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Invasive *Aspergillus* Tracheobronchitis Emerging as a Highly Lethal Complication of Severe Influenza

Invasive aspergillosis is increasingly being recognized as a secondary infection in hospitalized patients with influenza. A recent cohort study from Belgium and the Netherlands showed that in ICU patients, influenza was an independent risk factor for invasive pulmonary aspergillosis (1). Influenza-associated pulmonary aspergillosis was associated with a 33% mortality rate in previously healthy individuals and 71% ICU mortality in the subgroup of patients with underlying host factors according to the European Organization for Research and Treatment of Cancer/Mycosis Study Group Education and Research Consortium consensus definitions for invasive mycoses. Fatal outcome may further be associated with delayed initiation of antifungal therapy (2) if an aggressive diagnostic approach is not pursued.

In this issue of the *Journal*, Nyga and colleagues (pp. 708–716) (3) report 10 cases of invasive *Aspergillus* tracheobronchitis in a cohort of 35 (28.6%) patients with severe influenza and invasive pulmonary aspergillosis. The mortality rate of patients with invasive tracheobronchitis was significantly higher compared with those without tracheobronchitis (90% vs. 44%; $P = 0.02$) (3). Although invasive *Aspergillus* tracheobronchitis is a recognized *Aspergillus* disease entity, it is considered a rare manifestation of pulmonary aspergillosis or confined to specific host groups such as patients with chronic obstructive pulmonary disease and lung transplantation recipients. Invasive *Aspergillus* tracheobronchitis has been reported in rare cases in association with influenza (2, 4), but this study shows that invasive tracheobronchitis is a more common manifestation of influenza-associated pulmonary aspergillosis and carries a very high mortality rate in comparison with other pulmonary forms of influenza-associated pulmonary aspergillosis.

Histopathological studies show that influenza causes focal or extensive tracheitis and bronchitis, in addition to diffuse alveolar damage (5). Disruption of the epithelial barrier of the airways is likely to facilitate fungal colonization and infection. Furthermore, influenza virus can exhibit a direct immunomodulatory effect through suppression of the NADP oxidase complex, which might cause a temporary disease status resembling chronic granulomatous disease with impaired host defense against *Staphylococcus aureus* and *Aspergillus* species and excessive innate inflammation. Indeed, influenza viral antigen was found in the tracheobronchial epithelium and submucosal glands, and to a lesser extent in bronchiolar epithelium, alveolar epithelial cells, and macrophages (5), supporting a link between cellular tropism of influenza virus and *Aspergillus* tracheobronchitis. In addition, other factors such as active smoking could further increase the risk for airway disease (3). Invasive *Aspergillus* tracheobronchitis may be a less common disease manifestation in other severe viral infections. Now with the coronavirus disease (COVID-19) pandemic, it is a very timely question whether invasive tracheobronchitis is a frequent *Aspergillus* disease manifestation, similar to influenza. Unlike influenza, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters the cell via ACE2 (angiotensin-converting enzyme 2), a receptor that is present in type 2 pneumocytes and ciliated cells but not in the epithelial layer of the larger airways (6). Although invasive pulmonary aspergillosis is increasingly reported in patients with severe COVID-19 (7, 8), cases of invasive *Aspergillus* tracheobronchitis have not yet been reported, which

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may be due to the different cell tropism of SARS-CoV-2. However, further clinical evidence is needed to determine the full spectrum of *Aspergillus* disease manifestations in critically ill patients with COVID-19.

The high mortality rate of invasive *Aspergillus* tracheobronchitis could be due to numerous factors including delayed diagnosis, rapid disease progression, and inadequate antifungal drug exposure at the site of infection. Extensive disruption of the epithelial barrier and intraluminal sporulation of *Aspergillus fumigatus* may facilitate rapid disease progression and extreme fungal burden in the lungs, which is supported by high biomarker levels (3). The trachea and bronchial lumen may be regarded a sanctuary site for adequate drug exposure, and administration of nebulized antifungal agents in addition to systemic therapy is recommended (9). However, there is no clinical evidence that supports nebulized antifungal therapy in ventilated patients, and many factors may have an impact on local drug delivery including physicochemical properties of the antifungal drug, particle size, ventilation settings, and patient-related factors (10). Although high concentrations of liposomal amphotericin B (L-AmB) were found in BAL samples from lung transplant recipients receiving nebulized L-AmB (11), other studies suggest that most of the drug is deposited in the lung alveoli rather than in the trachea and bronchi. Other treatment options include combining systemic antifungal therapy with bronchoscopic interventions such as mechanical debridement or intraluminal instillation of antifungal agents (12). As the bronchoscopic appearance of intraluminal lesions may differ, varying between superficial infiltration to full-layer involvement (12), the need and effectiveness of bronchoscopic interventions may also vary. However, there is currently no broadly accepted classification system for *Aspergillus* airway disease, which is needed to develop and validate new therapeutic strategies (12). Research pathways similar to those proposed for nebulized antibiotics may be useful to optimize delivery and deposition of antifungals (10). In addition to L-AmB, novel triazole compounds specifically designed for inhaled administration are under development (13). Excessive innate inflammatory responses and host defects caused by influenza might be another target to overcome fulminant *Aspergillus* infection. Immunomodulatory drugs that increase host defense, such as recombinant IFN-γ, or dampen excessive inflammation, such as the IL-1 blocker anakinra, might restore the dysregulated immune response (14). However, in the setting of influenza, there is a note of caution regarding the use of recombinant IFN-γ, which has been used in the clinics as rescue therapy for invasive aspergillosis, because it can carry the risk of inducing a detrimental innate inflammatory response. The exploration of immunotherapeutic strategies for influenza-associated pulmonary aspergillosis will be investigated in a clinical trial by the HDM-FUN (Host-directed Medicine in Invasive Fungal Infections) consortium.

Recently, an expert panel proposed a case definition for influenza-associated pulmonary aspergillosis, which distinguishes between invasive *Aspergillus* tracheobronchitis and other pulmonary forms (15). This case definition will facilitate clinical research and epidemiologic studies of influenza-associated pulmonary aspergillosis. The study of Nyga and colleagues nicely adds evidence to this case definition and lowers the threshold in clinical practice to perform early bronchoscopy in critically ill patients with severe influenza. Given the high mortality of invasive *Aspergillus* tracheobronchitis and the challenges regarding successful management, more studies are urgently needed to improve our understanding of this disease and to design effective treatment interventions. ■

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A New Role for CXCL4 in Respiratory Syncytial Virus Disease

Although the impact of coronavirus disease (COVID-19) in future years is hard to predict, it seems certain that the ongoing morbidity and mortality caused by respiratory syncytial virus (RSV) (1, 2) is set to continue. Although many RSV vaccines are in advanced development (3), none have yet succeeded. Until there is a good vaccine or a specific treatment, the healthcare impact of RSV (4) and other respiratory viruses is set to continue unabated.

In this issue of the *Journal*, Han and colleagues (pp. 717–729) describe a hitherto unknown inhibitory effect of CXCL4 (previously known as platelet factor 4) on RSV infection, replication, and disease (5). The authors screened a complementary DNA library of human genes to identify factors that promote or inhibit transcription of RSV nucleoprotein in HeLa cells. They identified 122 proviral and 233 antiviral genes, of which 9 and 49, respectively, were of special interest to the authors. This screen reassuringly identified several known restriction factors (e.g., *IFNA1*, *IFNG*, and *IRF3*) but delivered the unexpected finding that CXCL4 was among the factors showing the greatest antiviral effect.

They confirmed this by overexpressing CXCL4 in culture, showing that RSV protein expression after infection was inhibited by CXCL4 and that only the secreted form of CXCL4 was effective. They went on to show that CXCL4 also inhibited replication of EV71 and HSV1 but not influenza A virus. In BALB/c mice, they showed that intranasal administration of CXCL4 prior to RSV challenge inhibited viral replication and lung inflammation but again had no effect on influenza. Taking samples from RSV-infected pediatric patients, they showed that CXCL4 in plasma, nasopharyngeal aspirate, and BAL fluid were increased after RSV infection. The airway CXCL4 level correlated positively with viral load and disease severity, but the increase in plasma levels was paradoxically negatively associated with more severe disease.

These are very interesting and novel data. CXCL4 has been previously shown to offer some protection against influenza and HIV (6, 7), but to our knowledge this is the first time it has been shown to have a protective effect against RSV infection and disease severity. The authors demonstrate that CXCL4 protects against RSV infection through inhibition of RSV attachment to heparan sulfate (HS) on the surface of target cells. This attachment step is considered to largely be mediated by the RSV attachment glycoprotein, although the RSV fusion (F) protein can also interact with HS (8). Antibodies can prevent the attachment of RSV to target cells; such neutralizing antibodies commonly target the F protein and are most effective when specifically directed against the pre-F conformation (9).

Directing antibodies against pre-F through vaccination has proved challenging, although exciting progress is being made (10). An alternative approach may be to increase resistance to RSV binding sites via HS, as demonstrated here with studies of CXCL4. Indeed, families of small-molecule antagonists of the HS–RSV interaction have been reported (11). Presumably, such approaches will be most effective prophylactically; clinical studies would be needed to determine the optimal time of intervention and to discover undesirable off-target effects.

Historically, CXCL4 was described as a secreted chemokine produced by platelets (leading to the synonym "platelet factor 4"), and the lung is an important source of platelets (12). This does not imply that the same is true of the nasal mucosa, but Han and colleagues' demonstration of elevated CXCL4 levels in nasal samples from severe cases of pediatric RSV bronchiolitis should direct attention to the study of megakaryocytes and platelets at other mucosal sites. Release of CXCL4 by platelets and associated cells might act to recruit monocytes and neutrophils to the airway (13), which is characteristically seen in severe RSV bronchiolitis. The elevation of CXCL4 in severe RSV bronchiolitis reported by Han and colleagues may indicate that the positive effects of suppression of viral replication are offset by the chemotactic recruitment of neutrophils, which may contribute to pathogenesis. Other platelet-derived chemokines such as CCL5 (C-C chemokine ligand 5) are also elevated during RSV infection (14). Despite this potential function in the early innate respiratory immune response,

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