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“The relentless and awful growth of Aspergillus during systemic disease has been described as an ‘autocatalytic process’, with the hyphae having ‘the overall appearance of an army on the march’”.

Introduction

COVID-19 and immunosuppression via corticosteroids. It COVID-19 pandemic irreversibly and irreversibly altered our lives forever. The pandemic showed just how many areas of our public health system are lacking and just how ill-prepared we are, both as a nation and on a global scale, when it comes to tackling an unplanned viral emergency. Initial treatment of COVID-19 involved frequent use of corticosteroids, with some statistics showing corticosteroid use in the treatment of up to 46% of critically ill COVID-19 patients. Corticosteroid treatment has long proven to be an effective treatment method for autoimmune disorders, among others, as they are a fantastic immune suppressant in addition to having strong anti-allergic and anti-inflammatory effects on immune cells, tissues, and organs. Corticoid immunosuppression, however, is a significant risk factor for many diseases, and if COVID-19 is coupled with greater immune suppression, it makes for a potentially deadly recipe.

COVID-19 and immunosuppression via Aspergillus infection. Invasive pulmonary aspergillosis (IPA) is the most common invasive fungal infection leading to death in severely immunocompromised patients. Aspergillus is a slow-growing pathogen capable of compromising the lung, making it easier for viruses to infect. In turn, respiratory viruses tend to cause direct damage to airway epithelium, which favors a state of immunosuppression, or reduced macrophage activity, enabling fungal invasion of tissues. There is a remarkable synergy between respiratory viruses and Aspergillus, with many examples showing how the two together exacerbate the disease process.

In patients with viral pneumonia, various studies have shown that mortality rates may be 16-25% higher for those with aspergillosis versus those without, making IPA infection a severe complication of viral illnesses. In ICU patients, one study found survival rates to be 24% lower in patients with influenza-associated pulmonary aspergillosis as compared to patients with no evidence of aspergillosis. Furthermore, in 2012, the world was struck with the H1N1 virus, which caused severe influenza A infection in those who caught it. In patients with severe influenza over this period, some sources report influenza-associated pulmonary aspergillosis (IAPA) present in 17-29% of cases. Those with IAPA had a high mortality rate of up to 67%. One of the reasons for this high mortality rate is the significant complication that aspergillosis infection adds to critical patients already suffering from acute respiratory distress syndrome (ARDS).

In late 2019, another aspergillosis took the stage; COVID-associated pulmonary aspergillosis, or CAPA. The nonspecific nature of CAPA symptoms coupled with a lack of adequate imaging tools to diagnose made it extremely difficult to differentiate between COVID-19 pneumonias and invasive aspergillosis, impacting treatment and leading to statistically significant increases in mortality rates. The apparent similarities between IAPA and CAPA cannot be ignored. Immunosuppression, orotracheal ventilation (intubation), non-invasive ventilation, and corticosteroid use all proved to be risk factors increasing the susceptibility of patients to CAPA; preliminary statistics at the start of the COVID-19 pandemic showed that in ventilated COVID-19 patients, up to 30% were affected by CAPA. Immunosuppression, corticosteroids, and Aspergillus virulence via immunomodulators called oxylipins are intertwined in a complicated relationship that is explored herein.

Aspergillosis

Aspergillus exists as a common mold that lives both indoors and outdoors and is regularly inhaled by mammals, including humans. It is one of the most common molds on earth, with over 200 species grouped together due to their similar reproductive structures. Aspergillus reproduces asexually; it can produce vast
numbers of clonal conidiospores, with one sporulating colony of *Aspergillus* having the capability to make millions of genetically identical copies of itself\(^9\). As one paper puts it, the best adjective to describe *Aspergillus* is ubiquitous; *Aspergillus* conidia are small, hydrophobic, easily dispersed through the air, and found just about anywhere, from happily thriving on stone surfaces, to propagating in decaying biomaterial in Antarctic soil, to even popping up in the interior of spacecraft\(^3,6\).

Human lungs experience near-constant exposure to fungi; fortunately, most of us regularly inhale *Aspergillus* without incident. Immunosuppressed patients and those with debilitating diseases are historically much more likely to breathe in *Aspergillus* and develop fungal infection/disease. Only a few of the hundreds of existing species of *Aspergillus* are known to cause infection in humans, the most notable of these being *A. fumigatus*\(^4,5\). The species *A. fumigatus* is responsible for over 90% of aspergillosis infections. However, *A. flavus* and other *Aspergillus* species are also found in many infections\(^4\). Of note, *A. flavus* is often found to be the primary etiological agent in fungal eye and wound infections, and *A. niger* is commonly a culprit in otomycosis (a fungal infection of the ear canal), found growing on debris and cerumen\(^4\).

When it comes to respiratory *Aspergillus* illnesses, however, the primary method of infection is via inhalation of airborne conidia and their subsequent deposition in the lungs, where these spores adhere to lung tissue, germinate, and grow into filamentous hyphae, evoking inflammatory response and destroying lung and other tissues\(^5,6,15\). *A. fumigatus* is the most virulent of the *Aspergillus* species for multiple reasons: it grows readily at human body temperature, shows remarkable thermotolerance and its hyphae have an average diameter of 2 to 3 um, small enough for deep infiltration into the alveolar spaces and bronchioles of the lungs\(^6\). As *Aspergillus* invades preexisting lung cavities, it can form a fungal cavity called an aspergilloma. Aspergillomas, or fungal balls (mycetomas) are formed by *Aspergillus* hyphae combined with cellular debris and mucus. Specimens typically appear with an often-necrotic central core surrounded by a soft mesh of inflamed hyphae, blood clots, fibrin (the non-globular protein involved in blood clotting), mucus residues, and other cellular debris\(^5\). It is estimated that aspergilloma incidence occurs in up to 25% of aspergillosis patients\(^5\). In aggressively growing aspergillomas, the deprivation of oxygen to tissues, coupled with lung tissue architecture distortion and inflammation, increases the likelihood of further fungal lung exposure and tissue erosion\(^5\).

**Diagnosis**

In patients with respiratory illness, a chest X-ray is often the first diagnostic tool, as chest X-rays can reveal the overall health of the lungs and the presence of spots that might be mycetomas. Definitive diagnosis, however, requires biochemistry. Direct inspection of the trachea and bronchi via bronchoscopy can be done, which, beyond allowing visual identification of potential pulmonary aspergillosis, also allows for biopsy through the collection of lung tissue\(^4\). *A. fumigatus* has a carbohydrate-rich cell wall comprised of various polysaccharides, including galactomannan, or GM; the presence of GM in bronchoalveolar lavage fluid is highly indicative of IPA\(^4\). During active fungal growth, the GM antigen is released, making this antigen an ideal biomarker of active infection. Detection of the GM antigen in bronchoalveolar lavage fluid (bGM) or serum (sGM) is one of the primary methods used for microbial diagnosing of aspergillosis, with some studies even suggesting that these biomarkers are detectable even before the clinical onset of symptoms\(^4\). Thus, one method used to determine prevalence of aspergillosis is that of GM-Ag tests. From the first period of 2020 to the first period of 2021, the number of positive GM-Ag tests went from 2.5% to 12.3%\(^4\). However, at the height of the SARS-CoV-2 pandemic, the prevalence of bronchoscopies became severely reduced; bronchoscopies involve significant exposure of healthcare workers to aerosols, making this method of aspergillosis and aspergilloma detection less than ideal.

*Immune response is altered by oxylipins during *Aspergillus* infection.*
The critical step in aspergillosis pathogenesis is the transition from an inhaled *Aspergillus* spore to hyphal growth, which subsequently penetrates host tissues, causing organ damage and host mortality\(^3\). One signal for the transition from dormant spore to hyphal growth is that of cyclooxygenase (COX) products\(^5\). Free polyunsaturated fatty acids are oxygenated into oxylipins by the enzymes cyclooxygenase (COX), lipooxygenase, and cytochrome P450. These oxylipins, or oxygenated fatty acids, can then diffuse through plasma membranes and signal to the fungal tissues as well as those of the host (in a paracrine or autocrine manner) through G protein-coupled receptors (GPCRs). Oxylipin structures are quite varied, as fatty acid backbones are highly variable in length, the various oxygenase or P450 enzymes that produce them can oxygenate different carbons on the fatty acid backbone, and modifying enzymes can further alter the structure\(^6\).

**Fungal oxylipins: biological roles.** Oxylipins are used as developmental signals by fungi\(^6\). In various *Aspergillus* species, oxylipin-mediated developmental shifts have been shown to regulate spore production\(^1\).

**Mammalian oxylipins: biological roles.** Mammalian oxylipins (eicosanoids) function as a part of the cellular immune response. Some examples of mammalian oxylipins include prostacyclins, which prevent blood clot formation by causing vasodilation, thromboxanes, which cause platelet aggregation via vasoconstriction (opposite function of prostacyclins), and leukotrienes, which are formed via lipooxygenase pathway and cause increased mucus secretion, eosinophil recruitment, bronchoconstriction, and increased vascular permeability. Another example is that of prostaglandins, which exist in practically every human cell type and are important for causing inflammation and maintaining homeostasis. Prostaglandins are of particular interest and importance, as they are remarkably conserved across mammalian and fungal species. During inflammation via COX enzymes, prostaglandin production is induced; these COX-synthesized prostaglandins function to recruit immune cells to infection sites\(^15\).

**Oxylipins as cross-kingdom signals in pathogenesis.** Oxylipins, through various cell-signaling pathways, control cell response to a wide range of stress conditions and have emerged as signaling molecules playing a potent and significant role in orchestrating mammalian-fungal interactions\(^9,17\). The human immune system consists of the innate immune response and the adaptive immune response. The innate immune response represents the first line of defense against pathogens and includes both chemical and physical barriers, such as the skin. The adaptive immune response represents the second line of defense and is characterized by various cells, including lymphocytes, which recognize and destroy pathogens\(^14\). Prostaglandins produced by cyclooxygenase (COX) enzymes can have either anti-inflammatory or pro-inflammatory effects on innate immune cells, working to modulate both the recruitment and functioning of phagocytes. However, innate immune cells cannot distinguish native from fungal-produced prostaglandins, which are sometimes similar or identical in structure, due to a common evolutionary origin of prostaglandin cyclooxygenases\(^17\). It has been hypothesized that fungal prostaglandins play an important role in the stimulation of host immune systems and that without the prostaglandin-provided signal, the immune response is diminished or delayed\(^17\). During inflammation of a healthy host, infiltrating innate immune cells, such as macrophages and neutrophils (the major phagocyte that works to target and kill invasive hyphae), are the main source of prostaglandin synthesis, with COX enzymes catalyzing the main regulatory step of said synthesis. In other words, innate immune cells prevent hyphal growth and tissue destruction using cyclooxygenase signaling\(^15\). But during *Aspergillus* infection, a vicious cycle occurs in which decreased phagocyte control then leads to an inhibition of COX signaling; this inhibition has been shown to increase fungal spore germination and invasive hyphal growth, propagating aspergillosis infection and leading to subsequent decreased host survival\(^15\).

During infection, another key oxylipin species is eicosanoids, which are produced by host cells but also by the fungal pathogen. Because of this, fungal species can modulate pathogenesis and immune responses via the synthesis of their own lipid signaling molecules\(^13,15\).
Steroids and Immunosuppression

Steroids, lipophilic hormones, are broken down into several subdivisions, largely due to where they are produced in the body and what they do once they’ve been produced. Sex hormones, for instance, are produced in the zona reticularis, the innermost layer of the adrenal cortex, and the gonads and affect growth, development, and reproductive cycles. Glucocorticoids are another example; they are produced in the zona fasciculata, the middle/widest layer of the adrenal cortex, and link the endocrine and immune systems. Glucocorticoids ensure the correct function of inflammatory events throughout cell regeneration, pathogen elimination, and tissue repair. Some commonly known synthetic glucocorticoids are prednisolone and dexamethasone, used in the treatment of arthritis, among other pathologies, and prednisone, used to treat a variety of inflammatory processes, but notably, frequently used in the treatment of severe asthma episodes to decrease lung inflammation. The lipophilic outer layer and low molecular weight of glucocorticoids allow them to easily pass through cellular membranes and bind to the cytosolic glucocorticoid receptors within. Glucocorticoid receptors are found all throughout the body and mediate glucocorticoid action.

The two primary risk factors for Aspergillosis are neutropenia and corticosteroid-induced immunosuppression. Per the Cleveland Clinic, neutropenia, or an abnormally low blood count of neutrophils, a type of white blood cell, can be a result of underlying genetic conditions but is more often a result of either underlying infections, such as hepatitis, HIV, tuberculosis, etc., or a result of cancer and certain cancer treatments (i.e. chemotherapies). Several studies have shown that corticosteroid-induced immunosuppression impairs the functional abilities of phagocytes (cells responsible for engulfing and absorbing infective agents) to recognize and kill invasive A. fumigatus conidia and hyphae. During immunosuppression by corticosteroids and at the start of infection by A. fumigatus, neutrophils are still recruited to areas of infected lung tissue and are able to prevent initial hyphal invasion. However, in doing so, they create an inflammatory response and lung environment which results in tissue injury, making the lungs more susceptible to further infection.

At the start of the COVID-19 pandemic, corticosteroid therapies were used in up to 46% of critically ill COVID patients. Corticosteroid therapy initially seemed to be more effective than other techniques at treating COVID-19; however, its immunosuppressive factor made patients more susceptible to developing aspergillosis, leading to a rapid rise in CAPA infections associated with high morbidity and mortality. During the second quarter of 2021, Italy introduced the National Guidelines on COVID-19 Therapy which led to a rapid decrease in the use of corticosteroids to treat Aspergillosis; this coincided with a decrease in positive GM-Ag tests throughout this period.

Conclusions

A great deal of knowledge surrounding the mechanisms of glucocorticoid immune response effects is still incomplete, and a significant amount of information is still lacking around the subject of CAPA comorbidities and risk factors. There is a need, going forward, to better understand the mechanisms and interactions behind these. When it came to COVID-19, humans found themselves on the woefully ill-prepared side; when it came to containment, economic recovery, and, as this review focused on, treatment associated with dealing with global pandemics. While the usefulness of glucocorticoid therapies cannot be disputed, the adverse effects and exponential increase in risk cannot be understated. The cost-effectiveness, and literal effectiveness, make them irreplaceable and indispensable in the treatment of many immunopathologies. Unfortunately, because of the many benefits of glucocorticoid treatments, dosages and duration of administration are on the rise. And as dosage and duration increase, so does the likelihood of adverse events, such as immunosuppression leading to CAPA. The evolutionarily clever methods of fungal infection through the conserved protein domains of oxylipins make aspergillosis, as rare as it may be, a formidable foe.

Further Directions

Abnormal oxylipin signaling has been linked to a number of cardiovascular disease-relevant pathologies, including hypertension (high blood pressure), hyperlipidemia (high cholesterol), thrombosis, and
hemostasis\textsuperscript{12,16}. The opportunity to examine how oxylipins are involved in these pathologies would be quite fascinating to look at; oxylipins sure are funky little guys!

Additionally, there are questions of whether other risk factors beyond treatment methods further increase the disease progression risk of both CAPA and aspergillosis. Some data suggests possible correlations between/comorbidities with diabetes, asthma, COPD, cancer, and even, most interestingly, male sex\textsuperscript{4}. Asthma and COPD make clear sense regarding their impact on pulmonary function and association with pulmonary disease; diabetes makes sense as a chronic, systemic disease, and cancer makes sense due to the harsh effects treatment has on the body. For me, what is most peculiar and enticing here is the possibility of a sex-linked correlation; for my own personal interest, I would like to look more at sex as a comorbidity.
Citations


