Therapeutic Application of Alpha-1 Antitrypsin in COVID-19

To the Editor:

Coronavirus disease (COVID-19) is a novel illness and is a rapidly evolving pandemic with unprecedented impact on global health in the last 100 years (1). At the time of submission of this work, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused 130 million diagnosed infections and 2.8 million known deaths. Although vaccines are currently developed and are now broadly used in public health prevention measures, the treatment options for patients with COVID-19 are sparse and do not show sufficient efficacy (2). SARS-CoV-2 uses several host factors to infect target cells, including TMPRSS2 (transmembrane protease serine 2), for activation of the viral S (spike) protein and ACE2 (angiotensin-converting enzyme 2) for entry (3).

AAT (alpha-1 antitrypsin) is a multifunctional host-defense and acute phase protein and member of the serpin superfamily (4). Genetic deficiency results in increased susceptibility for early chronic obstructive lung disease or chronic liver injury (4). AAT has a broad antiprotease activity and has been implicated in several biological processes, such as the regulation of inflammation (5). Intravenous or inhaled AAT has been used in multiple clinical trials for chronic obstructive lung disease or cystic fibrosis and has a good safety profile (6). In the context of hepatitis C virus infection, it has been shown that AAT inhibits TMPRSS2 (7). Earlier results from patients with COVID-19 indicated a relative deficiency of AAT in relation to inflammatory markers (8). Based on potential inhibition of virus entry by AAT and its broad antiinflammatory activity, the aim of this study was to investigate whether AAT can be considered as a candidate for the treatment of COVID-19. Some of the results of these studies have been previously reported in preprint form (https://doi.org/10.1101/2021.04.02.21252580).

We applied two-dimensional (2D) submersed and three-dimensional (3D) organoid cultures of airway epithelium for high-throughput screening to identify possible TMPRSS2 inhibitors (9). Immunostaining was performed and showed expression of both TMPRSS2 and ACE2 in 3D organoids (Figure 1A). Next, we analyzed the effect of protease inhibitors on the activity of trypsin-like proteases, including TMPRSS2, in 2D and 3D cultures of airway epithelial cells using a fluorescence-based assay. The protease activity was measured by incubating cell cultures with the synthetic peptide analog Boc-Gln-Ala-Arg-7-amino-4-methylcoumarin, a substrate for trypsin-like proteases, including TMPRSS2. We found that AAT inhibits the protease activity in a concentration between 1 and 5 mg/ml in submersed, undifferentiated epithelial cells (data not shown) or organoids (Figures 1B and 1C).

Next, we investigated whether AAT inhibits SARS-2-S (SARS-CoV-2 spike protein)–driven entry into the human lung cell line Calu-3, which expresses endogenous ACE2 and TMPRSS2. We used vesicular stomatitis virus (VSV)–based vectors bearing either SARS-2-S or the glycoprotein of VSV (VSV-G) (3). The protease inhibitor camostat was included as positive control. Camostat reduced SARS-2-S but not VSV-G–driven entry efficiently in a dose-dependent manner, as expected (data not shown) (3). We next analyzed whether these results translate to human lung organoids and authentic SARS-CoV-2. Camostat efficiently inhibited SARS-CoV-2 infection of lung organoids, while an approximately 50% reduction of SARS-CoV-2 infection was observed at 10 mg/ml of AAT (Figure 1D).

Based on the antiviral and antiinflammatory activities of AAT, we offered inhaled or combined inhaled/intravenous application to nine patients with mild to moderate COVID-19 (NCT04799873, inhaled AAT from Kamada, the intravenous preparation was Prolastin from Grifols) (mean [SD] patient age, 61.11 [10.33] yr; 56% male patients, median World Health Organization severity class, 4 [oxygen by mask or nasal canula] [mean, 3.89]; two patients received remdesivir, two dexamethasone). Four patients were treated with inhaled AAT (100 mg/d for 7 d) and five patients were treated with inhaled (100 mg/d for 7 d) and intravenous (60 mg/kg body weight, Days 1, 3, and 5) treatment. For further analysis we matched patients with COVID-19 from the observational CORSAAR cohort based on age and disease severity recruited in the same time period (mean age, 62.28 [9.77] yr; 67% male patients; median World Health Organization severity class, 3 [noninvasive ventilation or high-flow oxygen] [mean, 3.67]; one patient received remdesivir, two dexamethasone). AAT genotyping (*SERPINA1* gene) was available for 23 patients and revealed one individual with PiMZ in the control group. AAT application was reported to the ethics committee of the Landesärztekammer as individual treatment and informed consent was obtained. All patients treated with AAT survived and were discharged from the hospital in good functional status (length of hospital stay, 18.67 [7.57] d). The respiratory status eventually improved in all patients; three patients experienced an initial deterioration (Figure 2A). Serum CRP (C-reactive protein) levels decreased over the days after initiation of the AAT application with individual variations (Figure 2B). Virus load was monitored at irregular intervals and turned negative in all patients (data not shown). We did not observe any drug-associated side effects such as allergic reactions. In the group of matched patients, three patients died, the decrease of CRP was delayed as compared with the AAT treatment group (Figure 2B), and the mean length of hospitalization was 16.78 $\pm$ 11.03 days. Other studies showed that between 21.1 and 24.5% of the patients hospitalized deteriorated and died (10).

The main finding of this work is that AAT is a candidate for treatment of COVID-19. Evidence from this work and from the literature shows that AAT has antiviral and antiinflammatory activity. This study has limitations that need to be addressed, the first of which being the lack of data from a controlled trial and the missing deep mechanistic characterization of the mode of action of AAT. AAT application might be beneficial in two settings in

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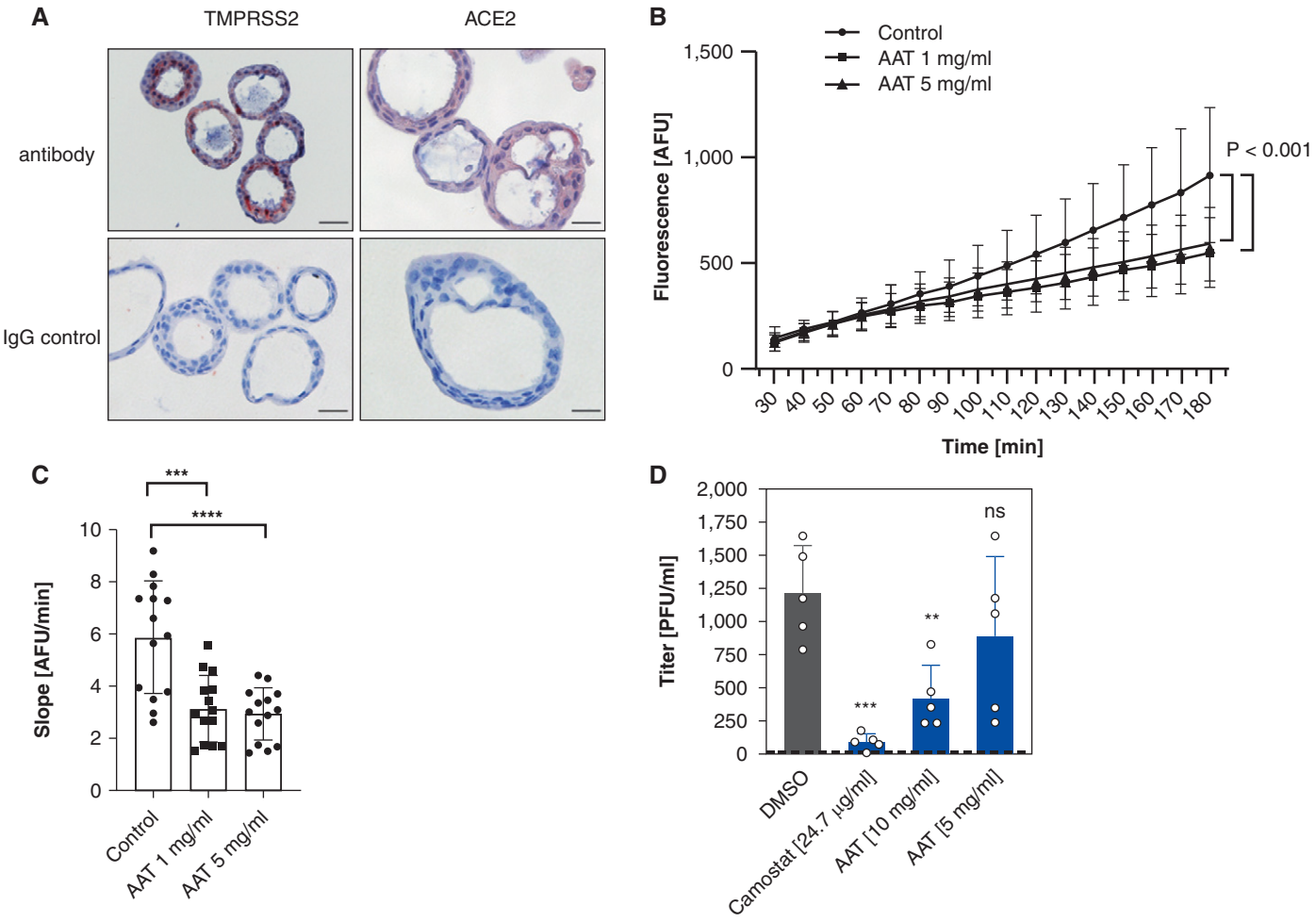

Figure 1. Human airway organoids as models to study SARS-CoV-2 infection. (*A*) TMPRSS2 and ACE2 are expressed in airway organoids as detected by immunohistochemistry. Representative images of organoids stained for TMPRSS2 and ACE2 with corresponding IgG controls are shown. Scale bars: TMPRSS2 column , 40 μm; ACE2 column, 20 μm. (*B*) AAT blocks the proteolysis of the synthetic peptide Boc-Gln-Ala-Arg-AMC added to airway organoids in culture. Final levels of fluorescence were compared using ANOVA with Tukey *post hoc* test and significance levels are displayed (***$P < 0.001$). Data are from three independent experiments. (*C*) The slopes of the graphs from *B* were calculated using a linear regression line model (Microsoft Excel, 2021). The slopes of the control group were significantly steeper as compared with the AAT-treated groups as calculated with ANOVA with Tukey *post hoc* test (***$P = 0.001$ and ****$P < 0.001$). (*D*) Human lung organoids were pretreated for 2 hours with the indicated concentrations of camostat or AAT, inoculated with SARS-CoV-2, and viral titers determined at 24 hours after infection using plaque assay. The infection of five cultures was analyzed. The experiment was repeated with equivalent results. Error bars indicate SD with **$P < 0.01$ and ***$P < 0.001$. AAT = alpha-1 antitrypsin; ACE2 = angiotensin-converting enzyme 2; AFU = artificial fluorescence units; ns = not significant; PFU = plaque-forming units; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TMPRSS2 = transmembrane protease serine 2.

COVID-19. The first is early disease stages, in which inhaled therapy could decrease local viral load and local inflammation. This type of therapy could also be applied in an outpatient setting. The other setting might be application in severe COVID-19, in which a systemic inflammatory response could be modified by combined systemic and inhaled AAT application. Inhaled application might result in high AAT concentrations at the upper and proximal airways, a region in which initial SARS-CoV-2 replications takes place. Although several trials are ongoing with intravenous application (NCT04547140 and NCT04495101), a trial with inhaled AAT is not registered. ■

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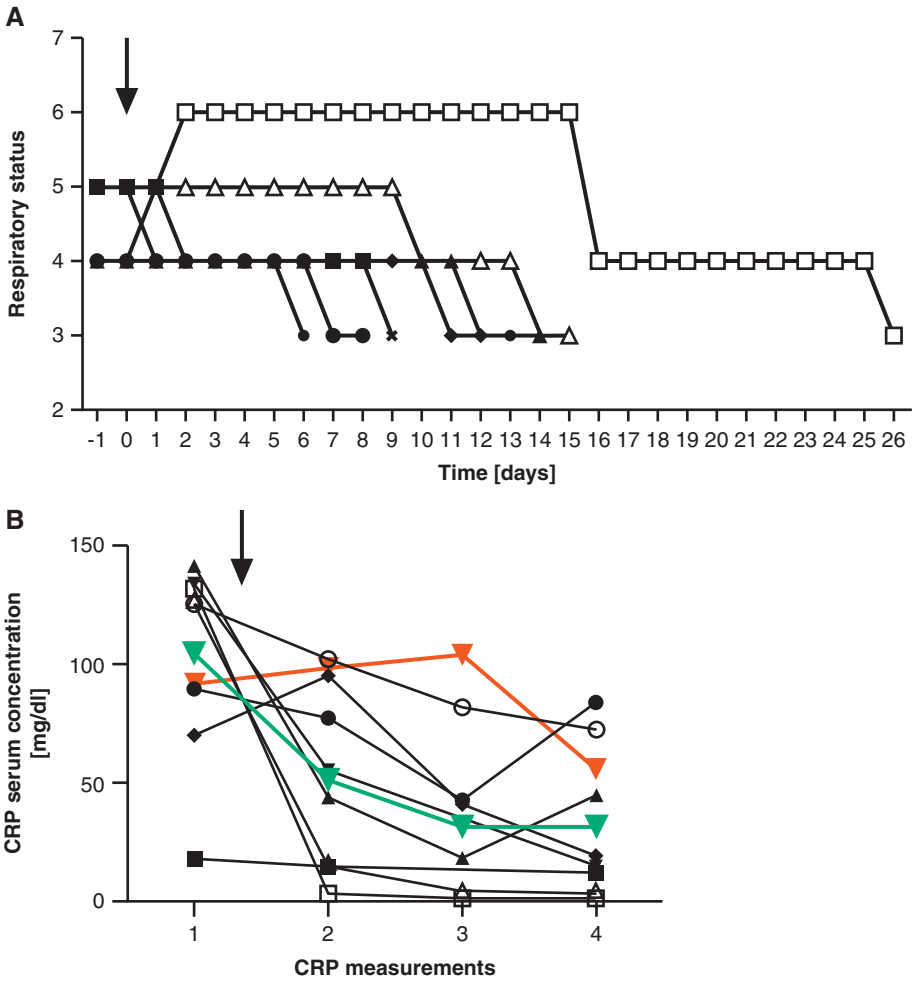

Figure 2. Application of AAT (alpha-1 antitrypsin) to patients with coronavirus disease (COVID-19). (*A*) Development of the respiratory status in all nine patients after the initiation of AAT treatment over time (arrow), World Health Organization severity class: 3 = no oxygen, 4 = oxygen by mask or nasal prongs, 5 = noninvasive ventilation or high-flow oxygen, and 6 = intubation and mechanical ventilation. (*B*) Overall CRP serum concentrations were decreasing after AAT application (arrow). The green line represents the mean of all nine patients with AAT treatment, and the red line represents the mean of 18 matched control patients with COVID-19. Time point 1 is the last CRP measurement before the initiation of treatment (in treated patients) or the first available CRP measurement in the control patients. Time points 2, 3, and 4 indicate subsequent measurements during the next 10 days. CRP = C-reactive protein.

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Lipofibroblasts in Structurally Normal, Fibrotic, and Emphysematous Human Lungs

To the Editor:

Lipofibroblasts were first described as a distinct cell type in the rat lung by Vaccaro and Brody, characterized by a large volume of lipid bodies and localization in the alveolar interstitium, often at the base of the septa and in close proximity to alveolar epithelial type 2 cells (1). Since then, lipofibroblasts have repeatedly been reported in the lungs of rodents at various developmental stages, whereas their occurrence in the human lung has only been reported rarely (2) and is even questioned by other studies (3). A definite answer to the question of whether lipofibroblasts are a (common) feature of human lungs is of importance, as lipofibroblasts have recently gained considerable recognition in lung research as part of the stromal cell population (4) because they store and synthesize vitamin A (5) and contribute to surfactant synthesis (6), thus playing an important role in alveolar development (7). Most importantly, newer studies suggest that a shift from lipofibroblasts to myofibroblasts is involved in the development of experimental lung fibrosis and in the reversal of extracellular matrix deposition (8), indicating that experimental modification of the fibroblast phenotype may serve as a novel therapeutic strategy. Because of the involvement of lipofibroblasts in fibroblast growth factor 10 signaling, they are potentially involved in various human lung diseases, such as bronchopulmonary dysplasia, idiopathic pulmonary fibrosis, and chronic obstructive pulmonary disease (9). The hope that identifying the contribution of lipofibroblasts to alveologenesis may help to (re)activate their potential in lung regeneration (10) critically depends on whether murine studies can actually be translated to humans. Therefore, we reinvestigated the presence of lipofibroblasts in the human lung.

Methods

The material investigated in this study was taken from explanted fibrotic ($n = 10$) and emphysematous ($n = 10$) human lungs as well as from the periphery of tumor resections ($n = 7$); it was then directly fixed in 4% buffered formaldehyde and embedded in

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