate the local renin-angiotensin system and induce inflammation.

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E. Takahashi, D.F. Bruley (eds.), *Oxygen Transport to Tissue XXXI*, Advances in Experimental Medicine and Biology 662,
DOI 10.1007/978-1-4419-1241-1_3,

1 Introduction

Reduction of alveolar PO_2 is commonly encountered in pulmonary disease and in healthy subjects exposed to altitude. Rapid ascent may trigger illnesses such as acute mountain sickness, high altitude pulmonary edema or high altitude cerebral edema [1]. The pathogenesis of these diseases is unknown; while inflammation is present, it is unclear whether it is a cause or an epiphenomenon of the disease. Altitude exposure is accompanied by increased levels of inflammatory markers [2–5] and leukocytosis [6], increased superoxide generation and CD18 expression by granulocytes, and enhanced inflammatory response of neutrophiles [7, 8]. Anti-inflammatory drugs such as dexamethasone are useful therapeutic agents [1]. Either acclimatization [9] or hypoxic preconditioning [6] prevent or attenuate these changes. These findings suggest that inflammation may play a role, either in the genesis or the development of altitude illness. These conditions are becoming increasingly common owing to the large number of individuals worldwide who reach altitudes higher than 2,500 m within a few hours for recreational, work, or military purposes [1].

Pulmonary diseases such as COPD may present systemic effects, including weight loss, skeletal muscle dysfunction, and cardiovascular and nervous system abnormalities [10]. While the systemic hypoxemia commonly present in COPD may influence these effects, it has been suggested that systemic inflammation may also contribute to the genesis or development of these systemic abnormalities [11]. As with diseases of altitude, the role of inflammation in COPD is still unclear.

We have shown that reduction of inspired PO_2 initiates a rapid and widespread inflammation in mesentery [9], skeletal muscle [12, 13] and pial microcirculations [14] of rats. The inflammation features increased levels of reactive O_2 species (ROS) [15], mast cell degranulation [12, 13, 16], local renin-angiotensin system (RAS) activation [17], leukocyte-endothelial adhesive interactions [15, 18], and albumin extravasation [18]. If hypoxia is maintained for several days, the inflammation resolves and more severe hypoxia is not accompanied by inflammation, suggesting that the vascular endothelium has "acclimatized" to prolonged hypoxia [9].

Recent observations support the hypothesis that the systemic inflammation of alveolar hypoxia is not triggered by the reduction of systemic PO_2, but by a mediator released into the circulation from alveolar macrophages. This paper will review the evidence that led us to formulate this hypothesis.

2 Dissociation Between Cremaster Microvascular PO_2 Values and Cremaster Inflammation

The first observation suggesting that local hypoxia was not the trigger of systemic inflammation was the dissociation observed between cremaster microvascular PO_2 (PmO_2) – estimated from the phosphorescence decay method [19],

and cremaster inflammation [12, 13]. Selective reduction of cremaster PmO_2 (local ischemia or selective cremaster hypoxia induced by equilibrating the cremaster with 95% N_2, 5% CO_2) in rats breathing room air did not produce leukocyte endothelial adherence (LEA) or mast cell degranulation (MCD). On the other hand, lowering alveolar (and systemic) PO_2 produced cremaster LEA and MCD, even when the cremaster PmO_2 was higher than normal. Accordingly, cremaster LEA and MCD occur only when alveolar PO_2 is reduced, independent of the value of cremaster PmO_2. One possible explanation for this phenomenon, among other alternatives, is that the inflammation is triggered by a substance released from a distant site.

3 Plasma from Hypoxic Rats Produces Inflammation in Normoxic Cremaster

We reasoned that if the putative mediator is carried by the circulation, plasma obtained from hypoxic rats should produce inflammation in normoxic tissues. Blood obtained from conscious rats breathing 10% O_2 was centrifuged and plasma separated allowing plasma PO_2 equilibration with room air [20]. Application of plasma onto the surface of normoxic cremaster elicited a rapid increase in LEA and substantial MCD. These responses are similar to those observed in various vascular beds during alveolar hypoxia [9, 12, 16]. The inflammation is specific of plasma from hypoxic donors, since it was not elicited by application of plasma from normoxic rats. The inflammatory agent is not generated by blood cells, since no inflammation developed in response to plasma separated after hypoxic in vitro equilibration. The inflammation of hypoxic plasma is not due to agents released by activated leukocytes or mast cells in the systemic microcirculation of the donor, since plasma obtained from hypoxic donors pretreated with cromolyn still elicited inflammation. Cromolyn blocks LEA and MCD in the hypoxic donor [20]. The combined data of these studies strongly support the idea that the inflammation of alveolar hypoxia is triggered by an agent(s) released from a distant site and transported by the circulation.

4 Alveolar Macrophages Are Necessary for the Inflammation of Alveolar Hypoxia

Several reasons suggest the lungs as the site of origin for the agent(s) responsible for triggering the inflammation of alveolar hypoxia. First, inflammation occurs only when the alveolar PO_2 is reduced, independent of systemic tissue PO_2; second, the lung receives the entire cardiac output so substances released by the lung rapidly reach the systemic circulation. Alveolar macrophages (AMØ) are ideally positioned to sense changes in alveolar PO_2. Additionally, AMØ store a number of substances which could trigger inflammation. While most known

effects of AMØ activation occur within the lung, examples of systemic inflammation secondary to AMØ activation have been documented [21, 22].

AMØ were depleted by tracheal instillation of disodium clodronate-containing liposomes [7]. This technique results in temporary depletion of AMØ [23]; in our hands, the number of AMØ recovered from each rat by broncho-alveolar lavage (BAL) decreased from a normal of $6.7 \pm 0.3 \times 10^6$ to $0.45 \pm 0.07 \times 10^6$ four days after treatment. Alveolar hypoxia (10% O_2 breathing) in AMØ-depleted rats failed to increase LEA, MCD, or albumin extravasation. This was not due to inability of the AMØ- depleted rats to mount an inflammatory response to Angiotensin II (Ang II) or mast cell secretagogues. Plasma obtained from hypoxic, AMØ-depleted donors did not produce increased LEA or MCD in normoxic cremaster; furthermore, supernatant of primary cultures of AMØ exposed to hypoxia produced inflammation in the normoxic cremaster [18].

5 Activation of Mast Cells by the AMØ-Generated Intermediary Stimulates the Local Renin-Angiotensin System (RAS)

The RAS plays a role in the inflammation of alveolar hypoxia: Ang II receptor blockade or Ang I-converting enzyme (ACE) inhibition attenuate LEA, MCD and increased vascular permeability of alveolar hypoxia [17]. The inflammatory effects of plasma from hypoxic rats [17] and of supernatant of hypoxic AMØ are also attenuated by blockade of the RAS [24]. The activation of the RAS in these cases is secondary to mast cell degranulation. Several lines of evidence support this: 1. Topical Ang II induces LEA but not MCD; 2. Ang II-induced LEA is not blocked by cromolyn, while this agent blocks the inflammatory responses to plasma from hypoxic rats and from supernatant from hypoxic AMØ; 3: The inflammation induced by the mast cell secretagogue C4880 is blocked by RAS blockade [24]. These findings indicate that the effects of Ang II in the leukocyte-endothelial interface are downstream from mast cell activation, and that MCD leads to generation of Ang II. The mechanism by which this occurs is not clear, mast cells of some species contain an angiotensin I converting chymase; also renin is contained in cardiac mast cells of several species.

The data summarized here indicate that the inflammation of alveolar hypoxia is initiated by a substance released into the circulation by AMØ stimulated by the reduced alveolar PO_2. This substance activates perivascular mast cells which, in turn, activate the local RAS and initiate the inflammatory response. The identity of the mediator is still unclear; the data suggest that it is a mast cell secretagogue either stored or rapidly assembled by AMØ. Identification of this substance and development of means to antagonize its effects may provide a useful tool for improving our understanding of the strategies employed by intact organisms to adapt to reduced alveolar PO_2.

Acknowledgments Supported by National Institutes of Health, USA, grant HL39443

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