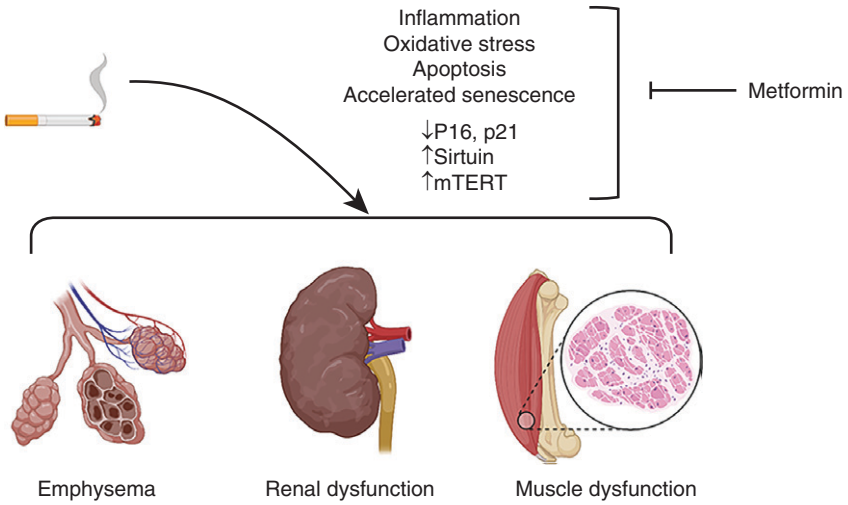

Figure 1. Schematic of effects of metformin in the mouse model of cigarette smoke–induced emphysema. mTERT = mouse telomerase. Created with Biorender.com.

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The Saga of Necroptosis in Chronic Obstructive Pulmonary Disease Continues

Chronic obstructive pulmonary disease (COPD) is a disease state in which chronic inflammation drives irreversible airway remodeling and airspace destruction, leading to chronic bronchitis and emphysema. COPD is predicted to be the third leading cause of death worldwide by the year 2030 and there remains a paucity of therapies (1). Cigarette smoke (CS) exposure is the primary risk factor for the development of COPD, and subsequent imbalances in oxidative stress, inflammation, and growth factor signaling support dysregulation and/or death of the epithelial, endothelial, and immune cellular compartments within the lung (2).

Regulated cell death (RCD) pathways are genetically encoded programs that support the maintenance of tissue homeostasis after cellular stress and/or injury. RCD can also represent aberrant responses in the pathogenesis of tissue injury, leading to deleterious consequences in human diseases (3). Apoptosis is the prototypical form of RCD in which caspase activation is associated with chromatin condensation, cell shrinkage, DNA fragmentation, and

Supported by NHLBI grant 5T32HL134629.

Originally Published in Press as DOI: 10.1164/rccm.202106-1378ED on August 3, 2021

eventual mitochondrial dysfunction. Remaining cellular fragments are encompassed in apoptotic bodies that are phagocytosed, allowing this form of RCD to be noninflammatory (4). Necroptosis relies on the formation of the necrosome, which is formed through the activation of RIPK1 and RIPK3 (receptor-interacting serine/threonine-protein kinase 1 and 3) with subsequent phosphorylation of MLKL (mixed-lineage kinase domain-like protein), which is the inducer of cell death. In contrast to apoptosis, necroptosis is a strong inducer of inflammation through the release of damage-associated molecular patterns from dying cells (4).

Dysregulation of cell death is a known feature of human COPD lung tissue and is associated with an emphysematous phenotype (5–7). Both the alveolar endothelial and epithelial cellular compartments undergo cell death, but whether one cell type is driving the disease remains unclear (8–11). Initial studies monitored cell death in diseased lungs through the assessment of DNA fragmentation, in which positivity represented an apoptotic state (6). Of note, positive findings in these assays are not specific to apoptosis and also identify cells undergoing necroptosis or even necrosis. Studies in which pharmacological modification of the caspase pathway impacted the development of emphysema have shed light on the relevance of apoptosis to the disease process (12, 13).

RCD via activation of necroptosis has also been described in experimental models of COPD (14) (15). In those studies, CS exposure led to induction of mitophagy and activation of necroptosis (14). Loss of key mitophagic protein, *PINK1*, reduced the activation of necroptosis through the loss of MLKL phosphorylation. In human COPD tissue, these proteins colocalized, but the degree of necroptosis and its correlation with disease severity remained unknown. Further studies have linked dysregulated sphingolipid metabolism to the activation of mitophagy and necroptosis in response to CS (15).

In this issue of the *Journal*, Lu and colleagues (pp. 667–681) report a series of studies that shed light on the contribution of necroptosis to the pathogenesis of COPD (16). Detailed assessment of this pathway in human lungs with COPD and the use of genetically modified mice in which necroptosis was ablated were performed. Elevated concentrations of active RIPK3 and MLKL in human COPD lung epithelium and alveolar macrophages were observed. The degree of impaired lung function, observed as changes in % D_{LCO}, directly correlated with increased necroptotic tissue activity. These data define the human COPD lung as a pronecroptotic environment where increases in activity correlate clearly with more advanced disease states.

In vivo studies revealed elevated levels of cell death and increased amounts of *Mlkl* expression in total lung tissue and alveolar macrophages, as well as higher protein levels of RIPK1 and RIPK3 after chronic CS exposure. The data presented show upregulation of key necroptotic proteins in response to CS, but in the absence of an assessment of the phosphorylated forms of RIPK3 and MLKL, it is not possible to ascertain the degree of necroptotic activation in these tissues. These studies are often limited by the availability of antibodies to the phosphorylated forms of these two proteins.

The authors use *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ mice in the acute and chronic CS models to dissect the role of necroptosis in airway inflammation, remodeling, and emphysema. These mice were used to distinguish specific necroptotic effects (*Mlkl*$^{-/-}$) from additional inflammatory and apoptotic functions performed by RIPK3. Airway inflammation was reduced in the acute and chronic models in *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ mice. The blunted inflammatory response coincided with reduced expression of MMP12, a key molecule associated with the development of emphysema through enzymatic digestion of lung tissue. Alteration of inflammatory mediators were measured through mRNA expression, and studies examining enzymatic activity are needed to validate and strengthen these conclusions. Of note, although measurable reductions in disease endpoints occurred in *Ripk3*$^{-/-}$ mice, global loss of MLKL seemed to blunt airway inflammation, remodeling, and the development of emphysema much more robustly. This was perhaps surprising, as RIPK3 loss would impact both the apoptotic and necroptotic pathways, whereas *Mlkl*$^{-/-}$ mice represent necroptotic-specific effects, which further highlights the complexity of the cross-talk between necroptosis and apoptosis in a disease model such as COPD.

Through the administration of pan-caspase inhibitor, the contribution of apoptosis to the COPD phenotype and the therapeutic potential of apoptosis and necroptosis inhibition (*Mlkl*$^{-/-}$) were tested. Airway inflammation was reduced under conditions of both necroptoic and apoptotic inhibition, but there was an absence of synergy in the combined conditions. In addition, neither airway remodeling nor the development of emphysema was significantly altered by the addition of the pan-caspase inhibitor. The authors took these findings to suggest that airway remodeling and the development of emphysema are necroptotic-specific events. Dissecting the contribution of different RCD mechanisms in the development of COPD poses great challenges and the authors should be commended on their approach. However, in the absence of further assessment of specific downstream apoptosis markers, the conclusions on the impact of the inhibitor must be made with caution. Dissecting out the degree of apoptosis blockade and identifying additional off-target consequences of the inhibitor are critical to fully answer the questions proposed.

The data presented by Lu and colleagues are highly compelling, as it is undeniable that necroptotic blockade reduces the murine COPD phenotype after only 8 weeks of CS exposure. Extended time points of CS exposure (4 or 6 mo) were not performed, so the full extent of the protection in the *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ may not have been realized through these studies. Future experiments whereby the impact of cell-specific ablation of necroptosis are assessed will be critical in understanding the molecular mechanisms underlying these initial observations. These studies position the necroptotic pathway as a potential therapeutic target for the treatment of COPD. Necroptotic inhibitors are available and their potential utility as a therapy for the treatment of COPD is of great interest. ■

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

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Nonpharmacological Interventions in Delirium: The Law of the Handicap of a Head Start

Numerous patients who have been admitted to an ICU show a disturbance of consciousness and cognition. Usually this is compatible with delirium, a clinical expression of acute encephalopathy (1). Important sequelae of delirium may include long-term cognitive impairment, discharge to a nursing home, and low quality of life (2, 3). Delirium also has an impact on society due to increased duration of ICU and hospital admission, which results in decreased medical capacity and increased costs (2, 4).

It is generally assumed that nonpharmacological measures may decrease the burden of delirium, both by prevention and by treatment. The current Society of Critical Care Medicine guideline for pain, agitation/sedation, delirium, immobility, and sleep disruption suggests application of multicomponent nonpharmacologic intervention programs to optimize modifiable delirium risk factors (5). However, evidence is limited, particularly in ICU patients. A meta-analysis on multicomponent nonpharmacological interventions in non-ICU patients showed that these could decrease the odds of delirium by 53% (6). These components included "improvement of cognition or orientation," "early mobility," "stimulation of the use of hearing aids and glasses," "sleep–wake cycle preservation," and "treatment of dehydration" (6). It is important that these measures are studied in ICU patients for at least two reasons. First, although delirium features are similar in patients across different settings, findings in non-ICU patients may not be generalizable to ICU patients, as these may differ from non-ICU patients with regard to risk factors as well as treatment. Second, application of nonpharmacological interventions is not for free, as successful implementation requires time and effort to change the work culture of healthcare professionals. The application of nonpharmacological interventions in the ICU should therefore be supported by solid scientific evidence.

The UNDERPIN-ICU (Nursing Delirium Preventive Interventions in the Intensive Care Unit) study, described in this issue of the *Journal* (pp. 682–691), is an important contribution (7). UNDERPIN-ICU investigated the impact of a multicomponent nursing intervention program with a multicenter stepped-wedge cluster-randomized clinical trial in 1,749 patients who were admitted to one of 10 participating ICUs and who were at high risk of developing delirium (7). A program targeting modifiable risk factors was implemented as standard of care, focusing on visual and hearing impairment, orientation loss, sleep deprivation, cognitive impairment, and immobility. These domains were customized for

Originally Published in Press as DOI: 10.1164/rccm.202106-1475ED on July 7, 2021