Correspondence

Defining Lung Injury in Animals

To the Editor:

We read with considerable interest the outstanding article “Official American Thoracic Society Workshop Report: Features and Measurements of Experimental Acute Lung Injury in Animals” by Matute-Bello and coworkers (1). The authors should be congratulated for establishing clear guidelines to define appropriate models of acute lung injury in animals. Because we think that the scientific community would benefit from some additional discussion, the following questions are posed.

Is It Possible to Postulate the Presence of ALI Based on Physiological and Biochemical Criteria?

We recently published a series of studies in which we showed that rats exposed to chlorine gas (Cl_2) and returned to room air developed arterial hypoxemia (PaO_2 in room air < 40 mm Hg, which was marginally increased while breathing 100% O_2), a 5- to 6-fold increase of protein in the bronchoalveolar lavage (BAL) and decreased ability of the concentrated BAL to reach a minimum surface tension upon dynamic compression, indicative of abnormal surfactant function (2–4). Upon return to room air, rats showed obvious signs of respiratory distress, as evidenced by flaring of the nostrils and use of accessory inspiratory muscles during inspiration and expiratory grunting. We also showed that mice exposed to Cl_2 and returned to room air had decreased levels of alveolar fluid clearance, another index of distal lung epithelial injury (5). Histologic and morphologic studies revealed the presence of significant damage to airways, and mice exposed to Cl_2 and returned to room air developed reactive airway disease syndrome characterized by abnormal airway resistance following the administration of methacholine (6). However, the alveolar regions were largely intact (although focal areas of pneumonitis were clearly present). We reasoned that damage to cellular junctions by reactive species may damage critical targets on distal lung epithelial cells, which will result in compromised vectorial sodium transport and efflux of plasma proteins from the plasma into the alveolar space; however, these changes may be focal and very difficult to detect using light microscopy. Thus we suggest that overt lung injury in animals, documented by the presence of physiological abnormalities, may occur before the appearance of morphologic and histologic changes in distal lung spaces. An alternate explanation is that protein leaked in the alveolar space due to injury to terminal airways.

Measurement of Intravascular Injected Labeled Proteins or Evans Blue in Lung Tissue Does Not Necessarily Reflect Changes in Vascular Permeability

We and others have shown previously that exposure of animals to oxidant gases increases the volume of distribution of albumin in the lung extravascular space (7). Thus steady-state measurements of radio-labeled albumin or Evans blue in lung tissue may simply reflect changes in its volume of distribution. We thus propose that conclusions concerning vascular permeability require measurements of the rate of albumin accumulation before the onset of steady state.

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

SOTIRIOS G. ZAROGIANNIS, PH.D.
SADIS MATALON, PH.D., DR.SC. (HON.)
University of Alabama at Birmingham
Birmingham, Alabama

References

1. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM; on behalf of the Acute Lung Injury in Animals Study Group. An Official American Thoracic Society Workshop Report: Features and measurements of experimental acute lung injury in animals. *Am J Respir Cell Mol Biol* 2011;44:725–738.
2. Yadav AK, Bracher A, Doran SF, Leustik M, Squadrito GL, Postlethwait EM, Matalon S. Mechanisms and modification of chlorine-induced lung injury in animals. *Proc Am Thorac Soc* 2010;7:278–283.
3. Leustik M, Doran S, Bracher A, Williams S, Squadrito GL, Schoeb TR, Postlethwait E, Matalon S. Mitigation of chlorine-induced lung injury by low-molecular-weight antioxidants. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L733–L743.
4. Yadav AK, Doran SF, Samal AA, Sharma R, Vedagiri K, Postlethwait EM, Squadrito GL, Fanucchi MV, Roberts LJ, Patel RP, et al. Mitigation of chlorine gas lung injury in rats by postexposure administration of sodium nitrite. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L362–L369.
5. Song W, Wei S, Zhou Y, Lazrak A, Liu G, Londino JD, Squadrito GL, Matalon S. Inhibition of lung fluid clearance and epithelial Na^+ channels by chlorine, hypochlorous acid, and chloramines. *J Biol Chem* 2010;285:9716–9728.
6. Song W, Wei S, Liu G, Yu Z, Estell K, Yadav AK, Schwiebert LM, Matalon S. Post exposure administration of a β_2 -agonist decreases chlorine induced airway hyper-reactivity in mice. *Am J Respir Cell Mol Biol* 2011;45:88–94.
7. Matalon S, Egan EA. Interstitial fluid volumes and albumin spaces in pulmonary oxygen toxicity. *J Appl Physiol* 1984;57:1767–1772.

Copyright © 2013 by the American Thoracic Society

Reply

From the Authors:

We thank the editors for giving us the opportunity to reply to the interesting issues raised in the letter by Zarogiannis and Matalon. Before addressing their specific questions, we would like to point out that our article (1) was meant to serve as the basis for a discussion on lung injury that would eventually lead to revised, improved criteria. Thus, we welcome the questions of Zarogiannis and Matalon.

Their first question is highly relevant in that it describes an animal model of Cl_2 exposure in which there is clinical, physiological, and biochemical evidence of lung injury, but with minimal evidence of inflammatory alveolitis. Interestingly, while there was evidence of injury to the airways epithelium on histologic examination, there was also evidence of leak of fluid and plasma proteins into the lung. This raises several important issues. First, injury predominantly to the airway epithelium can lead to flooding of the alveolar compartment with protein-rich edema fluid. This underscores the close interactions between the distal airways and alveolar space and how changes in one compartment can lead to alterations in the other. Second, the rodent model of Cl_2 -induced lung injury appears to display relatively little parenchymal inflammation (defined by “pneumonitis” on histology), yet has prominent physiological manifestations, including respiratory distress and severe hypoxemia. This model illustrates the dissociation between the acute

inflammatory response and lung injury that has also been reported in other circumstances. Thus, each animal model should be evaluated in terms of its intended purpose and the specific questions that it was intended to address. In this case, the authors have an excellent model of exposure to irritant gases that reproduces the evolution to reactive airways disease.

Regarding their second question, we thank the authors for pointing out the importance of potential changes in the volume of distribution when measuring labeled albumin or Evans blue dye extravasation that might have important effects on the measurements in animal models of lung injury.

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

GUSTAVO MATUTE-BELLO, M.D.
*University of Washington
Seattle, Washington*

GREGORY P. DOWNEY, M.D.
*National Jewish Health
Denver, Colorado*

Reference

1. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM; on behalf of the Acute Lung Injury in Animals Study Group. An Official American Thoracic Society Workshop Report: Features and measurements of experimental acute lung injury in animals. *Am J Respir Cell Mol Biol* 2011;44:725–738.

Copyright © 2013 by the American Thoracic Society

Erratum: Asthmatic Airway Epithelium Is Intrinsically Inflammatory and Mitotically Dyssynchronous

The article by Freishtat and colleagues (1), published in the June 2011 issue of *AJRCMB*, contains errors in the disclosures and grants sections.

Dr. Eric P. Hoffman was incorrectly reported to have had various relationships with commercial entities; in fact, he had none, but had received a sponsored grant from the National Institutes of Health. Dr. Dinesh K. Pillai (incorrectly listed as “P.D.”) had received a sponsored grant from the NIH of more than \$100,001; the statement incorrectly states the amount of the grant as being more than \$75,000.

Finally, two grants were omitted from a footnote. The complete footnote should have read as follows (omissions are in boldface):

Acknowledgments: Supported by grants K23RR020069 (**R.J.F.**) and **K12HL090020 (S.F.I., D.K.P, M.C.R., E.P.H.)**, from the National Institutes of Health, Bethesda, MD, **a grant from the Muscular Dystrophy Association USA (E.P.H.)**, and by institutional grants from Children’s National Medical Center, Washington, DC (R.J.F.).

Reference

1. Freishtat RJ, Watson AM, Benton AS, Iqbal SF, Pillai DK, Rose MC, Hoffman EP. Asthmatic airway epithelium is intrinsically inflammatory and mitotically dyssynchronous. *Am J Respir Cell Mol Biol* 2011;44: 863–869.

Copyright © 2013 by the American Thoracic Society