Interdepartmental Division of Critical Care Medicine
University of Toronto
Toronto, Ontario, Canada
and
Department of Medicine
University of Toronto
Toronto, Ontario, Canada

JESUS VILLAR, M.D., PH.D.
Keenan Research Centre
Li Ka Shing Knowledge Institute of St. Michael's Hospital
Toronto, Ontario, Canada
CIBER de Enfermedades Respiratorias
Instituto de Salud Carlos III
Madrid, Spain
and
Research Unit
Hospital Universitario Dr. Negrin
Las Palmas de Gran Canaria, Spain

ARTHUR S. SLUTSKY, M.D.
Keenan Research Centre
Li Ka Shing Knowledge Institute of St. Michael's Hospital
Toronto, Ontario, Canada
Interdepartmental Division of Critical Care Medicine
University of Toronto
Toronto, Ontario, Canada
and
Departments of Medicine and Surgery
University of Toronto
Toronto, Ontario, Canada

References

1. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: the Berlin Definition. *JAMA* 2012;307:2526–2533.
2. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000;342:1301–1308.
3. Abrams ST, Zhang N, Manson J, Liu T, Dart C, Baluwa F, Wang SS, Brohi K, Kipar A, Yu W, *et al.* Circulating histones are mediators of trauma-associated lung injury. *Am J Respir Crit Care Med* 2013;187:160–169.
4. Rittirsch D, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* 2008;8:776–787.
5. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr, Wrobleski SK, Wakefield TW, Hartwig JH, Wagner DD. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci USA* 2010;107:15880–15885.
6. Hogg JC, Doerschuk CM. Leukocyte traffic in the lung. *Annu Rev Physiol* 1995;57:97–114.
7. Grommes J, Soehnlein O. Contribution of neutrophils to acute lung injury. *Mol Med* 2011;17:293–307.
8. Redon C, Pilch D, Rogakou E, Sedelnikova O, Newrock K, Bonner W. Histone H2A variants H2AX and H2AZ. *Curr Opin Genet Dev* 2002;12:162–169.
9. Mosammaparast N, Shi Y. Reversal of histone methylation: biochemical and molecular mechanisms of histone demethylases. *Annu Rev Biochem* 2010;79:155–179.
10. Chen X, Barozzi I, Termanini A, Prosperini E, Recchiuti A, Dalli J, Mietton F, Matteoli G, Hiebert S, Natoli G. Requirement for the histone deacetylase Hdac3 for the inflammatory gene expression program in macrophages. *Proc Natl Acad Sci USA* 2012;109:E2865–E2874.
11. Dokmanovic M, Clarke C, Marks PA. Histone deacetylase inhibitors: overview and perspectives. *Mol Cancer Res* 2007;5:981–989.
12. Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, Taylor FB, Esmon NL, Lupu F, Esmon CT. Extracellular histones are major mediators of death in sepsis. *Nat Med* 2009;15:1318–1321.
13. Kleine TJ, Gladfelter A, Lewis PN, Lewis SA. Histone-induced damage of a mammalian epithelium: the conductive effect. *Am J Physiol* 1995; 268:C1114–C1125.
14. Huang H, Evankovich J, Yan W, Nace G, Zhang L, Ross M, Liao X, Billiar T, Xu J, Esmon CT, *et al.* Endogenous histones function as alarmins in sterile inflammatory liver injury through toll-like receptor 9. *Hepatology* 2011;54:999–1008.
15. Ranieri VM, Thompson BT, Barie PS, Dhainaut JF, Douglas IS, Finfer S, Gårdlund B, Marshall JC, Rhodes A, Artigas A, *et al.*; PROWESS-SHOCK Study Group. Drotrecogin alfa (activated) in adults with septic shock. *N Engl J Med* 2012;366:2055–2064.

Copyright © 2013 by the American Thoracic Society
DOI: 10.1164/rccm.201211-2025ED

The Yin and Yang of Cystic Fibrosis Transmembrane Conductance Regulator Function

Implications for Chronic Lung Disease

The cystic fibrosis (CF) transmembrane conductance regulator (CFTR) is a cAMP-activated anion channel, primarily expressed in ciliated epithelial cells that have an endoluminal lining of mucus. Genetic mutations in CFTR are known to induce its peripheral and/or endoplasmic reticulum–associated degradation affecting its cell surface expression and/or stability, resulting in the pathological manifestations of CF lung disease, including hypohydration and increased viscosity of mucus, predisposing affected individuals to recurrent infections. Recent studies have identified low levels of CFTR expression in inflammatory cells such as T cells, neutrophils, and macrophages (1–3), suggesting an additional role for CFTR in the pathogenesis of chronic inflammatory CF lung disease. CFTR has also been suggested to mediate internalization and phagocytosis (4, 5) of *Pseudomonas aeruginosa* (*Pa*), a common CF pathogen. A recent study suggests that a bacterial toxin, Cif (PA2934) secreted in outer membrane vesicles by *Pa*, reduces CFTR-mediated chloride secretion by human airway epithelial cells (6). In this issue of the *Journal*, Le Gars and colleagues (pp. 170–179) describe a novel mechanism for proteolytic cleavage of CFTR that disables the channel function (7).

Several recent studies demonstrate that membrane-CFTR expression/activity is modulated by cigarette smoke (CS) exposure (3, 8–10). The CFTR-dependent nasal potential difference is also suppressed in cigarette smokers (9, 11). These studies provide experimental evidence supporting the concept of acquired CFTR dysfunction induced by CS and its role in pathogenesis of chronic obstructive pulmonary disease (COPD). The CS-induced CFTR dysfunction has been suggested to modulate lipid-raft platforms initiating inflammatory-apoptotic responses and aberrant autophagy (3, 12). The mechanisms by which CS modulates membrane-CFTR levels and activity may include the induction of unfolded protein response, as the *Cftr* gene is a target of the unfolded protein response protein ATF6 (13, 14). In addition, CS may induce accumulation of

Author Contributions: N.V. and G.P.D. jointly wrote the manuscript.

CFTR as an immature B-form in the endoplasmic reticulum (3) by inducing its misfolding. Oxidants and CS may also interfere directly with CFTR protein function by altering key cysteine residues in CFTR to affect its potentiation or open channel probability (15).

The current study by Le Gars and colleagues describes an important and novel observation that neutrophil elastase promotes CFTR degradation (7), raising the intriguing possibility that this mechanism contributes to the pathogenesis of chronic obstructive lung disease. Recent studies demonstrating CS-induced acquired CFTR dysfunction in COPD support this notion. Additionally, the authors suggest that *Pa* infection may similarly impact CFTR ion channel activity in both CF and COPD. Conversely, membrane-CFTR may regulate bacterial pathogenesis and inflammatory-apoptotic signaling (3, 8, 12). It is not clear if the decrease in membrane-CFTR (genetic or acquired) activity is sufficient to trigger chronic lung disease or whether it requires an additional stimulus such as subsequent *Pa* infection (CF/COPD) and/or CS exposure (COPD).

The current study uses a carcinoma cell line and an acute infection animal model to clearly demonstrate the role of neutrophil elastase and calpains in CFTR dysfunction, suggesting an additional mechanism whereby neutrophil elastase contributes to the pathogenesis of CF lung disease. The chief function of neutrophils in host defense is to restrain and destroy invading microbial pathogens (16). Neutrophils accomplish this task by binding and internalizing the pathogens via a complex process termed phagocytosis, eventually killing the organisms through the combined actions of potent antimicrobial compounds including reactive oxygen and nitrogen species, antimicrobial peptides, and proteinases such as elastase that are delivered to the nascent phagosome (17). Although these antimicrobial functions are usually performed without injury to host tissues, in pathological circumstances such as during progressive bacterial infection, these potent antimicrobial compounds can be released extracellularly where they can induce a spectrum of responses in host cells ranging from activation to injury and death. Unregulated release of these neutrophil-derived cytotoxic compounds, particularly elastase, is believed to contribute to inflammatory injury to the gastrointestinal tract (18) and lungs (19, 20).

As indicated by their name, proteinases were originally identified as protein-degrading enzymes that can degrade a diverse range of substrates including various components of the extracellular matrix such as collagen (collagenases) and elastin (elastases) (21). In contrast to the widely held view that proteinases function primarily as simple degradative enzymes, it is now appreciated that proteinases control diverse physiological processes including immune responses, cell proliferation and death, wound repair, digestive processes, and recycling of critical proteins and organelles (22, 23). With respect to inflammatory processes, proteinases such as elastase and matrix metalloproteinases are able to activate cytokines, growth factors, and cell surface receptors by limited proteolytic processing (24). In contrast to signal transduction pathways initiated by traditional receptor–ligand interactions, proteinase-mediated signals are transmitted through the cleavage of protein substrates resulting in their activation, inactivation, or alteration of function (22). One example of such proteinase-mediated signaling relevant to inflammatory lung diseases involves proteinase-activated receptors (PARs) such as PAR-1, which can be activated by limited proteolytic cleavage by elastase, resulting in apoptosis of lung epithelial cells (25). Notably, elastase-mediated apoptosis of lung epithelial cells has been implicated in the pathogenesis of the acute respiratory distress syndrome (26, 27) and COPD (28).

In addition to PARs, neutrophil elastase has been shown to degrade numerous receptors involved in control of innate and adaptive immune responses such as CD2, CD4, and CD8 on T lymphocytes (29), and phagocytic receptors such as CD16 (30) expressed by neutrophils and macrophages. Neutrophil elastase is able to degrade receptors that are involved in recognition and clearance of apoptotic cells, which has significant implications for the resolution of neutrophilic inflammation in diseases such as CF (31). Neutrophil elastase also can directly cleave and activate the epithelial sodium channel, thus altering fluid and electrolyte transport across the pulmonary epithelium, a process that results in mucous dehydration and contributes to the pathogenesis of CF lung disease (32, 33).

In the current study, Le Gars and colleagues describe a novel mechanism by which neutrophil elastase induces proteolytic cleavage of CFTR (7). The authors demonstrate that the CFTR protein is degraded in a neutrophil elastase–dependent manner. Unexpectedly, as opposed to direct degradation of CFTR by neutrophil elastase, the authors describe a mechanism whereby neutrophil elastase activates intracellular calpains that, in turn, are directly responsible for the proteolytic degradation of CFTR. Notably, this proteolytic degradation of CFTR abrogates the chloride transport function of the CFTR protein, which has crucial pathophysiological consequences for inflammatory lung diseases such as CF and COPD as described above. Importantly, the authors demonstrate the importance of this pathway in an animal model of bacterial lung infection, underscoring the relevance of this pathway to more complex model systems. These observations have important implications for the pathogenesis of CF as well as COPD. Hence, as discussed above, further studies are warranted to determine if a decrease in functional membrane-CFTR (via genetic mutation or CS exposure) and/or *Pa* infection (via neutrophil elastase and calpains) is a critical step(s) in the initiation of *chronic* inflammatory responses in the lung contributing to the pathogenesis of obstructive lung disease.

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

Neeraj Vij, Ph.D.
Department of Pediatrics
Johns Hopkins School of Medicine
Baltimore, Maryland
and
Division of Pulmonary Medicine
Johns Hopkins School of Medicine
Baltimore, Maryland

Gregory P. Downey, M.D.
Department of Medicine
National Jewish Health
Denver, Colorado
Department of Medicine
University of Colorado
Denver, Colorado
and
Integrated Department of Immunology
University of Colorado
Denver, Colorado

References

1. Mueller C, Braag SA, Keeler A, Hodges C, Drumm M, Flotte TR. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am J Respir Cell Mol Biol* 2011;44:922–929.
2. Bonfield TL, Hodges CA, Cotton CU, Drumm ML. Absence of the cystic fibrosis transmembrane regulator (Cftr) from myeloid-derived cells slows resolution of inflammation and infection. *J Leukoc Biol* 2012;92:1111–1122.
3. Bodas M, Min T, Mazur S, Vij N. Critical modifier role of membrane-cystic fibrosis transmembrane conductance regulator-dependent ceramide signaling in lung injury and emphysema. *J Immunol* 2011;186:602–613.
4. Bajmoczi M, Gadjeva M, Alper SL, Pier GB, Golan DE. Cystic fibrosis transmembrane conductance regulator and caveolin-1 regulate epithelial cell internalization of Pseudomonas aeruginosa. *Am J Physiol Cell Physiol* 2009;297:C263–C277.

5. Di A, Brown ME, Deriy LV, Li C, Szeto FL, Chen Y, Huang P, Tong J, Naren AP, Bindokas V, *et al.* CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. *Nat Cell Biol* 2006;8:933–944.
6. Bomberger JM, Ye S, Maceachran DP, Koeppen K, Barnaby RL, O'Toole GA, Stanton BA. A Pseudomonas aeruginosa toxin that hijacks the host ubiquitin proteolytic system. *PLoS Pathog* 2011;7:e1001325.
7. Le Gars M, Descamps D, Roussel D, Saussereau E, Guillot L, Ruffin M, Tabary O, Hong S-S, Boulanger P, Paulis M, *et al.* Neutrophil elastase degrades cystic fibrosis transmembrane conductance regulator via calpains and disables channel function *in vitro* and *in vivo*. *Am J Respir Crit Care Med* 2013;187:170–179.
8. Bodas M, Min T, Vij N. Critical role of CFTR-dependent lipid rafts in cigarette smoke-induced lung epithelial injury. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L811–L820.
9. Cantin AM, Hanrahan JW, Bilodeau G, Ellis L, Dupuis A, Liao J, Zielenski J, Durie P. Cystic fibrosis transmembrane conductance regulator function is suppressed in cigarette smokers. *Am J Respir Crit Care Med* 2006;173:1139–1144.
10. Clunes LA, Davies CM, Coakley RD, Aleksandrov AA, Henderson AG, Zeman KL, Worthington EN, Gentzsch M, Kreda SM, Cholon D, *et al.* Cigarette smoke exposure induces CFTR internalization and insolubility, leading to airway surface liquid dehydration. *FASEB J* 2012;26:533–545.
11. Sloane PA, Shastry S, Wilhelm A, Courville C, Tang LP, Backer K, Levin E, Raju SV, Li Y, Mazur M, *et al.* A pharmacologic approach to acquired cystic fibrosis transmembrane conductance regulator dysfunction in smoking related lung disease. *PLoS ONE* 2012;7:e39809.
12. Teichgraber V, Ulrich M, Endlich N, Riethmuller J, Wilker B, De Oliveira-Munding CC, van Heeckeren AM, Barr ML, von Kurthy G, Schmid KW, *et al.* Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. *Nat Med* 2008;14:382–391.
13. Bartoszewski R, Rab A, Twitty G, Stevenson L, Fortenberry J, Piotrowski A, Dumanski JP, Bebok Z. The mechanism of cystic fibrosis transmembrane conductance regulator transcriptional repression during the unfolded protein response. *J Biol Chem* 2008;283:12154–12165.
14. Rab A, Bartoszewski R, Jurkuvenaite A, Wakefield J, Collawn JF, Bebok Z. Endoplasmic reticulum stress and the unfolded protein response regulate genomic cystic fibrosis transmembrane conductance regulator expression. *Am J Physiol Cell Physiol* 2007;292:C756–C766.
15. Harrington MA, Kopito RR. Cysteine residues in the nucleotide binding domains regulate the conductance state of CFTR channels. *Biophys J* 2002;82:1278–1292.
16. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 2006;6:173–182.
17. Flannagan RS, Jaumouille V, Grinstein S. The cell biology of phagocytosis. *Annu Rev Pathol* 2012;7:61–98.
18. Hokari R, Miura S. Neutrophil elastase in colitis: more than a marker of disease activity? *J Gastroenterol* 2006;41:395–396.
19. Abraham E. Neutrophils and acute lung injury. *Crit Care Med* 2003;31:S195–S199.
20. Zemans RL, Colgan SP, Downey GP. Transepithelial migration of neutrophils: mechanisms and implications for acute lung injury. *Am J Respir Cell Mol Biol* 2009;40:519–535.
21. Mykles DL. Proteinase families and their inhibitors. *Methods Cell Biol* 2001;66:247–287.
22. Turk B, Turk du SA, Turk V. Protease signalling: the cutting edge. *EMBO J* 2012;31:1630–1643.
23. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat Rev Mol Cell Biol* 2007;8:221–233.
24. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001;17:463–516.
25. Suzuki T, Yamashita C, Zemans RL, Briones N, Van Linden A, Downey GP. Leukocyte elastase induces lung epithelial apoptosis via a PAR-1-, NF-kappaB-, and p53-dependent pathway. *Am J Respir Cell Mol Biol* 2009;41:742–755.
26. Albertine KH, Soulier MF, Wang Z, Ishizaka A, Hashimoto S, Zimmerman GA, Matthay MA, Ware LB. Fas and fas ligand are up-regulated in pulmonary edema fluid and lung tissue of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Pathol* 2002;161:1783–1796.
27. Matute-Bello G, Liles WC, Steinberg KP, Kiener PA, Mongovin S, Chi EY, Jonas M, Martin TR. Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). *J Immunol* 1999;163:2217–2225.
28. Shapiro SD, Goldstein NM, Houghton AM, Kobayashi DK, Kelley D, Belaaouaj A. Neutrophil elastase contributes to cigarette smoke-induced emphysema in mice. *Am J Pathol* 2003;163:2329–2335.
29. Doring G, Frank F, Boudier C, Herbert S, Fleischer B, Bellon G. Cleavage of lymphocyte surface antigens CD2, CD4, and CD8 by polymorphonuclear leukocyte elastase and cathepsin G in patients with cystic fibrosis. *J Immunol* 1995;154:4842–4850.
30. Tosi MF, Zakem H. Surface expression of Fc gamma receptor III (CD16) on chemoattractant-stimulated neutrophils is determined by both surface shedding and translocation from intracellular storage compartments. *J Clin Invest* 1992;90:462–470.
31. Vandivier RW, Fadok VA, Hoffmann PR, Bratton DL, Penvari C, Brown KK, Brain JD, Accurso FJ, Henson PM. Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. *J Clin Invest* 2002;109:661–670.
32. Caldwell RA, Boucher RC, Stutts MJ. Neutrophil elastase activates near-silent epithelial Na+ channels and increases airway epithelial Na+ transport. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L813–L819.
33. Adebamiro A, Cheng Y, Rao US, Danahay H, Bridges RJ. A segment of gamma ENaC mediates elastase activation of Na+ transport. *J Gen Physiol* 2007;130:611–629.

Copyright © 2013 by the American Thoracic Society
DOI: 10.1164/rccm.201211-2011ED

The Elusive "Gold" Standard for Detecting *Mycobacterium tuberculosis* Infection

When introduced in 2001, IFN-γ release assays (IGRAs) were seen as a potential breakthrough in tuberculosis (TB) control because they could be completed with one patient visit and because they might avoid the subjectivity and variability associated with placing and reading the tuberculin skin test (TST) (1). Variability of the TST has been well described in both qualitative and quantitative terms during its long history of use (2). However, increased test–retest reliability of IGRAs compared with TST has been difficult to demonstrate. Lack of a gold standard for diagnosing *Mycobacterium tuberculosis* infection, the potential for TST to boost subsequent TST and IGRA results, the complexity of IGRAs, and the shortcomings of statistical methods limit assessment and comparisons between the tests. IGRA variability has been assessed in relatively few studies, and in most cases using different "yardsticks."

Author Contributions: Conception and design: J.D.M., G.H.M.; analysis and interpretation: J.D.M., J.B., G.H.M.; drafting the manuscript for important intellectual content: J.D.M., J.B., G.H.M.

This material has been reviewed by the Walter Reed Army Institute of Research and the Centers for Disease Control and Prevention. There is no objection to its publication. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, the Department of Defense, or the United States Army.