Chlorine Gas, Airway Inflammation, and Cysteinyl Leukotrienes: The Neutrophil Does Not Work Alone

Chlorine (Cl_2) is a chemical widely used in industry, in cleaning products, and as a germicide in swimming pools. Acute exposure to high levels of Cl_2 gas in industrial accidents can cause severe airway injury and/or life-threatening acute lung injury, whereas lower-level exposures may result in chronic airway inflammation and airflow obstruction (1). Examples of the latter include reactive airway dysfunction syndrome in women exposed to Cl_2 released by a chemical mixture used for household cleaning (2) and highly prevalent bronchial hyperreactivity in competitive swimmers who train in chlorinated indoor pools (3). Notably, bronchial biopsy specimens in such swimmers demonstrate airway inflammation and remodeling similar to that shown in asthma (4).

The mechanism by which Cl_2 gas injures the airway involves oxidants, including hypochlorous acid generated from Cl_2 in aqueous solution. This is supported by observations that antioxidants mitigate lung injury and airway hyperresponsiveness (AHR) in Cl_2 gas–exposed rats (5) and mice (6). Neutrophils play a critical role, as blocking Cl_2-induced neutrophilic infiltration of the airways abrogated AHR in Cl_2-exposed mice (7). The cysteinyl leukotrienes (LTs), LTC_4, LTD_4, and LTE_4, are also important, as exposure to Cl_2 gas triggers release of these lipid mediators in the lungs of mice (8), and blockade of the $cysLT_1$ receptor reduced Cl_2-induced airway inflammation and AHR (9).

Cysteinyl LTs are metabolites of arachidonic acid, which is deesterified from phospholipids by phospholipase A_2 and metabolized to bioactive eicosanoid metabolites via cyclooxygenase or lipoxygenase pathways. Synthesis of LTs requires 5-lipoxygenase (5-LO), expression of which is largely restricted to cells of myeloid origin. The product of 5-LO action on arachidonic acid is LTA_4, an unstable epoxide intermediate that is further metabolized by LTC_4 synthase to LTC_4 or by LTA_4 hydrolase to LTB_4. LTC_4 and its peptidase-derived metabolites, LTD_4 and LTE_4, collectively signal via $cysLT_1$ and $cysLT_2$ receptors to produce bronchoconstriction, mucus cell hyperplasia, airway smooth-muscle thickening, and subepithelial fibrosis (10), bioactivities highly relevant to Cl_2 gas–induced airway injury.

The dependence of Cl_2-induced AHR in mice on both neutrophils and cysteinyl LTs poses an apparent conundrum, as neutrophils are alone among myeloid cells in not expressing LTC_4 synthase. In this issue of the *Journal*, McGovern and colleagues (pp. 681–689) address this problem in their work presented (11). As in their previous studies, the authors exposed mice to Cl_2 gas at 100 or 400 parts per million for 5 minutes, which elicited influx to the lungs of large numbers of inflammatory cells and release of cysteinyl LTs into BAL fluid. To determine the cellular source of the cysteinyl LTs, they selectively depleted each of the major inflammatory cell types recruited to the lungs after Cl_2 exposure. Macrophages were depleted by three different strategies, instillation of clodronate liposomes, diphtheria toxin administration to CD11c-DTR mice, and use of $CCR2^{-/-}$ mice, each resulting in an ~50% decrease in BAL macrophages after Cl_2 exposure but resulting in no reduction in cysteinyl LTs. Likewise, depletion of eosinophils with an anti–IL-5 antibody had no effect on lung cysteinyl LT levels. On the other hand, treatment with an antibody to Ly6G, which blocked neutrophil influx (without decreasing macrophages), fully abrogated the Cl_2-induced release of cysteinyl LTs.

Because neutrophils are incapable of synthesizing cysteinyl LTs, the authors considered the possibility that they cooperate with other cells in producing these mediators. Thus, they cocultured mouse neutrophils and tracheal epithelial cells and found that these cocultures produced significant quantities of cysteinyl LTs, whereas neither cell type did so alone. Furthermore, when neutrophils from 5-LO–knockout mice were cultured with wild-type tracheal epithelial cells, no increase in cysteinyl LT synthesis occurred. The implication is that 5-LO–expressing neutrophils synthesize LTA_4, which they then transfer to LTC_4 synthase–expressing epithelial cells for conversion to cysteinyl LTs. The authors propose this as the mechanism by which cysteinyl LTs are produced in the lung after exposure to Cl_2 gas.

Transcellular biosynthesis of eicosanoids is a well-recognized phenomenon, and synthesis of LTC_4 from LTA_4 released by neutrophils, then taken up by other cells expressing LTC_4 synthase, has been reported previously (12). McGovern and colleagues (11) expand these observations by demonstrating that neutrophils participate with tracheal epithelial cells in the formation of cysteinyl LTs. Although the finding that this occurs *in vitro* does not establish that neutrophil–airway epithelial cell transcellular LTC_4 biosynthesis accounts for production of cysteinyl LTs in the lungs of Cl_2-exposed mice, this mechanism appears highly plausible. Furthermore, it reconciles the dependence of Cl_2-induced AHR both on neutrophils, which lack LTC_4 synthase, and on cysteinyl LTs. A larger point to be drawn from the study is that even when controlled experiments in a well-defined animal model pinpoint a specific cell type as essential to a particular disease outcome, the cell type in question likely drives pathophysiology in concert with other cells. In the case of Cl_2-induced alterations in airway physiology, neutrophils are clearly essential, but they do not act alone.

The current study raises multiple questions for further investigation. What are the Cl_2-induced chemokines or other signals that elicit neutrophil influx to the lung? Besides airway epithelial

Supported by U.S. National Institutes of Health grant R01 HL131745.

Originally Published in Press as DOI: 10.1165/rcmb.2020-0347ED on August 28, 2020

cells, do endothelial, mesenchymal or other LTC_4 synthase–expressing lung parenchymal cells metabolize neutrophil-derived LTA_4 to cysteinyl LTs after Cl_2 exposure *in vivo*? What mechanisms are involved in transfer of LTA_4 from the neutrophil to other cells?

LTA_4 is synthesized at the nuclear membrane, where 5-LO and 5-LO activating protein colocalize in stimulated neutrophils (13), so how is LTA_4 trafficked to the cell surface and released? Are a carrier protein, lipid vesicle, and/or molecular motor involved? McGovern and colleagues (11) cocultured neutrophils and tracheal epithelial cells on opposite sides of microporous filters, indicating that cell–cell contact is not required for LTA_4 transfer. As an unstable intermediate, is LTA_4 shielded from degradation in the extracellular milieu by binding to a protective protein (14) and/or inside a vesicle? How is LTA_4 taken up by the receiving cell and trafficked to the lumen of the nuclear envelope and endoplasmic reticulum, where LTC_4 synthase is localized (15), to be converted to LTC_4?

In summary, McGovern and colleagues (11) advance our understanding of Cl_2-induced airway injury by showing that although neutrophils are essential for Cl_2 to induce AHR, they do not work alone. Instead, tracheal epithelial cells or other lung cells play a critical cooperative role by participating in transcellular biosynthesis of cysteinyl LTs, the likely mediators of AHR. The results raise multiple scientific questions requiring further laboratory investigation. In addition, the obvious clinical implication of the work, that targeting the LT pathway may be beneficial in treating patients with Cl_2-induced airway disease, may be tested in clinical studies using currently available anti-LT drugs (16). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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