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Review

# SARS-CoV-2 infection, COVID-19 pathogenesis, and exposure to air pollution: What is the connection?

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**Exposure to air pollutants has been previously associated with respiratory viral infections, including influenza, measles, mumps, rhinovirus, and respiratory syncytial virus. Epidemiological studies have also suggested that air pollution exposure is associated with increased cases of SARS-CoV-2 infection and COVID-19–associated mortality, although the molecular mechanisms by which pollutant exposure affects viral infection and pathogenesis of COVID-19 remain unknown. In this review, we suggest potential molecular mechanisms that could account for this association. We have focused on the potential effect of exposure to nitrogen dioxide ($NO_2$), ozone ($O_3$), and particulate matter (PM) since there are studies investigating how exposure to these pollutants affects the life cycle of other viruses. We have concluded that pollutant exposure may affect different stages of the viral life cycle, including inhibition of mucociliary clearance, alteration of viral receptors and proteases required for entry, changes to antiviral interferon production and viral replication, changes in viral assembly mediated by autophagy, prevention of uptake by macrophages, and promotion of viral spread by increasing epithelial permeability. We believe that exposure to pollutants skews adaptive immune responses toward bacterial/allergic immune responses, as opposed to antiviral responses. Exposure to air pollutants could also predispose exposed populations toward developing COIVD-19–associated immunopathology, enhancing virus-induced tissue inflammation and damage.**

**Keywords:** air pollution; coronavirus; particulate matter; ozone; nitrogen dioxide; viral infection

## Introduction

Coronaviruses are classified in the family *Coronaviridae,* subfamily *Orthocoronavirinae*, which is further divided into the following genera: *Alpha-, Beta-, Gamma-*, and *Deltacoronaviruses*.[1] Common circulating coronaviruses include HKU1, NL63, OC43 (all *Betacoronaviruses*), and 229E (an *Alphacoronavirus*) and are associated with mild respiratory symptoms.[2] Severe infections with highly pathogenic coronaviruses are characterized by acute lung injury and acute respiratory distress syndrome (ARDS), leading to pulmonary failure and death.[3] An outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV-1), which was traced to horseshoe bats in Southern China, resulted in 8097 confirmed cases and 774 deaths in 29 countries from November 2002 to July 2003.[4] An outbreak of another pathogenic coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), originally identified in a 60-year-old Saudi man in 2012, resulted in 834 confirmed cases and 288 deaths.[5]

In December 2019, the Chinese Center for Disease Control and Prevention identified a novel coronavirus in lower respiratory tract samples of patients in Wuhan, China, as the cause of a pneumonia characterized by fever, dry cough, and fatigue.[6] This novel coronavirus was later named severe acute

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respiratory coronavirus-2 (SARS-CoV-2) and classified as a *Betacoronavirus* because it showed 85% genomic identity with a bat SARS-like CoV (bat-SL-XoVZC45).[7] The World Health Organization has termed the resulting disease as Coronavirus Disease 2019 (COVID-19).[4] As of September 8, 2020, SARS-CoV-2 has resulted in over 26 million cases worldwide and ∼900,000 deaths (https://covid19.who.int/), although analysis of death rates suggests that COVID-19 has a lower case fatality rate than MERS and SARS.[8] However, there are no currently approved vaccines available, as of September 8, 2020.

It is estimated that 91% of the world's population breathes polluted air, resulting in almost 9 million premature deaths every year.[9–11] The most common air pollutants consist of ozone ($O_3$), particulate matter (PM), carbon monoxide (CO), lead, sulfur dioxide ($SO_2$), and nitrogen dioxide ($NO_2$), according to the United States Environmental Air Protection Agency (https://www.epa.gov/criteria-air-pollutants). Epidemiological studies have demonstrated associations between pollution exposure and increased mortality and morbidity.[9–11] Consequences of pollution exposure include premature skin aging and dysfunction of the nervous, urinary, cardiovascular, and digestive systems, as well as ocular irritation.[12–16] Additionally, the lungs are constantly exposed to ambient air pollution, which can result in the development/exacerbation of lung conditions, such as chronic obstructive pulmonary disease (COPD), asthma, and lung cancer.[17–24]

Respiratory infections caused by viruses, such as respiratory syncytial virus (RSV), influenza, adenoviruses, and coronaviruses, can promote morbidity and mortality in otherwise healthy individuals, in addition to exacerbating preexisting chronic lung diseases, such as asthma and COPD (reviewed in Refs. 25–27). Previous studies have indicated an association between outdoor air pollution exposure and incidences of respiratory viral infections, transmissibility of viruses, healthcare encounters (clinic visits, emergency room visits, and hospitalizations) caused by viral infections, and disease severity of a variety of respiratory viruses, including influenza viruses, measles, mumps, rhinovirus, and RSV.[6,28–55] Studies have also linked outdoor air pollution exposure to coronavirus infection and virus-induced mortality.[56–67] The mechanisms by which pollution exposure is linked to SARS-CoV-2 infection and severity of COVID-19 disease remain unknown.

In this review, we discuss how outdoor pollution exposure could potentially affect the viral life cycle of SARS-CoV-2 and pathogenesis of COVID-19, which has also been recently reviewed by another group.[68] We believe that pollutant exposure contributed to the early, rapid spread of SARS-CoV-2 in polluted urban areas (e.g., Wuhan, Lombardy, etc.), before lockdown procedures reduced industrial emissions and limited population exposure to pollutants. The potential effects of pollutant exposure on SARS-CoV-2 infection and COVID-19 pathogenesis will remain relevant as countries began to reduce lockdown procedures, promoting an increase in industrial emissions and urban exposure to pollutants. However, the only studies correlating SARS-CoV-2 infection and COVID-19–associated mortality with outdoor pollutant exposure are mainly epidemiological studies, which have been rapidly published,[69] some without peer review;[58,60,70] therefore, to address a direct connection, studies need to directly test the effects of pollutant exposure on SARS-CoV-2 infection and COVID-19 *in vitro* and *in vivo*. The purpose of this review is to discuss the molecular effects of pollutant exposure on the viral life cycle of other RNA viruses in order to enable the focusing of future studies testing a connection between SARS-CoV-2 and COVID-19 and pollutant exposure on specific events/factors in the viral life cycle of SARS-CoV-2 and pathogenesis of COVID-19. Since antivirals target specific viral life cycle stages, we have organized this review into a discussion of the effects of pollutant exposure on specific stages of the viral life cycle, followed by the discussion of the effects of pollutants on adaptive immune responses and pathogenesis of other RNA viral infections. The studies discussed in this review indicate that pollutant exposure impairs host immunity and alters cellular responses, promoting viral infection. However, this review is limited by the fact that very few studies have compared acute versus long-term effects of pollutant exposure on viral infection *in vitro* or *in vivo*. In addition, because of direct effects of pollutants on virions themselves (i.e., adsorption, peroxidation, inactivation, etc.), in addition to immune responses, it is difficult to speculate how the differences between acute versus

long-term exposure would, therefore, affect viral infectivity and pathogenesis. Thus, although pollutant exposure results in the development/exacerbation of lung conditions, such as COPD, asthma, and lung cancer, which can promote the susceptibility to viral infections, connection between these comorbidities and COVID-19 is beyond the scope of this review, although this topic has been discussed/reported elsewhere.[71–74]

### *Coronavirus biology*

Coronaviruses are positive-sense, single-stranded (ss)RNA viruses that are approximately 80–220 nm in diameter and carry the largest genome (∼30 kb) of currently known RNA viruses.[75] They are enveloped and characterized by crown-like spike proteins protruding from viral surfaces that resemble the corona of the sun when viewed by electron microscopy.[76] The viral membrane (M), envelope (E), and spike (S) structural proteins are anchored in the envelope. The S protein is cleaved by host cell proteases into two separate polypeptides: S1 (receptor binding) and S2 (forms spike stalk).[77] Some *Betacoronaviruses* also have a hemagglutinin esterase embedded in the envelope.[78] The nucleocapsid, inside the envelope, consists of the nucleocapsid protein (N) bound to the viral genome in a beads-on-a-string conformation.[76]

To initiate entry, the S1 subunit of the SARS-CoV and SARS-CoV-2 viral spike (S) glycoprotein attaches to the host receptor, angiotensin-converting enzyme 2 (ACE2; Fig. 1).[79] ACE2 is an enzyme typically involved in regulating blood pressure, although it also regulates inflammation. In the absence of SARS-CoV-2 infection, ACE2 blocks the proinflammatory activity of the angiotensin II type 1 receptor by converting angiotensin II into the anti-inflammatory peptide angiotensin (1–7).[80] However, binding of SARS-CoV-2 to ACE2 alters functionality of the enzyme, which is discussed later.[80] Viral entry requires priming of the S protein by cellular proteases, which cleave the S protein at the S1/S2 and S2′ site, allowing fusion of viral and cellular membranes.[77] SARS-CoV-1 and SARS CoV-2 both utilize the cellular serine protease TMPRSS2 and cathepsins B/L for S protein priming.[77] After fusion of the viral and host cell membranes, viral RNA is released into the host cell and translated into viral replicase polyproteins pp1a and pp1ab via ribosomal frameshifting (Fig. 1),[76] which are cleaved by viral proteases into 11 or 16 individual nonstructural proteins (nsps; Fig. 1).[81] Some of these nsps form the replication/transcription complex (RTC).[81] Coronavirus replication and transcription are regulated by the RTC in rearranged internal host membranes or double-membranous vesicles (DMVs; Fig. 1).[82] nsp12 encodes the viral RNA-dependent RNA polymerase (RdRp) domain,[83] which produces genomic RNA and nested subgenomic RNAs (via discontinuous transcription) using negative-sense intermediates.[84] Subgenomic mRNAs are are translated into viral proteins, including structural proteins (S, E, and M), which are inserted into the endoplasmic reticulum (ER) and enter the secretory pathway to move into the ER-Golgi intermediate compartment (ERGIC; Fig. 1).[76] Newly encapsidated viral genomes bud into the ERGIC with the viral structural proteins, forming mature virions (assembly mediated by E and M proteins; Fig. 1). SARS-CoV-2 S protein has a putative furin cleavage site at the S1–S2 boundary, which is cleaved during viral assembly in the Golgi compartment, rendering the virus infectious without activity by additional cellular proteases.[79] Mature virions are transported in vesicles and released from the cell via exocytosis (Fig. 1).[76]

### *Innate immune responses in coronavirus infections*

To prevent pathogen infection, epithelial cells lining the airways function as a barrier using tight junctions and a mucociliary escalator. These cells are equipped with pattern recognition receptors (PRRs) that recognize conserved pathogen-associated molecular patterns or damage-associated molecular patterns (DAMPs). PRRs include Toll-like receptors (TLRs), nucleotide-binding domain and leucine-rich repeat containing (NLRs) receptors, and intracellular cytoplasmic retinoic acid–inducible gene-I (RIG-I)–like receptors (RLRs). TLR3 resides in endosomes and recognizes double-stranded (ds)RNA, which is produced during replication of ssRNA viruses.[85] TLR7 and TLR8 also reside in endosomes and recognize ssRNA.[85] Activation of TLRs triggers signaling cascades that stimulate translocation of the transcription factors nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) and interferon regulatory factors (IRFs) 3 and 7 to the nucleus where they

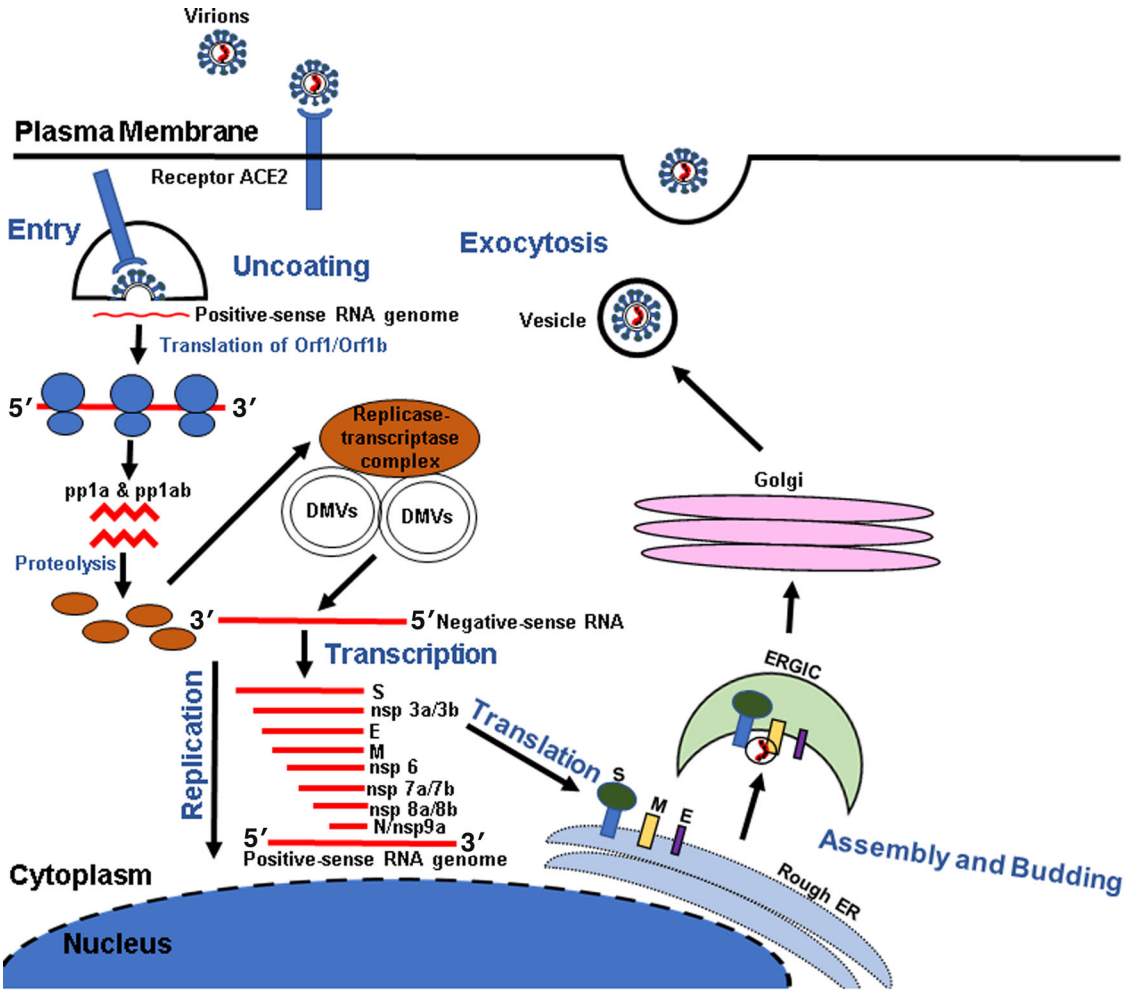


**Figure 1.** Coronavirus life cycle. To initiate entry, the spike (S) protein attaches to the host receptor angiotensin-converting enzyme 2 (ACE2). Entry requires cleavage of the S protein by the cellular serine protease TMPRSS2 and cathepsins B/L. After fusion, viral RNA is released into the cell and translated into the polyproteins pp1a and pp1b, which are cleaved into nonstructural proteins, some of which form the replicase complex. Coronavirus replication and transcription are regulated by the replication-transcription complex in rearranged internal host membranes or double-membranous vesicles (DMVs). Genomic RNA and nested subgenomic mRNAs are produced using negative-strand intermediates. Subgenomic mRNAs are translated into viral proteins, including the spike (S), envelope (E), and membrane (M) structural proteins. The structural proteins are translated and inserted into the endoplasmic reticulum (ER) and move into the ER-Golgi intermediate compartment (ERGIC). Newly encapsidated viral genomes bud into the ERGIC, forming mature virions, which are transported in vesicles and released from cells via exocytosis.

stimulate antiviral interferon (IFN) production. NLRs, such as NLRP3, can detect RNA viruses, and SARS-CoV-1 E and 3a proteins have been shown to activate NLRP3 inflammasomes.[86] The cytoplasmic RLRs RIG-I and melanoma differentiation-associated gene 5 (MDA5) also recognize viral RNA and promote activation of NF-κB and IRFs to produce IFN.[85] However, like many RNA viruses, SARS-CoV encodes strategies to evade antiviral immune responses. For example, the N protein of SARS-CoV-1 can inhibit ubiquitination and activation of RIG-I, preventing the induction of IFN.[87] The SARS-CoV-1 M protein can also inhibit IFN signaling by preventing the activation of NF-κB.[88]

Binding of type I IFNs to the type I IFN receptor induces activation of signal transducer and activator of transcription (STAT) 1 and 2, which can interact with IRF9, translocate as a complex to the nucleus,

and induce transcription of interferon-stimulated genes (ISGs). These proteins are the effectors of the antiviral response and include a variety of proteins. SARS-CoV-1 nsp1, ORF3b, ORF6, and ORF9b have been shown to suppress IFN signaling and ISGs.[3]

In addition to TLRs, NLRs, and RLRs, the collectins are a group of soluble PRRs that bind to sugars or lipids on the surfaces of microorganisms and that include surfactant proteins (SP) A and D, which are found in the respiratory tract–lining fluid. These proteins can bind to sugars on viral glycoproteins on virions to limit viral attachment to epithelial cells[89] and enhance phagocytosis by immune cells.[90] SP-D has been shown to bind the SARS-CoV-1 S protein.[91]

Antimicrobial peptides (AMPs) are another group of endogenous defensive proteins found in the respiratory tract–lining fluid that can act as innate immune antiviral effectors.[92] AMPs are classified into different groups, depending on their secondary structures.[93] These groups include beta-sheet–containing defensins, which can be further classified depending on the arrangement of disulfide bonds, and cathelicidins, which are amphipathic, alpha-helical AMPs.[93] The other classes of AMPs and the various functions of AMPs have been nicely reviewed by Zhang and Gallo.[93] Humans only express one cathelicidin gene, of which the mature proteolytically processed peptide is named LL37.[93] Defensins and LL37 have been previously shown to inhibit viral infection by acting on influenza virions and interfering with viral internalization and replication.[94–96] In addition, AMPs also regulate immune responses by regulating TLR activation, cytokine production, immune cell chemotaxis, and neutrophil and mast cell degranulation.[93] Whether endogenous AMPs can directly disrupt SARS-CoV-2 infection is unknown, although this effect may partly account for the large number of asymptomatic SARS-CoV-2 infections (Fig. 2).

### *Coronavirus pathogenesis*

Most SARS-CoV-2 infections are not severe.[97] The incubation period for SARS-CoV-2 ranges from 2 to 14 days.[98] Respiratory symptoms appear 3–7 days after exposure.[99] These symptoms include fever, dry cough, and fatigue, along with non-respiratory symptoms, such as palpitations, diarrhea, or headache.[99,100] Risk factors for COVID-19–associated severe pneumonia or death include age 60 or older, smoking, and the presence of comorbidities, such as diabetes mellitus, hypertension, cardiovascular disease, chronic pulmonary disease, and cancer.[101–103] Transmission is believed to occur through inhalation of respiratory droplets and/or aerosol particles, contact with fomites, and person-to-person contact.[104,105] Droplets are deposited in the upper airways, while smaller, aerosolized particles can penetrate lower airways and deposit in alveoli.[104] Smaller particles can also stay airborne longer, prolonging the amount of time people might be exposed to the virus.[106]

Although not much is known about the pathogenesis of SARS-CoV-2, knowledge about the pathogenesis of SARS-CoV infection can be used to predict pathogenesis of COVID-19. In addition, recent studies have illuminated some facets of SARS-CoV-2 biology. Nasal epithelial cells are now believed to be the primary initial site of SARS-CoV-2 infection because of expression of high levels of ACE2, while infection in the lower respiratory tract could be due to aspiration-mediated virus seeding to the lung.[107] Once in the lung, SARS-CoV-2 can infect type II pneumocytes.[107] Rapid viral replication due to immune evasion results in delayed activation of lung-resident macrophages, causing extensive local inflammation and increased vascular permeability, attracting monocytes/macrophages and neutrophils and leading to fluid accumulation in alveoli.[3,108] Furthermore, inactivation of ACE2 by binding to SARS-CoV-2 prevents the conversion of proinflammatory angiotensin II to anti-inflammatory angiotensin (1–7), further exacerbating virus-induced inflammation and damage.[80] Fluid accumulation prevents the lungs from filling with air, resulting in shortness of breath and pneumonia, causing lung injury and death. Lung damage also allows the virus to enter the circulatory system, resulting in viremia and attacking other organs expressing ACE2, including the heart, kidney, and gastrointestinal tract.[109] Furthermore, increased circulating inflammatory mediators activate coagulation cascades, promoting microcirculatory thrombi formation[110] in addition to promoting acute kidney injury, gastrointestinal inflammation, cardiovascular injury, and cerebrovascular injury.[109–115] Severe complications observed in COVID-19 patients include respiratory failure, myocardial injury,

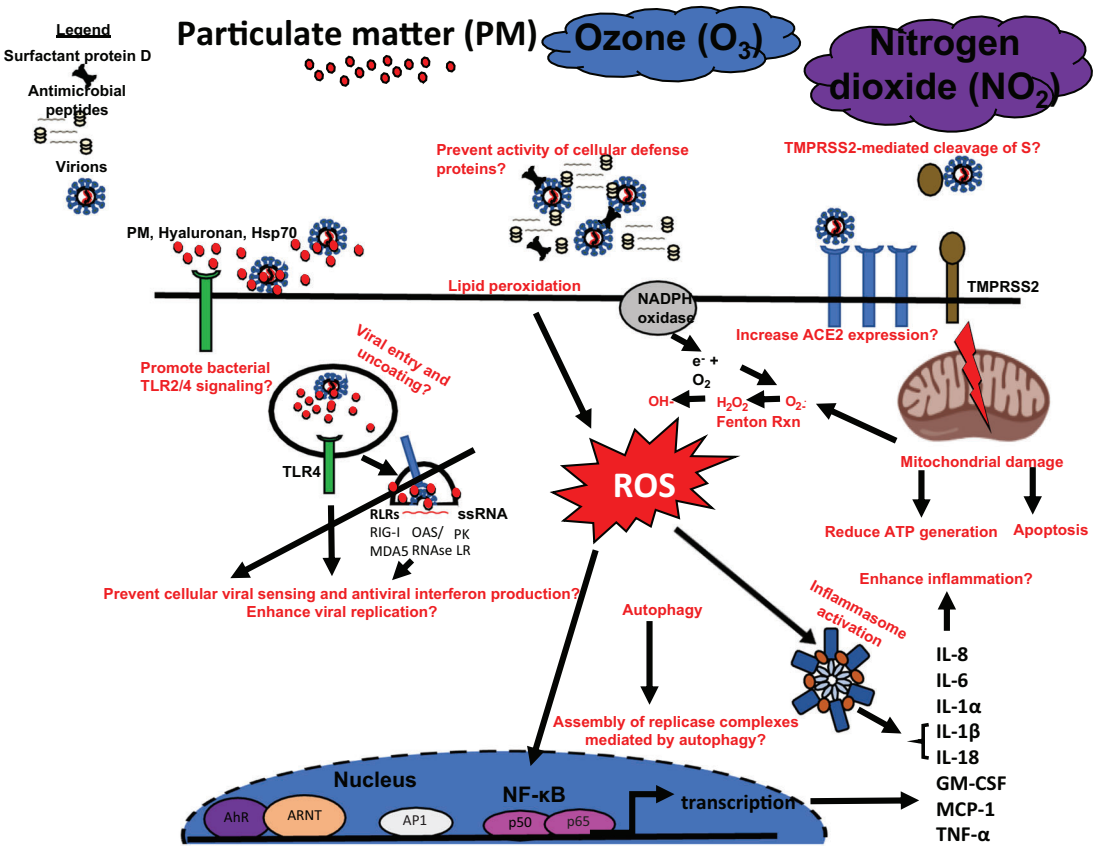


**Figure 2.** Potential effects of pollutant exposure on the viral life cycle of SARS-CoV-2. Exposure to air pollutants may promote viral entry through a variety of mechanisms, including preventing antiviral activity of SP-D and antimicrobial peptides, increasing levels of receptor ACE2, and promoting cleavage of TMPRSS2. The effects of exposure on viral uncoating are unknown. However, pollutants may promote TLR2 and TLR4 signaling, which is involved in bacterial immune responses, allowing evasion of antiviral responses. The effects of pollutant exposure on antiviral sensing mechanisms, including RIG-I–like receptors, are unclear. Exposure to pollutants induces lipid peroxidation and production of ROS through activation of NAPDH oxidase and mitochondrial damage, reducing ATP generation, stimulating apoptosis, and inducing autophagy. Coronaviruses may use autophagy to generate DMVs for replication. Thus, pollutant exposure may promote viral replication. Moreover, pollutant-induced ROS results in activation of proinflammatory redox-sensitive transcription factors NF-κB and AP-1, promoting transcription of proinflammatory genes. PAHs also stimulate AhR activation, which can crosstalk with NF-κB. Pollutant exposure also stimulates inflammasome activation. Thus, pollutant exposure enhances inflammation during viral infections.

arrhythmias, stroke, kidney failure, coagulopathy, secondary bacterial infections, and gastrointestinal disease.[111–115]

In the early stages of the pandemic, the primary cause of death was thought to be due to ARDS, caused by an excessive and prolonged production of proinflammatory cytokines—a cytokine storm—that caused extensive tissue damage.[6,116–118] However, levels of cytokines, such as IL-1β, IL-1RA, IL-6, IL-8, IL-18, and TNF-α, in patients with severe COVID-19 have been shown to be elevated at levels expected in critically ill patients, including those with severe cases of COVID-19, and to not be different from those observed in patients with ARDS or sepsis.[119] In fact, studies indicate that levels of IL-6 in severe COVID-19 patients are lower than those previously reported in patients with ARDS.[119–122] More recent studies indicate that the causes of death in COVID-19 patients include respiratory failure, stroke, myocardial injury, arrhythmias, coagulopathy, kidney failure, and secondary bacterial infections.[111–115] In fact, it is

now believed that COVID-19–induced mortality and morbidity is due to an "immunological collapse"[111] characterized by a loss of immune cells (B and T cells) in the spleen and secondary lymphoid organs.[111,123] Thus, an inability to control viral replication in the early stages of disease results in severe disease and death as a result of impaired and/or delayed immune responses, which is why the elderly are severely affected by COVID-19.[109]

### *Introduction to air pollutants*

Air pollution in urban environments is a mixture of anthropogenic and natural pollutants, which include PM, $O_3$, $SO_2$, $NO_x$, CO, and lead. Anthropogenic pollutants are created by industrial emissions, air/road/ship traffic, residential heating, and construction. $NO_2$ is a nitrogen-centered free radical, mostly produced in urban areas by traffic.[124] $O_3$ is a secondary air pollutant composed of three oxygen atoms and is formed at ground level by reactions of $NO_x$ and volatile organic compounds with sunlight. PM is a mixture of liquid, solid, or solid and liquid particles suspended in the air and is composed of a carbonaceous core with various organic (polycyclic aromatic hydrocarbons, PAHs), inorganic (transition metals, sulfates, and nitrates), and biological (bacteria, fungi, and viruses) components. PM is categorized according to size. $PM_{10}$ (thoracic) and $PM_{2.5}$ (respirable) are mass concentrations of particles with aerodynamic diameters of less than 10 and 2.5 microns, respectively. Ultrafine particles (UFPs) consist of PM of less than 0.1 microns and are generated by combustion and biogenic processes. $PM_{10}$ particles can be removed from the lungs through mucociliary clearance, whereas $PM_{2.5}$ can invade more deeply into the lungs and deposit into alveoli. UFPs can enter the circulation and reach other organs, resulting in adverse effects (as seen in the heart); however, this idea is still controversial.[125] It is believed that exposure to smaller particles ($PM_{2.5}$ and UFPs) is of greater health concern since they can deposit deeper into the lungs and adsorb more chemicals onto their surfaces because of larger surface-to-volume ratios. In fact, the toxic effects of PM exposure on human health are believed to be largely mediated by UFPs because of their ability to activate inflammation and oxidative stress at the cellular and systemic levels[126–129] Diesel exhaust particles (DEPs) are composed of $PM_{2.5}$ and UFPs. Below, we focus on mechanisms by which PM, $NO_2$, and $O_3$ could affect SARS-CoV-2 infection and COVID-19 pathogenesis.

### *Mechanisms involved in toxicity to air pollution*

Increased oxidative stress is thought to be the key mechanism of pollutant-induced toxicity. $O_3$ reacts directly with unsaturated fatty lipids in the respiratory tract–lining fluid and cell membranes to produce reactive oxygen species (ROS), such as hydrogen peroxide ($H_2O_2$), and lipid ozonation products, such as lipid peroxides and reactive aldehydes.[130] Like $O_3$, $NO_2$ reacts with substrates in the respiratory tract–lining fluid, producing oxidized species that can initiate inflammation.[124] By contrast, increased oxidative stress due to PM exposure is likely caused by various components. Transition metals present in PM (Fe, Zn, Ni, and V) are believed to undergo Fenton or Haber–Weiss reactions, generating ROS and promoting lipid peroxidation.[131] In the Fenton reaction, $Fe^{2+}$ reacts with $H_2O_2$ to produce a hydroxyl radical ($OH^{\cdot}$).[132] Thus, the metal content of particles is a factor in the cellular toxicity of PM. Furthermore, PAHs, which are lipophilic, can be converted by cytochrome p450 CYP1A1B into redox-active quinones that can stimulate ROS production.[133] The extent to which PM can cause oxidative stress depends on the type and amount of PAHs adsorbed on the surfaces of the particles, which depends on the source of the particles.[134] Oxidative stress can cause DNA damage, formation of protein adducts, and promote apoptosis through inducing mitochondrial dysfunction. Furthermore, oxidative stress stimulates activation of the redox-sensitive proinflammatory transcription factors NF-κB and AP-1 and the master antioxidant regulator Nrf2.[135] Therefore, oxidative stress induced by pollutant exposure results in increased inflammation, which is discussed further in the context of antiviral immune responses in the following sections. PAHs contained on PM also act as ligands for the aryl hydrocarbon receptor (AhR), triggering its nuclear translocation, which results in the expression of proteins like cytochrome P450 that are involved in the metabolism of xenobiotics.[135] AhR can also crosstalk with inflammatory and antioxidant transcription factors, such as NF-κB, STAT1, and Nrf2.[135]

## How can air pollution contribute to SARS-CoV-2 infection and COVID-19 pathogenesis?

### *Pollutant exposure alters respiratory epithelial barrier function*

One mechanism by which pollutant exposure could promote SARS-CoV-2 infection and COVID-19 pathogenesis is by increasing pulmonary epithelial permeability, allowing the pathogen and proinflammatory mediators access to the basolateral side of the epithelium, promoting viral spread and inflammation (Fig. 3). Multiple studies have demonstrated that $O_3$ exposure is associated with increased pulmonary permeability due to altered tight junctions, resulting in neutrophil infiltration into the lungs.[136–140] In addition to $O_3$ exposure, $NO_2$ exposure also disrupts tight junctions in the lungs.[141–143] Moreover, PM exposure also reduces levels of tight junction proteins.[144]

In addition to tight junctions, the mucus layer in the respiratory tract is a host-restriction factor for viruses.[145] In the respiratory tract, mucus maintains hydration and acts as a protective barrier by trapping particulate matter and pathogens, which can then be expelled by the beating of cilia bundles and is referred to as mucociliary clearance. However, mucus also functions in regulating immune responses, presenting inhibitory pathogen molecules, regulating cell proliferation and differentiation, and maintenance of the epithelial barrier function.[145] Mucus is composed of a gel layer of on top of a liquid layer (where cilia are located), and mucus itself is composed of heavily glycosylated mucins, which can be secreted or membrane-tethered. In addition, substances, such as lactoferrin (iron-binding glycoprotein), oxidants (nitric oxide), hydrogen peroxide, proteases and protease inhibitors, and lysosomes, are found in mucus.[145] The composition of mucus can prevent viral infection. For instance, lactoferrin demonstrates antiviral activity.[145] Furthermore, influenza virus encodes a surface glycoprotein that cleaves sialic acids to prevent the virus from being trapped in mucus by heavily sialylated mucins.[145] It has also been suggested that mucus hypersecretion is an underlying cause of hypoxia in COVID-19 patients.[146] Interestingly, PM exposure can suppress mucociliary clearance and promotes mucus hypersecretion,[147,148] although whether effects of PM exposure on mucus production and mucociliary clearance affect SARS-CoV-2 infection and COVID-19 pathogenesis is unclear. Pollutant exposure also decreases levels of various antioxidants in the respiratory tract–lining fluid and tissues, increasing the susceptibility to further oxidative damage mediated by inflammation and/or continued pollutant exposure.[135]

### *Direct effects on virions*

It has been suggested that the association between pollutant exposure and COVID-19 is due to atmospheric PM facilitating viral survival in the air, promoting atmospheric transport.[149] SARS-CoV-2 RNA has been found on PM collected from February to March 2020 in polluted areas of Northern Italy; however, this study did not investigate whether infectious virions were present.[70] Studies have shown variability on infectivity when virions are incubated with PM. For instance, incubation with urban PM decreased infectivity of bacteriophage Φ6, an enveloped virus, and slightly enhanced infectivity of $\Phi X_{174}$, a nonenveloped phage, possibly because of damage of the lipid membranes of the enveloped virus.[150] Particles prevented time-dependent decreases in RSV infectivity, because of shielding by particles, but conjugation of RSV to particles inhibited syncytia formation and rate of infection.[151] It has also been suggested that absorption to particulate matter protects viruses during disinfection.[152] Whether enough infectious virus is adsorbed onto PM to promote transmission is unclear. Inhalable microorganisms contained on $PM_{2.5}$ and $PM_{10}$ pollutants collected from Beijing were mostly soil-associated bacteria; DNA viruses accounted for only 0.1%.[153] Another study demonstrated that microbes in air carried on $PM_{2.5}$ and $PM_{10}$ particles collected in Beijing primarily consisted of bacteria (95.45% and 93.04%, respectively) and a small proportion of viruses, including porcine type C oncovirus and avian endogenous retrovirus EAV HP (2.8% and 4.52%, respectively).[154] These studies are limited because of the complexity of isolating viral RNA from particles.[106] However, in the context of the pandemic, when adequate social distancing practices are in place, most transmissions are likely occurring indoors by nosocomial spread, close person-to-person interactions, and fomite transmission. Instead, it is likely that the damaging effects on host immunity and cellular responses (i.e., systemic inflammation, etc.)

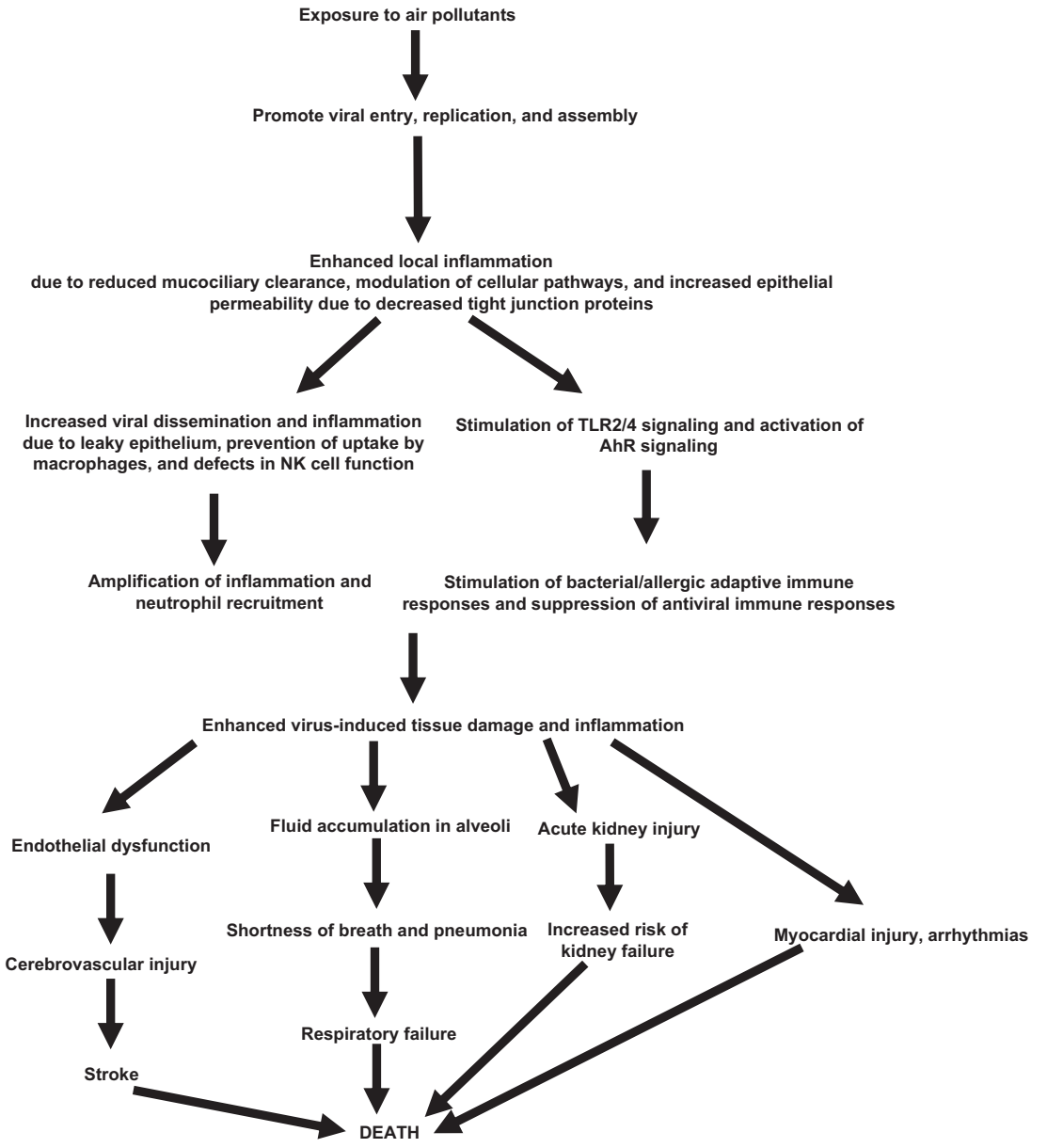


**Figure 3.** Potential effects of pollutant exposure on SARS-CoV-2 infection and COVID-19 pathogenesis. As illustrated in Figure 1, exposure to air pollutants may promote viral entry, replication, and assembly, and activate proinflammatory transcription factors, resulting in enhanced local inflammation. Furthermore, pollutant exposure reduces mucociliary clearance and decreases levels of tight junction proteins, promoting epithelial permeability, which can result in increased viral spread and inflammation because of the leaky epithelium. Prevention of macrophage uptake and defective natural killer (NK) cell function also promotes viral spread. Subsequent enhanced inflammation can stimulate neutrophil recruitment and further amplify inflammatory processes. Moreover, since pollutant exposure is believed to skew adaptive immune responses toward allergic/bacterial responses instead of antiviral immune responses, exposure may result in enhanced virus-induced tissue damage and inflammation, promoting dysfunction of a variety of organs, including the lungs, heart, kidney, and brain, resulting in death.

resulting from the long-term outdoor exposure of urban populations to high levels of PM, regardless of current pollutant emission levels, are likely responsible for the association between PM levels and SARS-CoV-2 infection and COVID-19–associated mortality.

$NO_2$ has been suggested to be utilized as a disinfectant in low-resource environments and can inactivate enveloped and nonenveloped viruses.[155] However, $O_3$ is commonly used as a wastewater disinfectant and inactivates poliovirus (nonenveloped, positive-sense ssRNA virus) by damaging the viral genome.[156] $O_3$ exposure has also been shown to reduce infectivity of nonenveloped bacteriophages and of a variety of enveloped viruses because of lipid and protein peroxidation.[157,158] Extremely high concentrations of $O_3$ (over 100 ppm) have also been shown to inactivate animal RNA viruses.[159] It is likely that direct exposure of SARS-CoV-2 to $O_3$ inactivates the enveloped virus. However, studies with another respiratory virus, influenza A, showed that exposure of human nasal epithelial cells to 0.4 ppm of $O_3$ 24 h before infection resulted in increased influenza A entry and replication. $O_3$ exposure increased levels of airway surface liquid–associated secreted TMPRSS2 and human airway trypsin-like protease but decreased levels of secretory leukocyte proteinase inhibitor, increasing cleavage of viral hemagglutinin protein, which is required for cellular entry. These effects of $O_3$ were inhibited with addition of antioxidants to cultures.[160] Thus, acute exposure to $O_3$ may potentially enhance SARS-CoV-2 infection, since TMPRSS2 cleaves the S viral protein to facilitate entry[77] (Fig. 2). Exposure of respiratory epithelial cells to PM has also been shown to increase influenza A viral attachment after exposure.[134] It is possible that PM and $NO_2$ exposure may affect SARS-CoV-2 entry by regulating proteases required for entry (Fig. 2), since exposure to PM, $NO_2$, and $O_3$ can all increase oxidative stress in the lungs.

In addition to pollutants directly interacting with virions, pollutant exposure can also alter the production/activity of cellular defensive proteins. However, the effects of pollutants on canonical antiviral effectors like ISGs have been sparsely investigated. However, there are studies demonstrating that pollutants can affect the production/activity of surfactant proteins and AMPs, which are found in the respiratory tract–lining fluid. For instance, incubation of nanoparticles (used as surrogate particles) with SP-A and SP-D prevents neutralization of influenza A infection.[161] *In vivo* studies also indicate that PM and $NO_2$ exposure decreases levels and/or activity of surfactant proteins.[162–164] As previously mentioned, SP-D has been shown to bind the SARS-CoV-1 spike protein,[91] so preventing SP-D activity could be one mechanism by which PM exposure promotes SARS-CoV-2 infection. PM has also been demonstrated to bind directly to cationic AMPs, interfering with antimicrobial activity.[165] Other studies have shown that PM prevents secretion and production of AMPs.[166–168] Furthermore, fine particles in air pollutants have been suggested to alter the charge and, subsequently, the activity of LL37 by promoting citrullination of the AMP.[169] As previously mentioned, AMPs may be involved in regulating SARS-CoV-2 infection by disrupting the viral envelope. In addition, AMPs also function in regulating immune responses by stimulating TLR activation, cytokine production, immune cell chemotaxis, and neutrophil and mast cell degranulation.[93] Thus, altering levels and/or activity of AMPs may be one mechanism that facilitates SARS-CoV-2 infection (Fig. 2).

### *Pollutant exposure may alter viral entry and surface levels of attachment receptors*

Another mechanism by which PM exposure could affect SARS-CoV-2 infection is by modifying viral entry (Fig. 2). UFPs (which are the majority of DEPs) are internalized as aggregates in membrane-bound vacuoles.[125,170] Thus, particle aggregation could interfere with receptor binding and uncoating of adsorbed viruses. Conversely, interaction of lignite fly ash (a component of coal particulate emissions) with cell membranes promoted influenza viral entry, increasing viral replication and suppressing influenza-induced interferon production.[171,172] Since SARS-CoV-1 entry utilizes pH-dependent endocytosis similarly to influenza,[173] it is also possible that PM exposure affects viral entry and uncoating in this manner. Both $PM_{2.5}$ and $PM_{10}$ have been demonstrated to enter trophoblasts via endocytosis,[174] and exposure to combustion-derived PM promoted lysosomal permeabilization.[175] Future studies should focus on whether pollutant exposure affects viral fusion and uncoating (Fig. 2). $O_3$ exposure has been shown to inhibit activity of lysosomal hydrolases.[176]

Since cathepsin B/L may also play a role in priming S viral protein,[77] $O_3$ exposure may also prevent SARS-CoV-2 entry. By contrast, $NO_2$ has been suggested to have no effect on lysosomal cathepsin D activity.[177] However, low doses of $NO_2$ increased the internalization of RSV, while higher doses decreased it.[178] Interestingly, higher doses of $NO_2$ also decreased the release of infectious virus.[178] Thus, the potential effects of $NO_2$ exposure on SARS-CoV-2 viral internalization are unclear.

Another potential mechanism by which pollutant exposure could regulate viral attachment and entry is by altering surface expression of viral receptors (Fig. 2). Whether $NO_2$ and $O_3$ exposure affects levels of ACE2, the receptor for SARS-CoV-2, is unclear. However, exposure to PM has been previously shown to affect levels of viral receptors for rhinovirus, a nonenveloped, positive-sense RNA virus.[179] Increased levels of ACE2 were observed in murine lungs 2 days after $PM_{2.5}$ exposure.[180] Thus, exposure to PM may alter viral entry by increasing levels of the SARS-CoV-2 receptor ACE2 (Fig. 2). Furthermore, pollutant-mediated oxidative post-translational modifications of ACE2 may alter its surface expression. Interestingly, ACE2 is involved in lung repair of $PM_{2.5}$-induced lung damage,[180] so binding of SARS-CoV-2 to ACE2 could prevent this activity, thereby exacerbating pollution-induced lung damage.

### *Pollutant exposure regulates pathogen-sensing mechanisms*

As previously mentioned, SARS-CoV-1 infection activates the NLRP3 inflammasome; however, PM exposure has been previously shown to activate NLRP3 inflammasomes in the lungs.[181] Human airway epithelial cells can uptake PM, which enhances the amount of inflammasome-associated IL-1β produced after influenza virus infection.[182] Our laboratory has also shown that skin exposure to $O_3$ activates the inflammasome.[183] Whether pollutant-induced inflammasome activation augments SARS-CoV–induced activation, which together contribute to inflammatory tissue damage, is unclear (Fig. 2).

Pollutant exposure has been shown to affect TLR signaling. TLR4 has been suggested to mediate $O_3$-induced epithelial permeability.[184] $O_3$ exposure may be able to stimulate TLR4 activation by inducing the release of DAMPs, such as hyaluronan or Hsp70.[184] $O_3$ exposure can also enhance TLR4 signaling *in vivo*.[185] Exposure of nasal epithelial cells to 0.4 ppm of $O_3$ 24 h before infection did not affect levels of TLR3, type I IFN, or RIG-I.[160] Thus, $O_3$ exposure may result in increased SARS-CoV-2 infection by skewing immune responses away from viral responses (Figs. 2 and 3).

Since bacteria are adsorbed on particles, TLR4 and TLR2 have been shown to be involved in PM-induced inflammation and cytokine production of IL-6 in alveolar macrophages.[186] In primary human airway epithelial cells, the production of IL-8 in response to PM depended on TLR2, possibly because of activation by the DAMP Hsp70.[187] Interestingly, different types of particulates differentially stimulate TLR2 and TLR4 activation in peritoneal macrophages.[188] PM exposure downregulated TLR2 and TLR4 in human dendritic cells, which correlated with a pro-$T_H2$ inflammatory profile, characteristic of allergic responses.[189] This idea is supported by Cao *et al.*, who observed that inhalable microorganisms found on PM included several microbial species known to cause allergies and bacterial pneumonia.[153]

Instead of directly acting as a ligand, PM exposure can also modify the ability of TLRs to respond to infection.[184] For instance, DEPs amplified responses to low levels of the TLR agonists LPS and flagellin, although exposure reduced IL-1β release.[190] By contrast, exposure of respiratory epithelial cells to aqueous-trapped diesel exhaust enhanced the susceptibility to influenza virus infections and increased levels and activity of TLR3, nuclear levels of IRF3, and expression of IFN-β.[191] Interestingly, we were unable to find any literature investigating whether pollutant exposure can alter activity of RIG-I, MDA5, or protein kinase R, which are canonical, cytoplasmic viral RNA sensors. However, in the context of coronavirus, it is possible that prior exposure to pollutants shifts innate TLR responses into allergic or antibacterial responses, as opposed to antiviral responses, preventing adequate antiviral signaling upon SARS-CoV-2 infection (Figs. 2 and 3). Alteration of the lung microbiome due to PM exposure may also skew immune responses in exposed individuals toward bacterial responses,[147] since alterations in the microbiome would likely trigger antibacterial TLR responses, causing the body to focus its efforts on fighting off a bacterial infection, rather than a viral infection, potentially promoting SARS-CoV-2 infection and

replication via failure to mount an adequate antiviral immune response.

### *Potential effects of pollutant exposure on viral replication and antiviral interferon production*

Although there is a lack of information in the literature about whether pollutant exposure regulates coronavirus replication, we believe that studies investigating the effects of pollutant exposure on antiviral IFN production and replication of other viruses may shed insight on this topic since antiviral mechanisms are evolutionarily conserved. However, it is necessary to observe that activities of viral IFN antagonists vary among different viruses.

In the context of $O_3$, exposure of mice to 0.8 ppm of $O_3$ for 11 days reduced the ability of respiratory epithelial cells, but not alveolar macrophages, to produce interferon *in vitro*.[192] Another study demonstrated that high concentrations of $O_3$ (1.5 or 2 ppm) exposure restricted enterovirus 71 (nonenveloped, positive-sense RNA virus) replication, prolonged cell survival, and increased levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α.[193] Exposure of airway epithelial cells to $O_3$ directly before infection reduced RSV (enveloped, nonsegmented, negative-sense RNA virus) replication in infected cells, although exposure after infection had no effect.[194] Continuous exposure of mice to 0.5 ppm of $O_3$ during murine influenza A (enveloped, segmented, negative-sense RNA virus) infection resulted in a less widespread infection of the lung, reduced immune responses (T and B cells and lower antibody titers), and reduced severity of the disease.[195] Thus, exposure to $O_3$ before or during infection may reduce viral replication (Fig. 2). By contrast, long-term exposure to $NO_2$ did not alter IFN production in mice.[196]

The effects of PM exposure on interferon production and viral replication are less clear. Exposure to Asian sand dust increased rhinovirus replication and production of IFN-γ, IL-1β, IL-6, and IL-18 in primary human nasal epithelial cells.[197] Coal dust suppressed virus-induced IFN induction.[198] Chronic aerosol exposure to diesel engine exhaust resulted in increased influenza viral growth in lungs and decreased IFN and antibody levels.[199] Ma *et al.* observed differential effects in mice acutely or chronically exposed to PM; chronic exposure reduced pulmonary resistance to influenza A virus infection and downregulated levels of histone demethylase KDM6A, altering histone methylation patterns on the IFN-β and IL-6 promoters, while acute exposure increased the production of IFN-β, IL-6, and OAS1.[200] Simultaneous infection with vesicular stomatitis virus (VSV, enveloped, nonsegmented, negative-sense RNA virus) and exposure to PM promoted viral entry and replication, stimulating apoptosis and decreasing levels of VSV-induced IFN-β, ISG1G, CCL5, and CXCL10 by reducing levels of phosphorylated IRF3.[201] Exposure of respiratory epithelial cells to PM increased influenza A viral attachment, which resulted in increased viral gene expression, IFN production, STAT1 phosphorylation, and ISG transcription because of pollutant-mediated oxidative stress.[134] In conclusion, it is likely that PM exposure may promote viral replication (Fig. 2). Future research is needed to assess whether $O_3$ and PM exposure alters antiviral interferon signaling and SARS-CoV-2 replication (Fig. 2).

### *Pollutant exposure interferes with mitochondrial function and autophagy*

Mitochondria are double-membranous organelles that synthesize ATP through oxidative phosphorylation, and mitochondrial damage leads to reduced generation of ATP and higher ROS production. These organelles are targeted by environmental pollutants, including PM, $NO_2$, and $O_3$, through a variety of mechanisms.[125,202–204] Pollutant-mediated mitochondrial dysfunction also promotes apoptosis, which could affect viral replication and immune surveillance in SARS-CoV-2 infection (Fig. 2). Internalization of $PM_{2.5}$ can stimulate ferroptosis, a form of cell death dependent on intracellular iron overload and lipid peroxidation,[205] which could also play a role in immune surveillance. In addition to harming mitochondrial function, pollutant exposure stimulates ROS production by NAPDH oxidase.[206] Increased ROS in mice caused mutations in the coxsackievirus B genome, which promoted virulence.[207] It has also been suggested that oxidative stress induced during infection promotes genome capping and replication of positive-sense RNA viruses.[208] Together, these data could lead to the prediction that pollutant-mediated interference with ATP generation could affect viral replication and assembly in host cells (Fig. 2), although this has not been studied.

Autophagy is a degradation process utilized by eukaryotic cells to dispose of misfolded proteins, protein complexes, and damaged organelles. During autophagy, cytoplasmic materials are enclosed by autophagosomes, which fuse with lysosomes and form autolysosomes, resulting in degradation of the contents by lysosomal hydrolases. Coronaviruses have been suggested to utilize autophagy as a way to generate the double membranous vesicles used in assembly of viral replication complexes[209] (Fig. 1). PM and $O_3$ exposure can regulate autophagy.[125,202,210–213] Whether effects of pollutant exposure on autophagy alter coronavirus assembly and replication is unknown (Fig. 2).

### *Effects of pollutant exposure on macrophage and natural killer cell function*

Another mechanism by which pollutant exposure could enhance SARS-CoV-2 infection and COVID-19 pathogenesis is by preventing the uptake of infected cells by macrophages (Fig. 3). SARS-CoV poorly replicates in monocytes/macrophages; reduced innate immune responses in these cells could promote viral spread.[214] Exposure of SP-A to $O_3$ reduced the ability of SP-A to mediate phagocytosis and superoxide production in alveolar macrophages.[215] Another study concluded that $O_3$ exposure likely does not alter the susceptibility of alveolar macrophages to infection or cytokine responses induced by infection.[216] Interestingly, alveolar macrophages from human volunteers exposed to $NO_2$ were less able to inactivate influenza.[217]

However, exposure of alveolar macrophages to PM decreased viral uptake and reduced RSV-induced production of MCP-1.[218] Another study did not observe an effect of PM on affecting RSV uptake; however, they observed decreased viral yield and decreased RSV-induced production of IL-6 in in alveolar macrophages exposed to PM.[219] By contrast, exposure to UFPs impaired macrophage phagocytosis.[220] Therefore, PM exposure may prevent macrophage uptake and phagocytosis of virus-infected cells, preventing antigen presentation and cytokine production by these cells, allowing uncontrolled viral growth (Fig. 3), as observed with influenza A.[221]

Furthermore, exposure to DEPs reduced natural killer (NK) cells' ability to increase levels of granzyme B and perforin and a variety of proinflammatory cytokines in response to a TLR3 agonist, suggesting that PM exposure may affect the ability of NK cells to kill virus-infected cells.[222]

### *Effects of pollutant exposure on adaptive immune responses*

Pollutant exposure also alters adaptive immune responses. Exposure to $PM_{2.5}$ in healthy, nonsmoking adults was associated with higher levels of endothelial microparticles (markers of apoptosis), increased levels of antiangiogenic and proinflammatory cytokines (MCP-1, MIP-1α/β, IL-6, and IL-1β), and increased circulating levels of immune cells.[223] Myeloid dendritic cells exposed to urban PM enhanced naive $CD8^+$ T cell priming, resulting in increased secretion of granyzme A and B.[224] Increased production of proinflammatory cytokines by $CD8^+$ T cells responding to a viral infection could result in damage to bystander cells and inflammation amplification.

Other studies indicate that PM exposure may suppress antiviral responses. Suppressed IL-2 and IFN-γ production was observed in $CD4^+$ and $CD8^+$ T cells exposed to DEPs.[125] Increased morbidity and decreased survival were observed in influenza A–infected neonatal mice exposed to combustion-derived PM, because of increased viral load, pulmonary $T_{reg}$ cells, and dampened protective T cell responses.[225] Infection with influenza A virus after exposure of neonatal mice to combustion-derived PM increased pulmonary $T_{reg}$ cells, and IL-10 levels, suppressing protective T cell responses.[226] Severe SARS-CoV infection is characterized by delayed development of adaptive immune responses and prolonged viral clearance caused by the dramatic loss of $CD4^+$ and $CD8^+$ T cells early in infection,[227] and overactivation of remaining $T_H17$ and cytotoxic $CD8^+$ T cells (perforin- and granulysin-positive cells) has been linked to severe immune injuries in COVID-19.[228] Thus, PM exposure may suppress antiviral adaptive responses, promoting SARS-CoV-2 replication and dissemination (Fig. 3).

Exposure to PM also skews adaptive immune responses toward allergic responses. Studies have demonstrated that DEP exposure promotes production of the allergy-associated antibody IgE.[229,230] In fact, *in vivo* studies indicate that PM exposure promotes allergic airway inflammation and increases viral replication in the lungs.[231–233] Thus,

researchers have concluded that exposure to PM potentiates $T_H2$ and $T_H17$ responses, which are observed in asthma and allergy, dysregulating antiviral protective $T_H1$ responses.[135,234] The mechanism by which PM exposure promotes allergic immune responses is unclear. PM-induced mucosal uric acid has been shown to mediate allergic sensitization and augment antigen-specific T cell proliferation.[235] PAHs, which stimulate AhR activation, may also be responsible for PM-induced allergic responses,[234] since AhR signaling drives allergic responses and regulates immune tolerance.[236] Pollutant-induced activation of AhR in the context of infection may enhance neutrophil recruitment to the lungs,[237] contributing to immunopathology. AhR activation during infection also suppresses expansion and differentiation of virus-specific $CD8^+$ T cells by affecting dendritic cells and $T^{regs}$ cells and affects antibody production by B cells.[237] Thus, PM-induced AhR activation may promote allergic immune responses and suppress antiviral adaptive immune responses (Fig. 3).

There is a lack of mechanistic studies investigating the effects of $O_3$ exposure on viral infection and adaptive immune responses. Chronic $O_3$ exposure has been suggested to activate AhR via production of lipoxin A4, which regulates lung inflammation and epithelial damage after exposure and promotes $T_H2$ and $T_H17$ responses.[238] In human subjects, $O_3$ exposure is believed to enhance pulmonary immunity and promote allergic responses.[48] Since $O_3$ is instantaneously consumed, unlike PM, allergic responses are believed to be triggered by secondary messengers, such as IL-33.[48] Experiments testing the effects of $O_3$ exposure alone on antiviral and allergic adaptive immune responses in response to SARS-CoV-2 function should be performed. $NO_2$ inhalation can induce imbalances in the ratio of $T_H1/T_H2$ differentiation and activate the JAK/STAT pathway, skewing immune responses toward allergic responses.[239] However, this effect has not been investigated in the context of viral infection.

### *Pollution exposure may enhance COVID-19–associated immunopathology*

An association between pollution exposure and increased COVID-19–associated mortality could be that exposure predisposes people toward developing SARS-CoV-2–related immunopathology and lung damage (Fig. 3). Exposure to PM increased the production of IL-6, TNF-α, IL-1β, MIP-1α, and GM-CSF by alveolar macrophages and in human subjects during acute exposure.[240] Exposure of respiratory epithelial cells to PM promoted the release of inflammatory cytokines, such as TNF-α, IL-8, and IL-7.[241] In addition to epithelial cells, neutrophils also contribute to inflammation. Chronic exposure to geogenic PM promoted the production of MIP-2, IL-6, and acute neutrophil infiltration in mice lungs.[242] PM exposure has also been suggested to enhance neutrophil extracellular trap formation,[243] which may be involved in COVID-19 pathogenesis.[244]

*In vivo* studies also indicate that PM exposure likely enhances virus-induced tissue damage and inflammation (Fig. 3). Preexposure of mice infected with coxsackievirus (nonenveloped, positive-sense RNA virus) resulted in increased lung and cardiac damage, inflammatory cell infiltration, and promoted $T_H17$ responses.[245] Preexposure of mice to diesel engine emissions (6 h/day for 7 days) increased viral gene expression, lung inflammation, and levels of TNF-α and IFN-γ in response to RSV infection.[162] Exposure of mice to diesel exhaust for 4 h/day for 5 days increased susceptibility to influenza A infection, neutrophil recruitment, IFN-β levels, and levels of IL-6.[163] However, Clifford *et al.* observed that exposure to geogenic PM reduced influenza-induced IL-6 and IFN-γ levels in murine bronchoalveolar fluid, but these authors did observe increased viral titers in exposed lungs, based on metal content of the particles.[246]

In addition to PM, *in vivo* studies also indicate that $O_3$ exposure may enhance virus-induced lung damage and inflammation (Fig. 3). Exposure of healthy adults to 0.06 ppm of $O_3$ for 6.6 h increased neutrophil inflammation in the airways,[247] suggesting that $O_3$ exposure may contribute to COVID-19 pathogenesis. Interestingly, exposure of mice to 0.5 ppm of $O_3$ did not alter influenza virus infectious titers in lungs but enhanced postinfection lung damage.[248] Another study observed increased mortality and morbidity in mice exposed to 1 ppm of $O_3$ 3 h/day for 5 days that were infected with influenza virus. The authors also observed more severe pneumonitis, necrosis, squamous metaplasia, and hyperplasia in exposed bronchial epithelia, although they did not observe an effect on virus titers in the lungs.[249] However, other studies have not observed differences in virus-induced

inflammation in mice or humans exposed to $O_3$.[250,251] In fact, exposure to 0.5 ppm of $O_3$ after viral infection decreased influenza virus mortality in mice, decreased virus titers in newly exposed mice, and prevented viral spread in the lungs.[250]

$NO_2$ exposure stimulates the release of IL-8, TNF-α, and IL-1β from human bronchial epithelial cells.[252] Moreover, synergistic increases in IL-8 production were observed when human nasal epithelial cells were exposed to $NO_2$ and infected with rhinovirus.[253] Exposure to $NO_2$ also promotes neutrophil adhesion to exposed airway epithelial cells, causing the death of exposed cells.[254] In human volunteers, $NO_2$ exposure increased neutrophil levels in the lungs and levels of IL-6 and IL-8 in lung fluid, although no differences in alveolar macrophage virus susceptibility were observed.[255] By contrast, another study observed that experimental exposure of human subjects to $NO_2$ increased susceptibility to influenza A viral infection.[256] Other studies indicate that $NO_2$ exposure promotes airway inflammation and increases susceptibility of airway epithelial cells to injury from RSV.[257] Exposure of mice to $NO_2$ did not alter infection with Sendai virus (negative-sense, ssRNA virus), but exposure enhanced virus-induced lung pathology.[258] Thus, it is possible that pollutant exposure may contribute to inflammation and exacerbate SARS-CoV-2–induced lung damage because of enhanced immune cell infiltration (Fig. 3).

## Conclusion

Currently, there are no approved vaccines that target human coronaviruses; treatment is primarily supportive. Although the U.S. Food and Drug Administration supports the use of the antiviral drug Veklury (remdesivir), which targets the viral RNA-dependent RNA polymerase, for treating hospitalized adult and pediatric patients with suspected or laboratory-confirmed COVID-19, irrespective of disease severity (https://www.fda.gov/news-events/press-announcements/covid-19-update-fda-broadens-emergency-use-authorization-veklury-remdesivir-include-all-hospitalized). Multiple clinical trials have observed that the antiviral drug shortens recovery time for COVID-19 patients; however, there is still high mortality among COVID-19 patients despite the use of the drug, so use of this drug alone is likely insufficient.[259–261] Therefore, the best way to prevent infection is to avoid exposure to the virus. Since air pollution exposure has been associated with increased SARS-CoV-2 infection and COVID-19–associated mortality,[56–62] reducing pollutant emissions and individual exposure may prevent pollutant-induced exacerbation of SARS-CoV-2 infection and COVID-19 disease. Because of the urgency of the current topic, we have also included nonpeer-reviewed preprints as references,[58,60,70,119,123] so the legitimacy of these findings should be confirmed by peer-reviewed studies.

However, on the basis of the studies reviewed in this paper, we believe that pollutant exposure may affect the viral life cycle of SARS-CoV-2 by preventing mucociliary clearance, enhancing viral entry by preventing activity of endogenous antimicrobial proteins (SP-D and AMPs), regulating levels of viral receptors and proteases required for viral entry (TMPRSS2 and ACE2), interfering with antiviral interferon production, and promoting viral replication and assembly (Figs. 2 and 3). However, none of these points have been experimentally tested. What stage and/or stages of the viral life cycle (i.e., entry, replication, assembly, etc.) that pollutant exposure influences should be identified to indicate potential targets for antiviral drugs. Furthermore, although we believe that virion adsorption to PM likely reduces SARS-CoV-2 viral infectivity and/or interferes with viral uncoating, this hypothesis has not been tested. In fact, the potential effects of exposure to the air pollutants $O_3$ and $NO_2$ on viral uncoating and fusion are also unclear. However, exposure to air pollutants may prevent the uptake of infected cells by macrophages and alter epithelial permeability, promoting viral spread and inflammation (Fig. 3). Furthermore, stimulation of TLR2 and TLR4 in response to pollutant exposure likely skews adaptive immune responses toward bacterial/allergic immune responses, dysregulating antiviral immune responses (Fig. 3). This would cause the body to focus its efforts on fighting off a bacterial infection rather than a viral infection, potentially promoting SARS-CoV-2 infection and replication via failure to mount an adequate antiviral immune response. However, maintaining sufficient antioxidant nutrients in the diet (vitamins C and E, etc.) can protect against pollutant-induced oxidative stress and virus-induced inflammatory tissue damage.[232,262] However, the hypotheses mentioned

here should be tested experimentally; thus, additional research on the effects of pollutant exposure on SARS-CoV-2 infection is required to better understand the current pandemic and combat future outbreaks.

## Future studies

To address the connection between pollution exposure and SARS-CoV-2 infection and pathogenesis, future studies should focus on addressing what specific stages of the viral life cycle are affected by pollutant exposure. Although gender-specific differences in COVID-19 susceptibility are controversial,[263] it should also be investigated whether there are gender-specific differences in pollution-induced exacerbation of SARS-CoV-2 infection caused by sex hormones or other factors.[135,264] Studies investigating the relationship between pollutant exposure and SARS-CoV-2 should also be performed in the context of age and comorbidities, since these are factors that determine the susceptibility to COVID-19.[265] Additionally, studies should focus on the effects of chronic, low levels of exposure in order to be representative of real-world pollution exposure. Moreover, in the everyday urban environment, people are exposed to multiple pollutants simultaneously, and there is experimental evidence demonstrating synergistic effects of combination exposure on pollution-induced inflammation,[253,266] so the effects of simultaneous exposure to multiple pollutants on SARS-CoV-2 infection and COVID-19 pathogenesis should be studied. It would also be interesting to study the involvement of neutrophils and AhR signaling in the context of pollution and SARS-CoV-2 infection and whether pollution exposure skews immune responses toward bacterial/allergic responses, promoting viral replication and dissemination. Another interesting idea is whether pollutant-induced oxidative posttranslational modifications of viral antigens could alter adaptive immune responses. It is also important, in the context of PM, to consider the source and composition of particles, which affects biological responses.[135] However, to study potential mechanisms by which pollutant exposure contributes to SARS-CoV-2 infection and COVID-19 pathogenesis, interdisciplinary collaboration between virologists, toxicologists, immunologists, clinicians, and public health officials will be necessary.

## Competing interests

The authors declare no competing interests.

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