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α1-Antitrypsin

Key Player or Bystander in Acute Respiratory Distress Syndrome?

Grace Hogan, M.Sc., Pierce Geoghegan, M.B., B.Ch., B.A.O., Tomás P. Carroll, Ph.D., Jennifer Clarke, M.B., B.Ch., B.A.O., Oisín F. McElvaney, M.B., B.Ch., B.A.O., Oliver J. McElvaney, M.B., B.Ch., B.A.O., Ph.D., Noel G. McElvaney, M.B., B.Ch., B.A.O., D.Sc., Gerard F. Curley, M.B., B.Ch., B.A.O., Ph.D.

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ABSTRACT

Acute respiratory distress syndrome is characterized by hypoxemia, altered alveolar–capillary permeability, and neutrophil-dominated inflammatory pulmonary edema. Despite decades of research, an effective drug therapy for acute respiratory distress syndrome remains elusive. The ideal pharmacotherapy for acute respiratory distress syndrome should demonstrate antiprotease activity and target injurious inflammatory pathways while maintaining host defense against infection. Furthermore, a drug with a reputable safety profile, low possibility of off-target effects, and well-known pharmacokinetics would be desirable. The endogenous 52-kd serine protease α1-antitrypsin has the potential to be a novel treatment option for acute respiratory distress syndrome. The main function of α1-antitrypsin is as an antiprotease, targeting neutrophil elastase in particular. However, studies have also highlighted the role of α1-antitrypsin in the modulation of inflammation and bacterial clearance. In light of the current SARS-CoV-2 pandemic, the identification of a treatment for acute respiratory distress syndrome is even more pertinent, and α1-antitrypsin has been implicated in the inflammatory response to SARS-CoV-2 infection.

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Increased alveolar–capillary permeability to fluid, proteins, neutrophils, and erythrocytes, resulting in their accumulation in the alveolar space and disruption of normal gas exchange, is the hallmark of acute respiratory distress syndrome (ARDS).[1] Despite advances in the supportive care of ARDS, an associated mortality rate of up to 40% remains, in part because of a lack of treatment options.[2] ARDS is diagnosed according to the Berlin definition, which includes (1) an acute onset, (2) bilateral infiltrates on chest imaging, (3) noncardiogenic pulmonary edema, and (4) a Pao_2:fractional inspired oxygen tension (Fio_2) of 300 mmHg or less with positive end-expiratory pressure of 5 cm H_2O or higher.[3] Although diagnosis and incidence tend to differ worldwide, a recent international observational study encompassing 50 countries reported that 10% of all intensive care unit (ICU) admissions over a 4-week period resulted in ARDS (n = 3,022).[4] Severe sepsis is the most common cause of ARDS, with both pulmonary and nonpulmonary sepsis accounting for up to 80% of cases,[5] while pneumonia is the main triggering factor in pulmonary sepsis.[6] Viral and/or bacterial pneumonia typically results in the accumulation of immune cells, protein, and erythrocytes in the lung because of disruption of the normally impermeable alveolar–capillary membrane. This in turn leads to pulmonary edema, which impairs gas exchange and results in respiratory failure.[7] Several other risk factors are also associated with the development of ARDS, including aspiration of gastric contents; major trauma; acute pancreatitis; transfusion of fresh frozen plasma, erythrocytes, and/or platelets (transfusion-associated acute lung injury); drug overdose; near drowning (inhalation of fresh or salt water); hemorrhagic shock or reperfusion injury (including after cardiopulmonary bypass and lung resection); and smoke inhalation.

Pathophysiology of ARDS

During the initial stage of ARDS, there is a significant increase in the production of proinflammatory cytokines and a pulmonary infiltration of myeloid leukocytes. A positive feedback loop between these cytokines and cells ensures the propagation of inflammation. Levels of tumor necrosis factor α and interleukins 1β, 6, and 8 have been shown to increase dramatically in the plasma and bronchoalveolar lavage fluid of ARDS patients[7] and are associated with poor prognosis.[8–10] Tumor necrosis factor α and interleukin 1β stimulate the production of interleukins 6 and 8 by surrounding cells and subsequently recruit monocytes and leukocytes into the air spaces.[11] In addition, tumor necrosis factor α has been shown to encourage pulmonary edema by altering the permeability and fluid transport capability of the alveolar–capillary barrier.[12] During ARDS, there is a rapid and sustained accumulation of neutrophils, particularly in the alveolar space, and a decrease in neutrophil apoptosis.[7,13] Reduced neutrophil infiltration has been shown to correlate with the resolution of lung injury in preclinical models of ARDS.[14–16] The degree of neutrophilia in the bronchoalveolar lavage fluid of ARDS patients also correlates with disease severity and clinical

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outcome.[7] Neutrophil-driven inflammation and tissue damage are in part due to the indiscriminate release of the protease neutrophil elastase, and increased concentrations of neutrophil elastase in critically ill patients have been strongly linked to the development of ARDS and the need for mechanical ventilation.[17] Intraalveolar macrophages play an important part in releasing chemotactic factors such as interleukin 8 and chemokines such as CC-chemokine ligand 2 (also known as MCP-1) that enhance the recruitment of neutrophils and monocytes into the lung, particularly in response to acute pulmonary infections. This uncontrolled inflammation in turn leads to pulmonary epithelial cell injury.[18] As a result, protein-rich fluid leaks into the interstitium and alveolar space from the capillaries.[19] Downregulation of pivotal sodium/potassium channels occurs on the injured epithelial cell membrane, alveolar fluid clearance is impaired, and pulmonary edema accumulates.[20] Damage to epithelial type II cells also leads to decreased surfactant production, which results in a loss of pulmonary compliance and alveolar lung collapse.[21] In addition to lung epithelial cell injury, a loss of barrier function at the level of the endothelium enables the influx of protein-rich fluid into the interstitium, through the epithelial lining of the lung, and ultimately into the alveoli.[22] It has been established that the endothelium undergoes significant structural changes during ARDS, in addition to releasing adhesion molecules in response to the associated inflammation.[23] Importantly, biomarkers of endothelial cell injury such as von Willebrand factor and intracellular adhesion molecule 1 are increased during ARDS, act as predictors of outcome, and play a role in the activation of neutrophils and recruitment to the interstitium and alveolar space.[24]

Failed Pharmacotherapies for ARDS

A number of pharmacotherapies have been investigated for the treatment of ARDS including aspirin, β_2 agonists, statins, and corticosteroids, but none have shown significant efficacy. One potential reason for this lack of success is the underlying heterogeneity of ARDS. Observational studies have demonstrated that patients on long-term aspirin therapy before hospital admission have a lower ARDS incidence[25] and a significantly reduced ICU mortality in patients who developed ARDS.[26] β_2 Agonists appeared to increase 28-day mortality in patients with ARDS in a large phase 3 trial,[27] despite having shown efficacy in an earlier phase 2 trial.[28] Early studies have shown the safety of the use of statins in ARDS[29] but have failed to demonstrate efficacy.[30] However, it is possible that one or more of these treatments might be effective if targeted toward specific subphenotypes, as illustrated by Calfee and others.[31–33] In an initial multicenter double-blinded randomized phase 3 clinical trial, simvastatin had no effect on the primary outcome of ventilator-free days or secondary outcomes of days free of nonpulmonary organ failure or mortality.[30] However, when secondary latent class analysis was performed, it became apparent that two distinct subphenotypes of ARDS were present, namely hypo- and hyperinflammatory groups.[31] Furthermore, patients in the hyperinflammatory simvastin-treated group showed significantly higher survival compared with their placebo-treated counterparts. Patients were stratified to the subphenotype classes primarily on the basis of levels of soluble tumor necrosis factor receptor 1 and interleukin 6, platelet counts, and vasopressor use. In line with previous studies examining the characteristics of hypo- *versus* hyperinflammatory ARDS, the hyperinflammatory subphenotype was also associated with decreased ventilator-free days and nonpulmonary organ failure-free days but increased 28-day mortality.[31,33] ARDS etiology is also a contributing factor when considering hypo- and hyperinflammatory patient cohorts. Although pneumonia, sepsis, and aspiration remain the main precipitating factors, the distribution of risk factors does differ significantly between the two subphenotypes in the literature.[31–33] Typically, pneumonia is the primary insult in the hypoinflammatory group, whereas sepsis is the predominant cause in the hyperinflammatory group.[31,33] Interestingly, trauma also appears to be more prevalent in the hypoinflammatory subphenotype.[32,33]

The use of corticosteroids as a pharmacotherapy for ARDS is a controversial topic. A number of different formulations, dosing, and timing regimens, as well as heterogenous ARDS patient etiologies, have been investigated, producing conflicting results. In 2008, Peter *et al.*[34] published a comprehensive review of nine randomized controlled trials to summarize the studies completed thus far. The authors found that there were no significant beneficial effects of corticosteroid treatment in patients with moderate-to-severe ARDS. When corticosteroids were administered after the onset of ARDS, there were trends toward reduced mortality and increased ventilator-free days, but both outcomes failed to reach significance. Furthermore, prophylactic corticosteroid use appeared to increase the risk of ARDS development and subsequent death, in addition to nosocomial secondary infection.[34] Nonetheless, in 2017, the Society of Critical Care Medicine (Mount Prospect, Illinois) and European Society of Intensive Care Medicine (Brussels, Belgium) recommended the use of methylprednisolone in patients with early moderate-to-severe ARDS.[35]

Outcomes of the most recent randomized controlled trial for corticosteroid use in patients with ARDS were published in February 2020 by Villar *et al.*[36] IV administration of 20 mg of dexamethasone daily for 5 days followed by 10 mg daily for days 6 to 10 to 139 patients with early moderate-to-severe ARDS has provided encouraging evidence. The primary outcome was ventilator-free days at 28 days and was significantly greater in the treated group. The secondary outcome was day-60 all-cause mortality, which was significantly reduced in the dexamethasone arm.[36] It should be noted that the major difference in this trial compared with previous trials was the provision of lung-protective ventilation. However, the precipitating factor for the

majority of cases was infectious ARDS, and as such, care must be taken when generalizing the results.

In July 2020, the Randomised Evaluation of COVID-19 Therapy (RECOVERY) trial demonstrated the efficacy of dexamethasone in the treatment of SARS-CoV-2. In this trial, 2,104 patients with SARS-CoV-2 infection received 6 mg of dexamethasone orally or IV daily for up to 10 days.[37] In the dexamethasone group, 28-day mortality was a third lower than in the control group in those also undergoing mechanical ventilation and a fifth lower in those receiving supplementary oxygen. Interestingly, dexamethasone had no beneficial effects in patients not receiving respiratory support.[37] The COVID-19 Dexamethasone (CoDEX) trial observed similar findings, albeit in a much smaller patient population.[38] Patients with SARS-CoV-2 treated with 10 to 20 mg of dexamethasone IV for up to 10 days had significantly higher ventilator-free days compared to controls. There was no mortality benefit noted, but the trial was stopped early because of the release of the results from the RECOVERY trial, which meant the study was underpowered.[38]

This body of evidence suggests that whereas corticosteroids may provide a degree of benefit in the treatment of ARDS and SARS-CoV-2, the efficacy depends on a number of factors including (1) the type of steroid used; (2) timing of administration; (3) dosing regimen; (4) severity and cause of ARDS (*e.g.*, in severe acute respiratory syndrome and Middle East respiratory syndrome, steroids are associated with increased mortality); (5) concomitant therapies; and (6) comorbidities. As such, the clinical feasibility is limited. Furthermore, steroids have multiple off-target effects that are highly undesirable in critically ill patients, including increased risk of delirium, hyperglycemia, and critical illness myopathy.[36]

Features of the Ideal Pharmacotherapy for ARDS

Despite the failure thus far to uncover an effective pharmacotherapy for ARDS, it is possible to speculate that one will be found. This therapy would ideally play a key role in immunomodulation and antiprotease activity. An additional role in preventing coinfection and perhaps enhancing bacterial clearance would also be attractive because sepsis and pneumonia are the main precipitating factors for development of ARDS.[5,6] A drug with a reputable safety profile, low possibility of off-target effects, and a well-known pharmacokinetic profile would be desirable. Furthermore, an approved drug with a wealth of supporting *in vitro* and *in vivo* evidence, the option of a number of administration routes, and the ability to alter its pharmacokinetics would offer undeniable advantages. Based on this, we propose that the endogenously produced glycoprotein α1-antitrypsin (AAT) is a potential novel treatment option for ARDS. A number of the main characteristics of ARDS pathology and corresponding beneficial actions of AAT are summarized in figure 1.

α1-Antitrypsin

α1-Antitrypsin is a 52-kd glycoprotein located on chromosome 14q3132.3.[39] It belongs to the serine protease inhibitor (SERPIN) superfamily and is produced mainly by hepatocytes in the liver but also by macrophages, neutrophils, and endothelial cells.[40] AAT acts as a protease inhibitor, most notably of neutrophil elastase, and plays a vital role in conserving the balance between levels of proteases and antiproteases in the lung. The plasma half-life of AAT is 4 to 5 days.[41] More recently, AAT has demonstrated an important function as a modulator of innate immune function and bacterial clearance.

Current Use of AAT

α1-Antitrypsin is Food and Drug Administration (Silver Spring, Maryland)–approved for the treatment of AAT deficiency. AAT deficiency is an autosomal codominant disorder first described in the early 1960s and is caused by mutations in the SERPINA1 gene.[42] The clinical manifestations of AAT deficiency are multifaceted and affect the lung, liver, and skin. In the lung, a loss of function occurs because of deficient production of AAT and disrupts the protease–antiprotease balance. Damage-inducing proteases are therefore unopposed, which results in lung tissue damage, emphysema, and chronic obstructive pulmonary disease.[40] In the liver, a toxic gain of function precipitates hepatic dysfunction because of abnormally folded and polymerized AAT aggregates.[40] Consequently, hepatocytes endure endoplasmic reticulum stress and ultimately apoptosis. Skin manifestations of AAT deficiency are less common but include necrotizing panniculitis[43] and vasculitis (typically positive for cytoplasmic–antineutrophil cytoplasmic autoantibodies [c-ANCA]).[44]

Formulations and Routes of Administration

The accepted standard route of administration of AAT since being introduced in 1981 is IV.[45] IV is also the only route currently approved by the Food and Drug Administration for use. Prolastin (Grifols, USA) was the first formulation approved in 1987, followed by Aralast (Baxter, USA), Zemaira (CSL Behring, USA), Trypsone (Grifols, Spain), Alfalastin (LFB Biomedicaments, France), Glassia (Kamada, Israel), and Respreeza (CSL Behring), respectively.[46] The widely accepted treatment strategy for AAT augmentation therapy in patients with AAT deficiency is a weekly infusion of clinical-grade AAT at a dose of 60 mg/kg.[47] This dosing regimen was found to maintain plasma concentration of AAT above a therapeutic threshold of 11 μM (50% of normal) and has become the mainstay of care ever since.[48] More recently, a study performed by Campos *et al.*[49] investigated the potential additive benefit of a 120 mg/kg weekly dosing regimen. The results showed that patients allocated to a double dose of AAT exhibited reduced protease activity, decreased elastin degradation, and an amelioration in inflammation compared

to the single-dose cohort.[49] The feasibility, safety, and efficacy of administering inhaled AAT are also under investigation. At the end of 2019, the pharmaceutical company Kamada began AAT deficiency patient recruitment into a phase 3 clinical trial (NCT00460096) investigating the efficacy of twice-daily inhaled AAT at a dose of 80 mg after a phase 2/3 trial met the primary and secondary endpoints of efficacy and safety, respectively.[50] Although AAT has been proven to be lung-protective in deficient states such as AAT deficiency, it would be plausible to investigate the administration of exogenous AAT to patients with ARDS where endogenous production would likely be insufficient to meet pulmonary demand because of a significant increase in neutrophil elastase.

Potential for Modification

The relatively short half-life of AAT (4 to 5 days) is one of the few limitations of its use and the reason patients with AAT deficiency receive weekly infusions. However, the stability and half-life of the molecule can be modified through processes such as glycosylation and pegylation. Glycosylation is a posttranslational process achieved by the addition of carbohydrate residues to proteins.[51] AAT undergoes *N*-glycosylation, whereby *N*-linked oligosaccharides, or glycans, attach to the nitrogen side chain of an asparagine residue.[52] This process occurs in the emergency room[53] and has been shown to improve the functional efficacy of the molecule at least partially by extending the plasma half-life and preventing aggregation.[54,55] Another method that has been shown to extend the half-life of AAT is polyethylene glycol conjugation, or pegylation.[56] In 2002, Cantin *et al.*[57] demonstrated how pegylation of AAT at Cys^{232} extended its half-life in mice compared with nonpegylated and recombinant human AAT. Importantly, the process of pegylation did not affect the ability of AAT to bind neutrophil elastase and offered prolonged protection against neutrophil elastase–induced lung injury.[57]

Safety Profile of AAT

The safety profile of AAT is long-established and offers an added advantage to repurposing the protein for ARDS. A large European study conducted between 1989 and 1995 recruited 443 patients with severe AAT deficiency and reported on mild side effects associated with IV protein administration in 65 patients.[58] This included symptoms such as pyrexia, rash, nausea/vomiting, and fatigue. A small proportion of patients (n = 22) experienced more severe side effects in the form of dyspnea (n = 17), anaphylaxis (n = 4), and an exacerbation of congestive heart failure (n = 1), but all of these patients ultimately recovered.[58] To circumvent the issue with anaphylaxis, a modified Prolastin called Prolastin-C (Grifols, USA) was introduced that has demonstrated bioequivalence to Prolastin with no serious adverse events documented thus far.[59] There have been no deaths associated with IV administration of AAT.

AAT-mediated Immunomodulation

Lung and systemic inflammation in ARDS is associated with worsening of lung injury and distal organ failure.[60] AAT has been shown to modulate not only the migration of neutrophils and monocytes but also the production of proinflammatory and anti-inflammatory cytokines. The immunomodulatory effect of AAT on neutrophils, monocyte/macrophages, and pulmonary endothelial cells are summarized in figure 2.

Neutrophils

α1-Antitrypsin has a significant influence on neutrophils, in particular on the interleukin 8/CXCR1 signaling axis.[61,62] Interleukin 8 is a proinflammatory mediator that also plays an important role in neutrophil chemotaxis. In 2010, Bergin *et al.*[62] reported that serum AAT binds to interleukin 8 and prevents subsequent interaction of interleukin 8 with CXCR1. Furthermore, this study showed that AAT also decreases soluble immune complex receptor–mediated neutrophil chemotaxis by controlling membrane expression of FcγRIIIb and inhibiting ADAM-17 activity.[62] In a more recent study, McCarthy *et al.*[61] demonstrated how the sialylation of AAT influences its ability to affect interleukin 8/CXCR1–mediated neutrophil chemotaxis. AAT purified from the plasma of patients during the resolving phase of community-acquired pneumonia had a larger number of sialic acids and significantly enhanced interleukin 8 binding to AAT while greatly reducing interleukin 8 binding to CXCR1.[61] In addition, AAT has also been shown to (1) decrease tumor necrosis factor α release from neutrophils and tumor necrosis factor α–induced neutrophil degranulation, (2) reduce reactive oxygen species production,[63] (3) regulate neutrophil apoptosis, and (4) enhance bacterial killing.[63]

Monocyte/Macrophages

Several reports have implicated AAT as playing an important part in the response of the macrophage to infection and inflammation. Exposure of healthy human-derived monocyte/macrophages to lipopolysaccharide (LPS) in the presence of AAT resulted in a significant increase in the proinflammatory cytokines tumor necrosis factor α and interleukin 8 when the supernatant was measured 2 to 4 h later.[64,65] However, when measured at later timepoints (17 to 24 h), AAT appears to undergo a switch to a more anti-inflammatory phenotype, decreasing the release of tumor necrosis factor α, interleukin 8, and interleukin 1β.[66–68] Of note, oxidized AAT failed to reproduce the same anti-inflammatory switch.[66] More recently, it has been reported that AAT reduces interleukin 1β release from monocytes by abrogating downstream adenosine triphosphate–induced activation of P2X7, a receptor involved in inflammasome assembly.[69] Elsewhere, Tilg *et al.*[67] observed a dose-dependent increase in the release of anti-inflammatory interleukin 1ra from macrophages exposed to AAT for 24 h, whereas other groups demonstrated increased production of

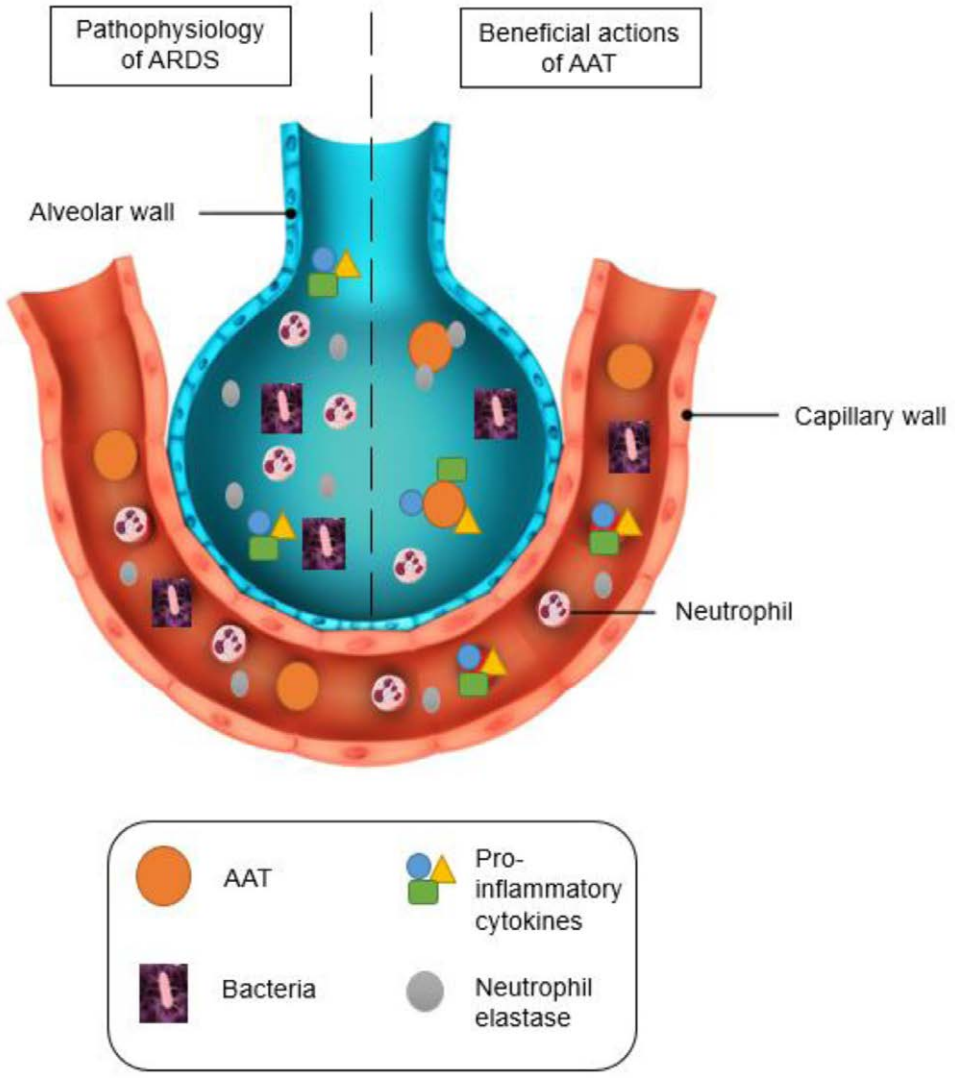

Fig. 1. Pathophysiology of acute respiratory distress syndrome (ARDS) and beneficial actions of α1-antitrypsin (AAT). ARDS is characterized, at least in part, by overwhelming inflammation of neutrophils, the release of neutrophil elastase, and an increase in proinflammation cytokine (interleukin 8, tumor necrosis factor α, and interleukin 1β) release. In sepsis- and pneumonia-related ARDS, there is also a significant bacterial burden in the lung. AAT targets each of these aspects of ARDS pathophysiology. AAT prevents neutrophil chemotaxis to the lung and decreases interleukin 8 release from neutrophils. AAT also directly binds and inactivates neutrophil elastase, a tissue-degrading enzyme released by activated neutrophils. In addition, AAT decreases the bacterial burden in the lung.

proresolving interleukin 10 from LPS-exposed macrophages in the presence of AAT when analyzed 2 to 18 h later.[65] This effect on interleukin 10 was attributed to increased cyclic adenosine monophosphate and activation of cyclic adenosine monophosphate–dependent protein kinase A.[65]

Pulmonary Endothelial Cells

The immunomodulatory action of AAT is not limited to macrophages and neutrophils, however, and has been shown to mediate pulmonary microvascular endothelial cell responses to tumor necrosis factor α in particular.[70] *In vitro*, AAT abrogated the expression of tumor necrosis factor receptor 1 on rat lung–derived endothelial cells in addition to significantly reducing tumor necrosis factor α release. The authors delineated that these effects occurred at least partially because of inhibition of tumor necrosis factor α–converting enzyme activity.[70] Considering that pulmonary microvascular endothelial cells are key players in ARDS pathophysiology, the effect of AAT on these cells provides even further evidence about its potential as a novel therapy for ARDS.

AAT-mediated Bacterial Clearance

Because the vast majority of ARDS cases are precipitated by sepsis and pneumonia,[5] a candidate therapy for ARDS would have to play a central role in bacterial clearance. A pharmacotherapy with the ability to prevent coinfection would also be of advantage. This is because coinfection has been associated with greater morbidity and mortality in critically ill patients with respiratory failure.[71] AAT has demonstrated this ability in cells such as neutrophils and monocyte/macrophages but also in pulmonary epithelial cells. The antibacterial effects of AAT on neutrophils, monocyte/macrophages, and pulmonary endothelial cells are summarized in figure 2.

Neutrophils

α1-Antitrypsin has been shown to enhance the clearance of bacteria such as *Pseudomonas aeruginosa in vivo*,[72] but the quintessential mechanism and cellular mediators remain to be fully determined. Early studies have suggested that AAT restores neutrophil-mediated phagocytosis and bacterial killing *in vitro* by reversing neutrophil elastase cleavage of the complement receptor C3bi on opsonized *P. aeruginosa* and CR1 on neutrophils.[73] Other groups have also highlighted the beneficial effect of AAT on bacterial killing by neutrophils and have shown that AAT deficiency patients have an inherent deficiency of said process.[63]

Monocyte/Macrophages

In terms of monocyte/macrophages, a recent article highlighted the ability of AAT to enhance phagocytosis and efferocytosis *via* both mannose and scavenger receptor pathways in alveolar macrophages isolated from the bronchoalveolar lavage fluid of smokers, whereas there was no such effect observed in alveolar macrophages from healthy nonsmokers.[74] In addition, polymerized AAT failed to increase efferocytosis in alveolar macrophages isolated from either smokers or nonsmokers, which infers the importance of the biochemical alterations to AAT. *In vivo*, short palate, lung, and nasal epithelium clone 1 (SPLUNC1), which is a protein secreted from epithelial cells involved in host defense, has been implicated as being partially responsible for the bactericidal activity of AAT.[75] Mice challenged with *P. aeruginosa* showed decreased levels of AAT, which was reversed by administration of aerosolized AAT. Treatment with AAT also enhanced bacterial clearance from the lung, decreased neutrophil elastase activity, and reduced proinflammatory cytokine release. None of these therapeutic effects were seen when AAT was administered to SPLUNC1 knockout mice.[75]

Pulmonary Epithelial Cells

In addition to influencing inflammatory cell-mediated bacterial clearance, AAT has demonstrated the ability to inhibit the internalization of *P. aeruginosa* into pulmonary epithelial cells *in vitro* and has also prevented *P. aeruginosa*– and neutrophil elastase–mediated disruption of the pulmonary epithelial barrier.[76] The knock-on effect of this particular function of AAT is twofold. First, because internalization is inhibited, bacteria would be subject to prolonged exposure to antipathogen and phagocytic mediators such as immunoglobulins, components of the complement cascade, defensins, and cathelicidins. Second, by preserving the integrity of the epithelial barrier, AAT could potentially facilitate more efficient bacterial clearance. Disruption to the pulmonary epithelial barrier is a key feature of ARDS pathophysiology and results in impaired alveolar fluid clearance and the formation of edema.[20] A therapy that preserves epithelial barrier function may reduce edema formation in ARDS (fig. 2).

Antiviral Effects of AAT

In addition to anti-inflammatory and bactericidal effects, AAT has also been shown to possess indirect antiviral capacities. This is of particular importance in light of the current SARS-CoV-2 pandemic. In 2001, a study by Shapiro *et al.*[77] highlighted the ability of AAT to inhibit human immunodeficiency virus type 1 (HIV-1) production and activity in a human monocytic cell line and in primary monocytes. This was achieved *via* AAT-mediated abrogation of nuclear factor κ–light chain enhancer of activated B cells (NFκB), which is known to induce the transcription of HIV-1. AAT has also been shown to significantly reduce viral load in airway epithelial cells infected with human rhinovirus.[78] Exposure of epithelial cells to rhinovirus strain HRV-16 resulted in an increase in intracellular adhesion molecule 1, which acts as a receptor for HRV-16. Treatment with AAT subsequently significantly decreased intracellular adhesion molecule 1 messenger RNA levels in human rhinovirus–infected cells.[78]

Supporting Preclinical Data

Numerous studies have been performed availing of preclinical animal models of pulmonary and nonpulmonary sepsis. Results have indicated that in addition to targeting neutrophil elastase *in vivo*, AAT is capable of reducing inflammation, enhancing bacterial clearance, improving epithelial cell disruption, and increasing survival. Jonigk *et al.*[79] have shown that AAT maintains anti-inflammatory and immunomodulatory properties even without neutrophil elastase inhibitory activity. Supporting preclinical data are summarized in table 1.

Initial Effects of AAT on Inflammation

Although ultimately the resolution of inflammation during ARDS requires a decrease in inflammatory white cell counts and proinflammatory mediators, an increase in these components is necessary to fight infection in the initial acute phase. In a mouse model of LPS-induced lung injury, intranasal administration of AAT resulted in an increase in

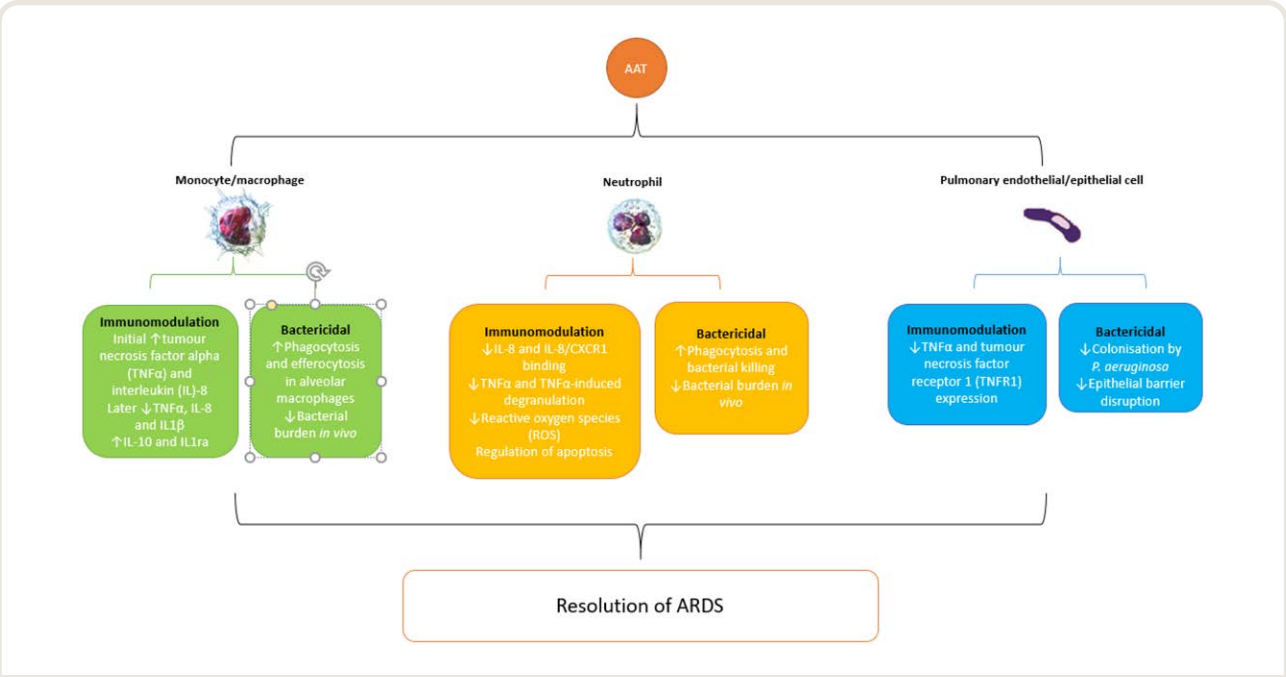

Fig. 2. Immunomodulatory and bactericidal actions of α1-antitrypsin (AAT) on neutrophils, monocyte/macrophages, and pulmonary endothelial/epithelial cells. AAT elicits a biphasic effect on proinflammatory cytokine release from monocyte/macrophages, with an initial increase in prolific cytokines in acute respiratory distress syndrome (ARDS) such as tumor necrosis factor α and interleukin 8, followed by a later decrease. AAT also enhances anti-inflammatory mediator release from monocyte/macrophages. Macrophage-mediated phagocytosis and efferocytosis is enhanced in alveolar macrophages after AAT treatment, and a decreased bacterial burden has been observed in preclinical models of infection. AAT decreases proinflammatory interleukin 8 release from neutrophils and forms a feedback loop, preventing subsequent binding of interleukin 8 with its ligand, CXCR1. AAT also reduces tumor necrosis factor α release from neutrophils and tumor necrosis factor–induced neutrophil degranulation. Reactive oxygen species production in neutrophils is abrogated by AAT, and normal apoptosis is restored. AAT also increases phagocytosis in neutrophils and is capable of reducing bacterial counts in small animal infection models. In pulmonary endothelial cells, AAT has been shown to abrogate both tumor necrosis factor α production and tumor necrosis factor receptor 1 expression. In pulmonary endothelial cells, AAT has been shown to prevent colonization of the cells by *P. aeruginosa*, which helps to avoid epithelial barrier disruption. Each of these actions is central to the resolution of ARDS.

cytokines such as interleukin 10, RANTES, granulocyte colony-stimulating factor, and granulocyte macrophage colony-stimulating factor in bronchoalveolar lavage fluid and lung homogenate when measured 4 h after injury.[64] These results suggest that AAT plays a vital role in the resolution of inflammation during the early stages of infection. However, it remains to be fully determined how the initial AAT-induced release of cytokines changes as infection progresses.

Therapeutic Effects of AAT in Pneumonia

Several *in vivo* preclinical studies have highlighted AAT as having a central role in the attenuation of pneumonia, in particular by enhancing bacterial clearance. In a rat model of chronic *P. aeruginosa* lung infection, aerosolized AAT (Prolastin) reduced the bacterial burden in the lung, as well as neutrophil elastase activity and neutrophil cell counts in bronchoalveolar lavage fluid.[72] The promising beneficial actions of AAT can also be seen in $AAT^{+/+}$ transgenic mice that express human AAT in the lung.[76] Pott *et al.*[76] demonstrated increased bacterial clearance in the lung and blood in addition to significantly reduced mortality, decreased lung tissue damage, and an attenuation of proinflammatory cytokine levels in $AAT^{+/+}$ mice infected with *P. aeruginosa* compared with wildtype mice.

Therapeutic Effects of AAT in Systemic Sepsis

The most commonly used animal models of systemic sepsis are cecal ligation and puncture and fecal peritonitis.[85–87] When $AAT^{+/+}$ mice were subjected to cecal ligation and puncture, observations similar to the study by Subramaniyam *et al.*[64] were made with serum proinflammatory cytokine concentrations showing an initial increase.[81] However, when measured again at 72 h after infection, there was a significant reduction in proinflammatory cytokine levels compared to controls. Interestingly, there was a significant increase in the number of circulating inflammatory white cells in the $AAT^{+/+}$ mice despite this attenuation of proinflammatory cytokine release. Results also showed enhanced bacterial clearance in peritoneal lavage fluid and

Table 1. Summary of Main Findings from Preclinical Lung Injury Models in Chronological Order

Article	Model	Main Findings
Cantin and Woods[72]	*Pseudomonas aeruginosa* (rat)	↓Neutrophil elastase activity and neutrophil infiltration ↑Bacterial clearance
Jie *et al.*[80]	Endotoxin (rabbit)	↑Oxygenation and pulmonary compliance
Subramaniyam *et al.*[64]	Endotoxin (mouse)	↑Interleukin 10, RANTES, granulocyte colony–stimulating factor, and granulocyte macrophage colony–stimulating factor in bronchoalveolar lavage fluid and lung homogenate
Pott *et al.*[76]	*P. aeruginosa* ($AAT^{+/+}$ transgenic mouse)	↓Mortality and lung tissue damage ↑Bacterial clearance ↓Interleukin 1α, interleukin 1β, interleukin 6, MCP-1, and granulocyte macrophage colony–stimulating factor
Kaner *et al.*[81]	Peritonitis and cecal ligation and puncture (human AAT transgenic mouse)	↑Tumor necrosis factor α, interleukin 6, and MCP-1 initially ↓Organ injury and later-stage inflammation ↑Bacterial clearance
Iskender *et al.*[82]	Ischemia–reperfusion injury (porcine *ex vivo* lung perfusion)	↑Gas exchange and compliance ↓Edema, permeability, and inflammation
Zhu *et al.*[83]	Ventilator-induced lung injury (rat)	↑Pao_2:FIo_2 ratio and alveolar–capillary barrier integrity ↓Wet-to-dry ratio, neutrophil infiltration, and proinflammatory cytokine release ↑Interleukin 10
Lin *et al.*[84]	Ischemia–reperfusion injury (porcine *ex vivo* lung perfusion)	↑Gas exchange and compliance ↓Peak airway pressure, pulmonary vascular resistance, edema, and interleukin 8 release

AAT, α1-antitrypsin; FIo_2, fractional inspired oxygen tension.

a significant decrease in markers of multiorgan failure in the $AAT^{+/+}$ mice compared with wildtype mice. When challenged with fecal peritonitis, the $AAT^{+/+}$ group also exhibited increased survival at 24 h after infection.[81]

Therapeutic Effects of AAT in Acute Lung Injury, Ventilator-induced Lung Injury, and Lung Transplant Models

In addition to pulmonary and nonpulmonary sepsis, AAT has demonstrated therapeutic potential in models of endotoxin-induced acute lung injury,[80] ventilator-induced lung injury,[83] and lung transplantation.[82] A study by Jie *et al.*[80] reported on the ability of AAT to improve oxygenation and pulmonary compliance when administered in a large animal model subject to LPS challenge. In recent years, the beneficial effects of AAT in a rodent model of ventilator-induced lung injury were evidenced by an amelioration of (1) the Pao_2:FIo_2 ratio; (2) the lung wet-to-dry weight ratio (a measure of lung water extravasation); and (3) alveolar–capillary barrier integrity.[83] Furthermore, AAT treatment significantly decreased neutrophil counts and inflammatory cytokine levels in bronchoalveolar lavage fluid. Notably, the concentration of anti-inflammatory interleukin 10 was significantly increased in AAT-treated animals.[83]

α1-Antitrypsin has also been investigated in the porcine lung using *ex vivo* lung perfusion to assess the benefit of administering AAT before reperfusion after transplant.[82,84] *Ex vivo* lung perfusion is a highly translatable technique that enables researchers to model the whole intact lung in as accurate a setting as possible without using human volunteers. The results of these studies demonstrated that AAT significantly improved gas exchange and pulmonary compliance. Porcine lungs that were randomized to the AAT group also showed decreased edema and lung permeability, as well as an attenuation of inflammation.[82,84] These results in particular have positive ramifications for the use of AAT in ARDS because deficient gas exchange, worsening pulmonary compliance, and lung edema are three important hallmarks of the disease. Moreover, it is encouraging to note that AAT has been investigated as a potential therapy for several diseases with an underlying inflammatory etiology including graft *versus* host disease,[88] inflammatory bowel disease,[89] diabetes,[90] and renal ischemia–reperfusion injury.[91]

Supporting Clinical Data

To investigate whether AAT could be a novel treatment option for ARDS, it is first important to understand what changes levels of AAT, neutrophil elastase, and AAT complexed to neutrophil elastase (AAT–neutrophil elastase) undergo during the course of ARDS. A small number of studies have been published in this area but span a period of almost 30 yr, avail of small cohorts, and often produced conflicting results. These studies are summarized in table 2.

AAT Levels in Bronchoalveolar Lavage Fluid and Plasma

The first study to quantify AAT levels in the bronchoalveolar lavage fluid of patients with ARDS was carried out by Lee *et al.* in 1981.[92] This study demonstrated that AAT levels were not significantly increased in the bronchoalveolar lavage fluid of a small cohort of eight ARDS patients, and the antiprotease activity of AAT was reduced.[92] Because of

Table 2. Summary of Main Findings from Clinical Data in Acute Respiratory Distress Syndrome Patients in Chronological Order

Article	No. of Patients	Main Findings
Lee *et al.*[92]	8	Nonsignificant ↓ in AAT activity in bronchoalveolar lavage fluid
Idell *et al.*[93]	13	↑ in AAT and neutrophil elastase in bronchoalveolar lavage fluid
Weiland *et al.*[94]	6	↑ Neutrophil elastase in bronchoalveolar lavage fluid
Wewers *et al.*[95]	7	↑ in AAT and neutrophil elastase in bronchoalveolar lavage fluid
Moalli *et al.*[96]	12	No change in AAT in plasma
Rocker *et al.*[97]	9	Correlation between AAT–neutrophil elastase (1) in plasma and worsening hypoxia, and (2) ↑ bronchoalveolar lavage fluid protein
Fujita *et al.*[98]	14	↑ AAT–neutrophil elastase complex in plasma
Groeneveld *et al.*[99]	13	↑ AAT–neutrophil elastase in plasma, correlation between AAT–neutrophil elastase and (1) oxygenation ratio and (2) interleukins 8 and 6
Sallenave *et al.*[100]	16	↑ in AAT in bronchoalveolar lavage fluid (portion inactive)
Hashimoto *et al.*[101]	32	↑ in AAT and AAT–neutrophil elastase in plasma

AAT, α1-antitrypsin.

these surprising results, Cochrane *et al.*[102] examined the issue of active *versus* inactive AAT in ARDS patient bronchoalveolar lavage fluid. The results showed that although AAT is inactivated by the process of binding neutrophil elastase, it is also subject to inactivation by oxidation and is an important consideration for AAT therapy.[102] Several years later, Idell *et al.*[93] reported conflicting results with levels of AAT found to be significantly elevated in the bronchoalveolar lavage fluid of 13 patients with ARDS compared with healthy controls. This was further evidenced by Wewers *et al.*,[95] who highlighted that seven patients with ARDS had levels of AAT in bronchoalveolar lavage fluid 60 times the amount found in healthy volunteers. In 1999, Sallenave *et al.*[100] advanced the field further by quantifying AAT in bronchoalveolar lavage fluid obtained from (1) patients at risk of developing ARDS and (2) patients with established ARDS. They showed that patients at risk of developing ARDS, regardless of whether they did or not, and patients with established ARDS had significantly increased levels of AAT compared to controls. Furthermore, concentrations of AAT were significantly lower in patients who did not develop ARDS *versus* patients with established ARDS. However, when the authors assessed the contribution of AAT to elastase inhibitory activity, they observed that a significant proportion AAT was inactive.[100] This appears to suggest that reduced AAT activity might play a role in ARDS pathogenesis but should be assessed in a larger patient cohort.

Levels of AAT in ARDS patient plasma are much less documented, with only one report indicating that plasma levels of AAT were not elevated in a cohort of 12 patients suffering from ARDS.[96] Recently, our group published the results of a study examining AAT levels in the plasma of 45 patients with ARDS.[103] We found that circulating AAT was significantly increased at baseline compared to healthy control levels. We also investigated the trajectory of AAT during ARDS pathogenesis and observed that AAT in plasma remained elevated from baseline through day 14 but was significantly decreased by day 28. Furthermore, decreased baseline levels of AAT appeared to affect the outcome and severity of lung injury. Patients who ultimately died had a significantly lower baseline circulating AAT compared to those who survived. In addition, 173 patients with ARDS were phenotyped for AAT deficiency. Although the prevalence of AAT deficiency was in line with what has been previously reported,[104] if not slightly lower, patients with the deficient phenotypes MS and MZ failed to mount a significant AAT response at baseline. Interestingly, patients who were determined to be MS also showed a trend toward lower baseline Pao_2:Fio_2 and higher positive end-expiratory pressure requirement.[103]

Neutrophil Elastase Levels in Bronchoalveolar Lavage Fluid and Plasma

There is a stronger consensus on alterations in neutrophil elastase levels during ARDS, with three studies publishing similar results between 1985 and 1988. In small cohorts of 13,[93] 6,[94] and 7[95] patients with ARDS, neutrophil elastase was significantly elevated in bronchoalveolar lavage fluid in each study compared to normal controls. The most recent study assessing levels of neutrophil elastase was performed by Hashimoto *et al.*[101] in 2008. The results showed a significant increase in neutrophil elastase in the plasma of 32 patients with acute lung injury or ARDS.

Neutrophil Elastase–AAT Complex in Plasma

The levels of neutrophil elastase–AAT in the bronchoalveolar lavage fluid of ARDS patients have not been quantified to date and present another knowledge gap. Authors have, however, quantified concentrations of the neutrophil elastase–AAT complex in the plasma because this has been previously shown to correlate with the microvascular injury and impaired gas exchange in patients with ARDS.[97,105] In a relatively large study (n = 79) assessing levels of the

complex in various inflammatory lung diseases, patients with ARDS indicated significantly greater levels of the neutrophil elastase–AAT complex in plasma and also substantially larger amounts when compared to patients with illnesses such as chronic obstructive pulmonary disease and pneumonia.[98] Groeneveld *et al.*[99] assessed one cohort of patients who were recovering from ARDS and a second cohort who were not recovering for plasma levels of neutrophil elastase–AAT. They found that although circulating neutrophil elastase–AAT was significantly higher in both groups compared to healthy controls, the levels were almost three times higher in the nonrecovering patients compared to the recovering patients. This highlights both the potential use of neutrophil elastase–AAT as a predictor of outcome and also the benefit of supplementing specific ARDS patient populations with exogenous AAT. The authors also found a positive correlation between the oxygenation ratio and the levels of proinflammatory cytokines interleukin 8 and interleukin 6, with neutrophil elastase–AAT.[99] Finally, when Hashimoto *et al.*[101] quantified levels of neutrophil elastase–AAT in the plasma of 32 acute lung injury/ARDS patients, they noted a significant elevation.

Collectively, the results of these ARDS patient studies present three lines of evidence. First, levels of AAT, neutrophil elastase, and neutrophil elastase–AAT appear to rise during the pathogenesis of ARDS. However, it would appear that despite the increase in AAT in the plasma and lung, (1) the protease–antiprotease balance remains perturbed, and (2) a certain amount of AAT is inactive. Second, AAT, either free or complexed to neutrophil elastase, may play a role as a novel biomarker for ARDS and predictor of outcome. Third, AAT is a potential commercially available and Food and Drug Administration–approved treatment option for ARDS. The field would therefore benefit from a larger and more thorough study to assess alterations in AAT, neutrophil elastase, and neutrophil elastase–AAT in patients with ARDS. This will provide a more accurate insight into the correlation between concentrations of AAT and neutrophil elastase–AAT with (1) the inciting stimulus, (2) progression to ARDS, and (3) mortality.

Identifying AAT-responsive ARDS Patient Cohorts

To avoid yet another failed pharmacotherapy for ARDS, it is imperative that ARDS phenotypes and/or etiologies that are mostly likely to be responsive to AAT are identified at the outset.

ARDS Etiologies and Subphenotypes

Because of its role in bacterial clearance, AAT would potentially be more effective in infectious as opposed to noninfectious ARDS. Considering pneumonia and sepsis account for approximately 80% of cases of ARDS,[5] this vastly broadens the clinical applicability of AAT. This also implies that AAT could be used to treat both hypo- and hyperinflammatory subphenotypes, because pneumonia is the predominant risk factor in the former and sepsis is the predominant factor in the latter.[31,33] In addition to increased interleukin 6 in the hyperinflammatory subphenotype, a number of studies also observed increased interleukin 8.[32,33] Based on the immunomodulatory effects of AAT, particularly in decreasing the release of proinflammatory cytokines such as interleukin 8,[66,68] it is rational to hypothesize that the hyperinflammatory subphenotype in particular would be likely to benefit from AAT treatment.

Decreased Plasma AAT

The recent data published by our group demonstrate that patients with ARDS concomitant with an underlying deficiency in AAT have a greater severity of lung injury and poorer outcome.[103] This infers that patients with a decreased level of circulating AAT at baseline, because of AAT deficiency or not, may benefit from supplementation with AAT. Although we identified the AAT deficiency phenotype MS as being at a disadvantage in the context of ARDS, this is the least severe phenotype, with a decrease in AAT of approximately 10%.[104] As such, it would be reasonable to infer that more severely deficient AAT deficiency phenotypes such as MZ (20 to 40% decrease in AAT production[104]) would also be predisposed to a greater severity of ARDS and thus an ideal target for AAT supplementation. The determination of a threshold therapeutic level of baseline plasma AAT would also offer valuable clinical significance.

Neutrophil Elastase and Functional AAT

Patients with ARDS experience a significant increase in neutrophil elastase release from activated neutrophils recruited to the lung, and higher levels are associated with worsening lung injury.[17] Therefore, ARDS patients with overwhelmingly high levels of neutrophil elastase would be another target population for exogenous AAT administration. Furthermore, it is possible to determine whether endogenous AAT is functional and therefore capable of inhibiting neutrophil elastase effectively. If not, this case too may be a candidate for AAT supplementation.

SARS-CoV-2–associated ARDS

Worldwide, more than 109 million individuals have been infected with SARS-CoV-2, the coronavirus causing COVID-19. As of February 23, 2021, almost 2.5 million deaths have been reported globally.[106] Infection causes destruction of alveolar epithelial cells, activation of the innate immune system, and dysregulation of adaptive immune responses, including release of proinflammatory cytokines and chemokines. This so-called "cytokine storm" might have an important role in the progression to ARDS and multiorgan failure.[107]

Cell entry of coronaviruses depends on binding of the viral spike (S) proteins to cellular receptors and on S protein

priming by host cell proteases. A recent study demonstrated that SARS-CoV-2 uses the SARS-CoV-2 receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming.[108] The authors reported that a TMPRSS2 inhibitor blocked viral entry. Additionally, elastase, a serine protease released by neutrophils, has been previously reported to play a role in enhancing severe acute respiratory syndrome–coronavirus 1 and Middle East respiratory syndrome entry.[109] Although the role of elastase in SARS-CoV-2 entry has not been determined, neutrophils are a key component of ARDS pathophysiology, and elevation of neutrophil counts in bronchoalveolar lavage fluid and serum have been consistently associated with severe COVID-19 cases.[110]

In addition to a role in inhibiting SARS-CoV-2 entry into host cells, AAT may play a role in modulating the immune response. Elastase is critical for the formation of neutrophil extracellular traps (NETs) in acute pneumonia, which can amplify inflammatory responses if not resolved by AAT. The cytokine storm noted in patients with late-stage SARS-CoV-2 infection may contribute to vascular hyperpermeability, multiorgan failure, and death, whereas exaggerated pulmonary inflammation and NETosis is an emerging theme in COVID-19 pathogenesis.[107,111] The protease ADAM17 is involved in several pathophysiologic processes, such as activation of tumor necrosis factor α and interleukin 6R cleavage in pulmonary inflammation.[63,112] AAT reduces ADAM17 activity,[63] which could help to control the release of interleukin 6R, the release of tumor necrosis factor α, and consequently, the excessive inflammatory response.

α1-Antitrypsin production is significantly increased during SARS-CoV-2 infection.[113] However, this acute phase response is overwhelmed in the most severe cases. Patients requiring ICU admission display a higher interleukin 6:AAT ratio, whereas an elevated interleukin 6:AAT ratio was also associated with a worse outcome.[113] Randomized trials of AAT augmentation therapy for patients with COVID-19–induced ARDS are ongoing (NCT04495101, EudraCT No. 2020-001391-15). Other immunomodulating agents currently being evaluated for severe COVID-19 include passive immunotherapy with convalescent plasma, intravenous immunoglobulin, and interleukin 1 and 6 pathway inhibition.[114] In the case of convalescent plasma therapies, the presence of AAT in blood plasma from donors may play a beneficial role because nonimmunoglobulin products are currently not excluded from transfused plasma.[115] Single anticytokine therapies can induce effective inhibition of individual cytokine pathways but can compromise host defense.[116,117]

Conclusions

A novel treatment option for ARDS is urgently required and is even more pertinent in light of the current SARS-CoV-2 pandemic. AAT represents an ideal candidate therapy for ARDS because of its role in targeting neutrophil elastase, ameliorating inflammation, enhancing bacterial clearance, and maintaining pulmonary epithelial and endothelial cell integrity *in vitro*. *In vivo*, AAT has been implicated in additional roles such as improving gas exchange, lung edema, and pulmonary compliance. Each of these effects is central to the pathophysiology of ARDS and thus indicates that AAT is a promising novel therapy. Furthermore, the safety profile of AAT is well established, and it can be administered *via* a number of routes and has demonstrated efficacy in a number of diseases with an inflammatory etiology. In patients with ARDS, levels of AAT have been shown to increase in the bronchoalveolar lavage fluid and plasma, indicating that AAT does indeed play a role during ARDS development and progression. This vast body of encouraging evidence supports the investigation of AAT for ARDS.

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Competing Interests

Dr. Curley and Dr. McElvaney are the chief investigators for an investigator-led trial of α1-antitrypsin for COVID-19–induced acute respiratory distress syndrome. The other authors declare no competing interests.

Correspondence

Address correspondence to Dr. Curley: Education and Research Centre, Royal College of Surgeons in Ireland, Dublin 9, Ireland, D09 YD60. gercurley@rcsi.com.

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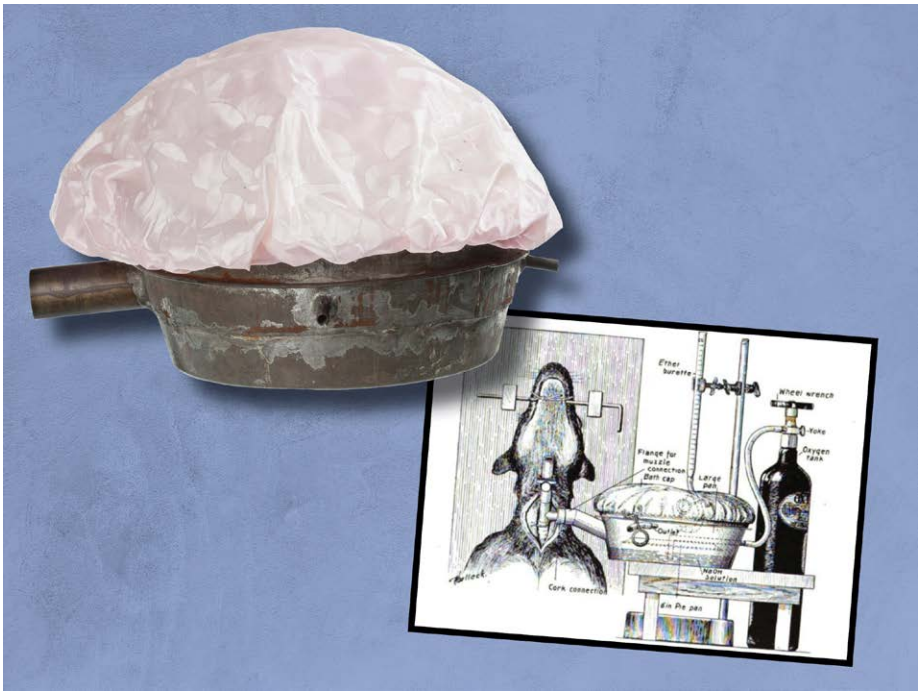
Before Soda Lime: Dennis Jackson's Cake-Pan Experiments



When not painting landscapes or carving violins, Dennis E. Jackson, Ph.D., M.D. (1878 to 1980), was baking up new anesthetic methods in his pharmacology laboratory at Washington University, St. Louis. Fashioned from everyday materials, Jackson's closed breathing system for experimental animals was highlighted in a 1916 article in *The Journal of Laboratory and Clinical Research*, now called *Translational Research*. Featuring a tinned-iron cake pan sealed by a rubber bathing cap (*left*), the confectionary apparatus was a visual treat. The cannulated trachea of an anesthetized dog was connected to a lye-layered chamber within the covered cake pan (*right*). As a potent carbon dioxide absorber, sodium hydroxide solution helped to prolong anesthesia, prevent hypercapnia, and contain noxious fumes. A separate inlet delivered oxygen for inhalation, and a burette dispensed precise drops of volatile anesthetic (*right*). Jackson's ingenious design would inspire future American carbon dioxide-absorbing devices like Ralph Waters' to-and-fro soda lime canister (1924) and Brian Sword's circle system (1936). With his easily replicable recipe for maintaining anesthesia, Jackson aspired to make physiological studies of ether, nitrous oxide, and chloroform anesthetics…a piece of cake. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology, Schaumburg, Illinois.)

Jane S. Moon, M.D., University of California, Los Angeles, California, and Melissa L. Coleman, M.D., Penn State College of Medicine, Hershey, Pennsylvania.