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Invasive pulmonary aspergillosis associated with viral pneumonitis

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The occurrence of invasive pulmonary aspergillosis (IPA) in critically ill patients with viral pneumonitis has increasingly been reported in recent years. Influenza-associated pulmonary aspergillosis (IAPA) and COVID-19-associated pulmonary aspergillosis (CAPA) are the two most common forms of this fungal infection. These diseases cause high mortality in patients, most of whom were previously immunocompetent. The pathogenesis of IAPA and CAPA is still not fully understood, but involves viral, fungal and host factors. In this article, we discuss several aspects regarding IAPA and CAPA, including their possible pathogenesis, the use of immunotherapy, and future challenges.

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Introduction

Invasive pulmonary aspergillosis (IPA), caused by the ubiquitous fungus *Aspergillus*, characteristically occurs in immunocompromised individuals, notably those with prolonged neutropenia, recipients of hematopoietic stem cell or solid organ transplants, or patients with hematological malignancies [1–4]. Recent outbreaks of respiratory viral diseases such as severe acute respiratory syndrome (SARS), H5N1 avian flu, the 2009 H1N1 influenza pandemic, and recently coronavirus disease 2019 (COVID-19), caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2) have underlined the importance of respiratory viruses as an important cause of severe pneumonia in adults [5]. In recent years, IPA has been described increasingly in these patients [1,6,7,8•,9,10•,11–17,18•]. IPA affects critically ill patients with viral pneumonia due to two viruses in particular: influenza and SARS-CoV-2.

Influenza-associated pulmonary aspergillosis (IAPA) occurs in critically ill patients in the intensive care unit (ICU) with severe influenza (predominantly influenza A), and is associated with a high ICU mortality of 45–61% compared to 20% in ICU patients with influenza without IAPA [1,8•,9,19]. Importantly, underlying classical risk factors for IPA are present in approximately only half of patients who develop IAPA [8•,9]. Furthermore, increasing acute physiology and chronic health evaluation (APACHE) II score at admission was an independent risk factor for IAPA in one study, indicating an association between influenza severity and risk of IPA [8•]. Besides IAPA, invasive aspergillosis has also been increasingly reported in patients with COVID-19 [10•,17,20]. The incidence of COVID-19-associated pulmonary aspergillosis (CAPA) among patients with COVID-19 is approximately 4–33%, and mortality is 44–71%, higher compared to patients with COVID-19 without CAPA (19–37%) [15–17]. Although IAPA and CAPA share certain clinical characteristics, such as the frequent absence of classical risk factors as defined by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC), absence of typical radiographic features and a high associated mortality, there are distinct differences as well, for example in terms of time of development and association with the use of corticosteroids. Whereas IAPA tends to develop early in the course of ICU admission (at a median of three days after ICU admission) [8•,9], CAPA develops after a median of 4–8 days after ICU admission or intubation [15,16]. Furthermore, whereas corticosteroids have been shown to constitute a clear risk factor for the development of IAPA, their role in CAPA risk varies between studies [8•,15–17].

IPA incidence in patients with influenza or COVID-19 in ICU is evidently higher than that in patients admitted to ICU with acute respiratory distress syndrome (ARDS) due to any cause (4%) [21], with community-acquired pneumonia (5%) [8•] or with severe respiratory syncytial virus (RSV) pneumonia (3.5%) [7]. This suggests a specific role of the causative viral agent in the increased susceptibility to IPA. In this review, we will elaborate on the possible pathogenesis of these two most common forms of IPA complicating viral pneumonitis, as well as possible and available adjunctive immune-based therapies.

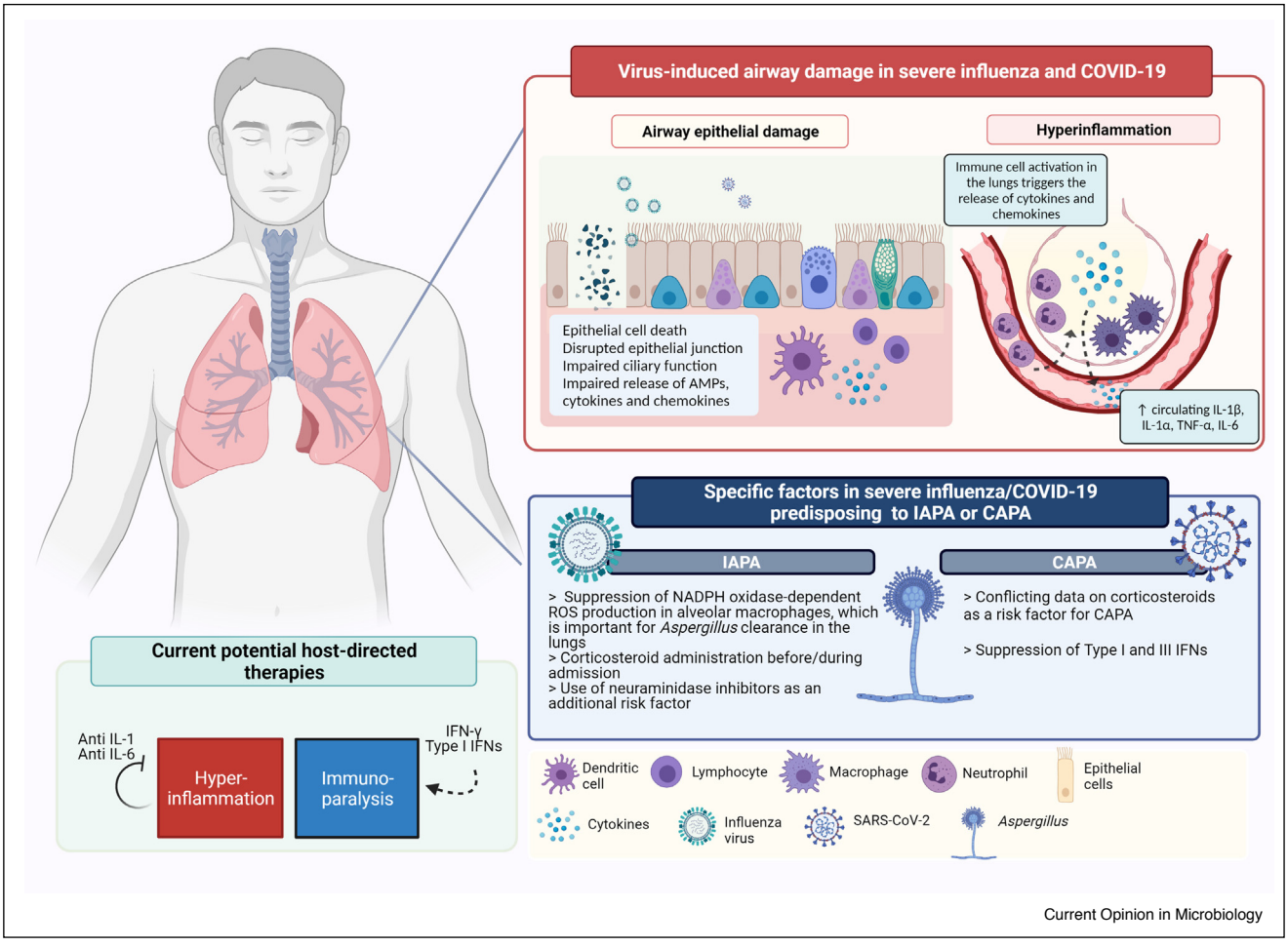
Pathogenesis of IAPA and CAPA

The pathogenesis of IAPA and CAPA is still not fully understood, but includes multiple factors caused by the viral infection, *Aspergillus* itself, and the host immune response. Influenza virus differs from SARS-CoV-2 in terms of cell tropism and viral replicative properties [22,23]. Influenza binds to sialic acids on respiratory epithelial cell surfaces via viral hemagglutinin (HA), providing cellular entry for replication. New virions are released only after cleavage of their connection with the host cell via sialic acids (SAs) by viral neuraminidase. Human adapted influenza virus strains preferably bind α-2,6-linked SAs, which are mainly present in the upper airways [24]. In contrast, avian influenza strains preferably bind α-2,3-linked SAs, which are expressed in birds' intestinal tracts, but also in the human lower respiratory tract. Therefore, increased HA binding affinity for α-2,3-linked SAs in human influenza strains (as in the pandemic H1N1 strain and the avian influenza H5N1 strain), and dual receptor specificity (as in the H7N9 avian influenza strain) are hypothesized to contribute to increased pathogenicity. However, receptor binding specificity is not the only determinant for pathogenicity [25•,26•]. Furthermore, although IAPA has predominantly been described in patients with influenza A or specific influenza A strains (H1N1, H3N2, H7N9) [1,8•,9,27,28], no direct associations between IAPA risk and specific influenza strains have been described thus far. Influenza's lytic effects on tracheobronchial epithelial cells may provide a portal of entry for *Aspergillus* to cause tissue invasion, which clinically presents as invasive *Aspergillus* tracheobronchitis. SARS-CoV-2, however, targets cells expressing angiotensin converting enzyme 2 (ACE2) and the transmembrane protease serine 2 (TMPRSS2), which facilitate its binding and entry [29]. These include airway epithelial cells, type 2 pneumocytes, vascular endothelial cells, and alveolar macrophages [30–33]. Although invasive *Aspergillus* tracheobronchitis has been described in both IAPA and CAPA, the reported frequencies in patients with severe influenza are higher compared to those with COVID-19 [18•].

Despite the differences in host cell-binding specificity, both influenza and SARS-CoV-2 cause airway epithelial damage, usually characterized by disrupted epithelial junctions, impaired ciliary clearance, and loss of cell functions, such as the release of antimicrobial proteins (Figure 1) [34,35]. In the lungs, virus-infected cells release danger-associated molecular patterns (DAMPs), which are detected by adjacent epithelial cells and resident alveolar macrophages. Subsequent activation of these cells results in the release of proinflammatory cytokines, including interleukin (IL)-6, interferon (IFN)-γ, IL-1β, and tumor necrosis factor (TNF)-α, and various chemokines. These immune mediators trigger influx of macrophages and neutrophils, further aggravating the local inflammatory response [36–39]. Influenza (strain A/PR/8/34; H1N1) has also been shown to induce apoptosis of alveolar macrophages, contributing to the increased susceptibility to secondary pneumococcal infection [40]. Hyperinflammation is a common feature of both influenza and COVID-19, characterized by high concentrations of circulating inflammatory cytokines, acute phase reactants and ferritin, and hemophagocytosis, some of which are typical features of macrophage activation syndrome (MAS) [41,42]. Lymphopenia is also observed in both influenza and COVID-19 [43,44]. The decreased $CD4^+$ and $CD8^+$ lymphocyte populations are often accompanied by impaired T cell mediated responses in the latter [45•].

Host defense against *Aspergillus* involves multiple immune system components. The first important layer is the airway epithelium. The release of various antimicrobial proteins, cytokines and chemokines by airway epithelial cells is essential for initial defense against *Aspergillus* [46,47]. Since the integrity of innate immune barriers in the airways is crucial to prevent invasive growth of *Aspergillus* into the pulmonary parenchyma, it is likely that airway damage induced by influenza and SARS-CoV-2 predisposes patients to viral pneumonitis-associated IPA. However, since ARDS in itself also causes loss of epithelial cell integrity, but is associated with a much lower incidence of IPA [21], other factors associated with the viral infection and antiviral immune response must be involved in susceptibility to viral pneumonitis-associated IPA. The second crucial pulmonary anti-*Aspergillus* defense layer involves alveolar macrophages and neutrophils [48,49]. These cells eliminate *Aspergillus* conidia by phagocytosis and release of reactive oxygen species (ROS), as well as by production of cytokines and chemokines [50–52]. Although ROS have been implicated in (X-31/H3N2) influenza-induced lung injury [53], influenza virus (strain A/PR/8) has been shown to suppress NADPH-oxidase-dependent ROS production in alveolar macrophages and neutrophils in an animal model, affecting their bactericidal capacity [54]. This transient suppression of ROS production mimics the phenotype of chronic granulomatous disease (CGD). CGD patients are genetically deficient in one of the components of the NADPH oxidase complex type

Figure 1



Proposed pathogenesis of IAPA and CAPA.
The pathogenesis of IAPA and CAPA share several common features, including the presence of airway epithelial damage and hyperinflammation (top right panel). However, different factors specific for influenza and COVID-19 might further underlie the increased susceptibility to IPA in these viral infections (bottom right panel). Potential host-directed therapies are highlighted in brief (bottom left panel). Figure created with Biorender.com (AMP, Antimicrobial protein. CAPA, COVID-19-associated pulmonary aspergillosis. COVID-19, Coronavirus disease 2019. IAPA, Influenza-associated pulmonary aspergillosis. IFN, Interferon. IL, Interleukin. IPA, Invasive pulmonary aspergillosis. NADPH, Nicotinamide adenine dinucleotide phosphate. ROS, Reactive oxygen species. TNF-α, tumor necrosis factor-alpha).

2 and are highly susceptible to invasive aspergillosis [55]. Furthermore, ROS deficiency in these patients leads to defective formation of a noncanonical autophagosome in macrophages, a process known as LC3-associated phagocytosis (LAP) [56]. LAP is crucial for *Aspergillus* elimination in phagocytic cells, whereby *Aspergillus*-containing phagosomes are efficiently targeted for lysosomal degradation [57,58]. Additionally, corticosteroids have been shown to inhibit recruitment of the LC3II protein, a crucial step in LAP [59]. Based on these findings, one could speculate that ROS suppression by influenza might lead to impaired LAP, resulting in uninhibited growth of *Aspergillus*, an effect worsened by use of corticosteroids.

Recent studies have explored the differences in immunological characteristics between patients with severe influenza and COVID-19. An important example is the decreased type I and III IFN response in COVID-19 patients [60••,61]. Bastard *et al.* described the presence of neutralizing autoantibodies against type I IFNs in approximately 10% of patients with life-threatening COVID-19. These autoantibodies could negate these type I IFNs' ability to block SARS-CoV2 infection *in vitro* [62•]. Type I IFNs are known to be key drivers of type III IFN (IFN-λ) production, which stimulates neutrophil actions against *Aspergillus fumigatus* [63]. Furthermore, addition of IFN-β to human dendritic cells is able to promote anti-*Aspergillus* T helper type 1 responses [64].

Whether a diminished type I IFN response in COVID-19 is associated with an increased susceptibility to *Aspergillus* infection is not yet known.

Another differential contributor to the pathogenesis of IAPA and CAPA could be the use of neuraminidase inhibitors. These agents are administered in patients with severe influenza to limit viral replication [65]. The possible role of neuraminidase inhibitors in the pathogenesis of IAPA has been studied by our group. We have described that neuraminidase is crucial for anti-*Aspergillus* defense *in vitro*, and that inhibition of endogenous neuraminidase by oseltamivir impairs the capacity of human monocytes and murine splenocytes to kill *Aspergillus* [66]. Additionally, oseltamivir-treated immunocompetent mice exhibited higher fungal burdens in the lungs and higher mortality after infection with *Aspergillus* compared with untreated controls. Therefore, although the use of neuraminidase inhibitors could prevent worsening of viral replication in patients with severe influenza, prolonged use might increase susceptibility to *Aspergillus* infection.

Implications of the pathogenesis for the clinical presentation of IAPA and CAPA

The abovementioned factors have important consequences for the clinical presentation and management of IAPA and CAPA. The combination of the lytic effects of influenza, inhibition of ROS production, immune dysregulation and presence of predisposing factors, such as EORTC/MSGERC host factors, result in a host highly susceptible to IPA. As a consequence, IAPA is frequent, occurs early after ICU admission and is a rapidly progressive infection. Furthermore, in patients with invasive *Aspergillus* tracheobronchitis a mortality of 90% was reported [67••]. The high proportion of patients with positive serum galactomannan (GM), that is, 90% in influenza patients with invasive *Aspergillus* tracheobronchitis and 65% in IAPA patients, indicates that angioinvasive growth occurs early in the disease process.

The course of infection is different in patients with CAPA, which may be due to less severe lytic effects of SARS-CoV-2 on respiratory epithelium and lack of (known) viral effects on fungal host defense pathways. However, immune dysregulation and possible EORTC/MSGERC host factors do contribute to susceptibility to IPA. The infection occurs at a median of seven days after ICU admission, indicating a less acute disease progression compared with IAPA. This is supported by the low frequency of serum GM detection in 15% of CAPA patients and lack of angioinvasion, as opposed to tissue invasion, in histopathological specimens of infected patients. Nevertheless, the CAPA ICU mortality rates of 52% are similar to those observed in IAPA [10•].

Immunotherapy: rebalancing the host response

The optimal management of viral pneumonitis-associated aspergillosis should not only include antiviral and antifungal treatments, but also a consideration of adjunctive host-directed therapy aimed at resolving the dysregulation of the immune response. Immunopathology in IAPA and CAPA is not only due to *Aspergillus*-induced inflammation, but also to collateral damage resulting from a dysregulated immune response against the fungus and direct effects of the virus. On the one hand, a strong immune response is important for effective pulmonary clearance of *Aspergillus*. On the other hand, excessive inflammation might induce tissue damage, and thus impair pulmonary function. In this regard, the timing and type of treatment are crucial to achieve a balanced immune response.

Immunomodulatory therapies aimed at reducing systemic inflammation have been explored in COVID-19 (Figure 1). Tocilizumab, an anti-IL-6 drug, improves clinical outcome in a subset of patients with COVID-19, but is not effective in all patients, particularly those with moderate to severe disease [68,69]. However, secondary infections as a possible immunological consequence of IL-6 blockade should be kept in mind, although current evidence does not strongly support this [70]. Furthermore, inhibition of the IL-1 receptor with anakinra has been shown to be beneficial in COVID-19 patients, resulting in ameliorated clinical symptoms and reduced inflammatory parameters [71,72•,73,74]. The role of corticosteroid use in viral pneumonitis is more complex. Corticosteroids are a well-known risk factor for the development of IPA. In ICU patients with influenza, corticosteroid use is associated with higher mortality [75]; in addition, it is independently associated with the development of IAPA [8•]. However, in COVID-19, dexamethasone has been shown to reduce mortality of patients receiving ventilatory support [76•]. In contrast to IAPA, literature on the effects of corticosteroid use on CAPA risk and outcome is conflicting. Apart from immunosuppressive agents, administration of immunostimulatory agents should also be considered during the course of COVID-19. In later disease stages with immune paralysis often present, administration of recombinant IFN-γ or type I IFNs could be beneficial [77]. IFN-γ has been shown to restore immune functions in patients with fungal sepsis, notably by improving antigen presentation and the capacity of immune cells to produce proinflammatory cytokines [78].

Summary and future perspectives

Viral pneumonitis is increasingly recognized as a risk factor for IPA, and the fact that the majority of cases occur in previously immunocompetent individuals is concerning. Understanding the pathogenesis of viral pneumonitis-associated IPA from a perspective of the virus,

the fungus, and the host, preferably in an integrated model system is imperative [79]. Mortality rates of IAPA and CAPA are high despite antifungal therapy, indicating that an integrated approach involving interventions aimed at diminishing the effects of the viral infection, early diagnosis and treatment of the fungal infection and rebalancing of the immune dysregulation, may improve patient outcome. Early screening for *Aspergillus* infection in critically ill patients with influenza is crucial in limiting disease progression, and administration of antifungal agents immediately following ICU admission of patients with severe influenza might be indicated in regions with high IAPA incidence. Given the variation in immune dysregulation, that is, hyperinflammation or immune paralysis, determining the prevailing immune status of the patient is critical in order to select the appropriate host-directed intervention. In this case, measurement of circulating immune markers at several time points could be a valuable approach. Integrated and personalized treatment strategies need to be incorporated into future clinical trials in order to determine which approaches are successful in reducing IAPA and CAPA mortality.

Conflict of interest statement

Nothing declared.

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