Carbonic Anhydrase IX: Scaring Away the Grim Reaper in Acute Lung Injury?

Endothelial injury is a major contributor to the pathophysiology of acute respiratory distress syndrome (ARDS) (1). Promoting survival of injured endothelial cells may confer a clinical benefit to critically ill patients with acute lung injury. Understanding molecular dynamics that promote survival of lung endothelial cells during infection may eventually translate to improved patient survival.

Patients with ARDS experience impaired gas exchange due to damage of alveolar units with protein-rich edema. Clinically, this is in part reflected by hypoxemia with low levels of circulating oxygen (Pa_{O_2}) from shunt and low ventilation–perfusion ($\dot{V}/\dot{Q}$) units as well as by hypercapnia with elevated levels of carbon dioxide (Pa_{CO_2}) from $\dot{V}/\dot{Q}$ mismatch, especially from high $\dot{V}/\dot{Q}$ units that contribute to an increase in pulmonary dead space (2). It is uncertain whether these abnormalities confer beneficial (3, 4) or harmful (5, 6) outcomes in patients with ARDS, although hypercapnia and hypoxemia impair alveolar epithelial fluid clearance and the resolution of alveolar edema (7, 8). Furthermore, the mechanism of either protective or detrimental effects of abnormal CO_2 levels on pulmonary microvascular endothelial cells (PMVECs) during infection is uncertain.

In vivo, CO_2 is in part regulated by enzymes termed carbonic anhydrases (CAs). In addition to catalyzing conversion of CO_2 to carbonic acid and thereby regulating pH, CA isoforms, which are abundantly expressed in PMVECs (9), may act as immunomodulators and contribute to improved PMVEC survival during infection. In this issue of the *Journal*, Lee and colleagues (pp. 630–645) report on the role of CA IX during *in vivo* human and rat pneumonia as well as CA IX isoforms on *in vitro* PMVEC survival during *Pseudomonas aeruginosa* infection under hypoxic and hypercapnic conditions (10).

The authors found that although plasma CA IX levels are similar in critically ill patients and rats with *P. aeruginosa* pneumonia relative to respective controls, levels of this enzyme in BAL fluid from patients with pneumonia and rat *P. aeruginosa* pneumonia lung tissue were higher, for the first time providing *in vivo* data suggesting pneumonia-specific CA IX differential expression in the lung. These observations together with this group's previous finding that PMVECs express CA IX (9) prompted detailed *in vitro* characterization of CA IX expression and function under stress conditions relevant to ARDS.

CA IX is composed of an N-terminal proteoglycan-like (PG) domain, a catalytic (CA) domain, a transmembrane domain, and an intracellular (IC) domain (11). To study the mechanism of domain-specific secretion in rat PMVECs, CRISPR-Cas9 was used to generate rat PMVECs with ΔPG, ΔCA, and ΔIC conditionally expressing CA IX functional mutants. The authors identified that ΔIC domain mediated the CA IX membrane localization in PMVECs.

Next, to understand whether increased lung tissue CA IX concentrations originate from pulmonary capillaries, the authors studied CA IX secretory mechanisms by measuring baseline CA IX concentration in PMVEC cell lysates and supernatants. After determining that CA IX release and cleavage at the ectodomain–transmembrane domain junction was present at baseline, metalloproteinase (MMP) inhibitors were used to demonstrate that MMPs mediate CA IX ectodomain cleavage in the extracellular space after unprocessed CA IX is released from cells. Interestingly, MMP inhibition prevented extracellular CA IX cleavage without affecting CA IX release from PMVECs. Thus, CA IX processing by MMPs warrants further investigation to test the potential protective role of CA IX during infections identified in this study.

Because *in vivo* data (patient BAL fluid and *P. aeruginosa* pneumonia rat lung tissue) identified increased tissue-specific CA IX levels, the authors next used an *in vitro* rat PMVEC *P. aeruginosa* infection model to study infection-induced changes in CA IX expression, demonstrating that *in vitro* infection increases both CA IX release and ectodomain cleavage. More specifically, *P. aeruginosa* infection of CA IX ΔIC PMVECs compared with CA IX wild-type PMVEC was associated with impaired cell survival, pointing to the critical role of the CA IX IC domain during *P. aeruginosa* infection.

Lastly, the authors tested host environmental conditions associated with pneumonia and ARDS (low O_2 and high CO_2) as well as the enzymatic properties associated with CA IX (CO_2 regulation) using the *in vitro* rat PMVEC model. These studies provided evidence that during *P. aeruginosa* infection, severe hypoxia protects PMVECs (less necrosis) while hypercapnia attenuates this protective effect. The negative impact of hypercapnia was more pronounced in CA IX wild-type relative to CA IX ΔIC PMEVCs, suggesting that the IC domain increases the cytotoxic effect of hypercapnia under *in vitro* infection-related hypoxic conditions, providing further evidence that the IC domain of CA IX influences *in vitro* pulmonary endothelial cell survival. These pathways are summarized in Figure 1.

Translating the authors' findings into clinical practice is challenging at this time. First, the authors postulate that the lack of a relationship between circulating plasma CA IX levels in patients and in the *in vivo* rat pneumonia model may be due to rapid degradation of CA IX released from the lung tissue before it reaches the systemic circulation. This could make it challenging to follow levels of this enzyme in response to potential future therapies. Second, the *in vitro* studies were performed in rat PMVECs, and how they translate to human microvascular responses to infection, hypoxia, and hypercapnia deserves further investigation. Third, although modest levels of hypoxemia (recommended oxygen saturation as low as 88%) and hypercapnia occur in the era of lung protective ventilation (12),

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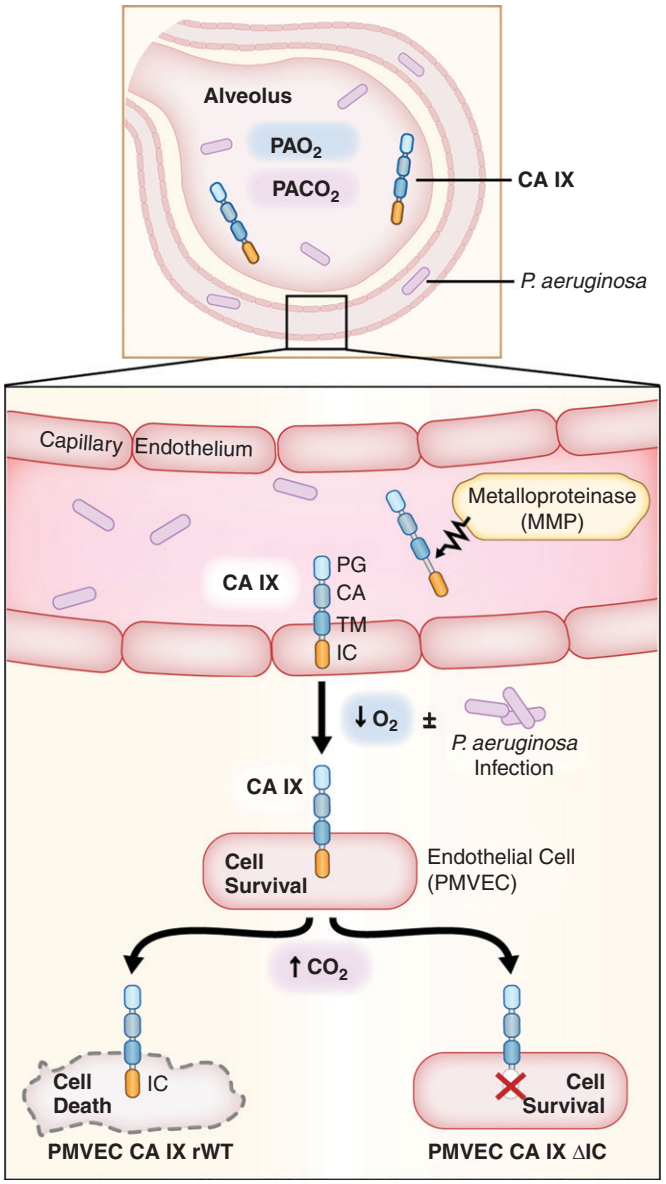

Figure 1. The role of carbonic anhydrase IX (CA IX) in regulating pulmonary endothelial cell survival during acute lung injury. CA IX in human BAL fluid and in rat lung tissue after *Pseudomonas aeruginosa* infection is elevated relative to uninfected controls. Metalloproteinases (MMPs) in the circulation cleave secreted CA IX in the extracellular compartment and MMP inhibitors prevent its degradation. Rat pulmonary microvascular endothelial cells (PMVECs) have greater survival after *in vitro* exposure to decreased levels of O_2 and *P. aeruginosa* infection. When hypercapnia (high CO_2) is applied to PMVECs exposed to hypoxia and *P. aeruginosa,* the protective effect of low O_2 and infection on PMVEC survival is negated in CA IX rWT PMVEC but not in ΔIC PMVECs. CA = catalytic; IC = intracellular; *P. aeruginosa* = *Pseudomonas aeruginosa*; PG = proteoglycan-like; rWT = wild-type; TM = transmembrane.

the levels chosen by the authors to represent these *in vivo* settings (1% O_2 and 10% CO_2 to simulate hypoxia and hypercapnia, respectively) may not reflect arterial oxygen tension/pressure and arterial carbon dioxide tension/pressure during infection. Furthermore, how CA IX expression in other nonpulmonary organs is affected *in vivo* by the conditions tested in this study should be studied before testing therapies targeting CA IX.

This study provides compelling and new evidence that CA IX, in addition to being an enzyme that sustains CO_2 metabolism, may be important for mediating endothelial cell survival during infection. Moreover, focusing on the intracellular domain may be particularly relevant. These interesting findings provide a strong rationale for future studies of this enzyme and its expression, processing, and metabolism as potential therapeutic targets for mechanisms of acute lung injury and translational issues in ARDS. ■

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