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BRIEF REPORT

Urban Particulate Matter Impairment of Airway Surface Liquid–Mediated Coronavirus Inactivation

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Air pollution particulate matter (PM) is associated with SARS-CoV-2 infection and severity, although mechanistic studies are lacking. We tested whether airway surface liquid (ASL) from primary human airway epithelial cells is antiviral against SARS-CoV-2 and human alphacoronavirus 229E (CoV-229E) (responsible for common colds), and whether PM (urban, indoor air pollution [IAP], volcanic ash) affected ASL antiviral activity. ASL inactivated SARS-CoV-2 and CoV-229E. Independently, urban PM also decreased SARS-CoV-2 and CoV-229E infection, and IAP PM decreased CoV-229E infection. However, in combination, urban PM impaired ASL's antiviral activity against both viruses, and the same effect occurred for IAP PM and ash against SARS-CoV-2, suggesting that PM may enhance SARS-CoV-2 infection.

Keywords. airway; viral infection; common cold; COVID-19; innate immunity.

Before the current coronavirus disease 2019 (COVID-19) pandemic, humans lacked virus-specific immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Therefore, in immunologically naive individuals, the airway's innate immune response remains critical to combating SARS-CoV-2 and other infections.

Airway surface liquid (ASL) is a thin layer of fluid lining the airway epithelium. ASL contains antimicrobial peptides and proteins (AMPs) that provide a critical first line of defense against incoming pathogens [1]. Specific AMPs in the ASL may inhibit viral cell binding or attachment or alter subsequent viral life cycle stages to enhance viral clearance [2]. In addition, AMPs within the ASL have been reported to have antiviral activity against enveloped and nonenveloped viruses [3]. On viral challenge, other innate immune mechanisms are also activated (eg, macrophages and neutrophils phagocytize infected cells and intracellular antiviral mechanisms interfere with viral replication, inactivating viruses).

Airway innate immunity is disrupted by air pollution. Stapleton et al [4] have previously demonstrated that indoor and ambient air pollution particulate matter (PM) inhibits ASL-mediated bacterial killing, in part owing to competition between cationic AMPs and negatively charged PM and bacteria. Ambient PM is also associated with increased coronavirus infection incidence and mortality rates in epidemiological studies [5]. However, the mechanisms underlying this association are unknown.

Endogenous ASL with abundant and diverse AMPs, derived from primary human donor airway epithelial cell (hAEC) cultures at the air-liquid interface, has not been assessed for anticoronavirus activity. In the current study, we hypothesized that ASL is antiviral and that PM impairs ASL antiviral activity (Supplementary Figure 1). If so, this effect likely contributes to the reported increased risk of SARS-CoV-2 infection related to urban air pollution exposures [6]. To test this, we assessed whether ASL from primary hAECs inactivates the causative agents responsible for COVID-19 (betacoronavirus SARS-CoV-2), and a common cold (human alphacoronavirus 229E [CoV-229E]) and then determined the effects of indoor and ambient PM on ASL antiviral activity in vitro.

METHODS

After informed written consent was obtained, human donor lungs, confirmed with polymerase chain reaction to be negative for SARS-CoV-2 infection at the time of tissue retrieval, were dissected to obtain bronchial and tracheal cells. Cells were seeded onto collagen-coated filters at the air-liquid interface by the University of Iowa (UI) Cell Culture Core Repository and then washed to obtain endogenous ASL ($n > 4$ donors/experiment), as described elsewhere [4]. For donor demographics see Supplementary Table 1. This study is approved by the UI Institutional Review Board (no. 199507432).

Washington strain human SARS-CoV-2 and CoV-229E (American Type Culture Collection VR-740) were quantified in Vero E6 cells (kindly provided by Dr Wendy Maury, UI) and MRC-5 cells (American Type Culture Collection CCL171), respectively, as described elsewhere [7]. Both viruses are coronaviruses and share considerable virion and genome structure and organization but represent 2 different genera. Epithelial Vero E6 and MRC-5 cells were maintained at 37°C with 5% carbon dioxide before viral infection (multiplicity of infection, 1), after which they were maintained at 33°C with 5% carbon dioxide. For cell viability and viral binding and entry methods, see the Supplementary Methods.

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SARS-CoV-2 work was performed in the UI biosafety level 3 facility, and CoV-229E studies in UI biosafety level 2 facilities.

Media

Vero E6 and MRC-5 cells were maintained in Dulbecco modified Eagle medium plus 5% fetal bovine serum and minimum essential medium plus 10% fetal bovine serum and 1% penicillin-streptomycin, respectively. Basolateral hAEC medium (1:1 Dulbecco modified Eagle medium/F12 plus 2% Ultroser G, 1% penicillin-streptomycin, and 0.1% gentamicin-fluconazole) was changed biweekly.

PM Determinations

Urban PM SRM 1648a, and NIST Trace Elements in Indoor Dust SRM 2584, hereafter indoor air pollution PM (IAP PM), was derived from the National Institute of Standards and Technology (NIST; https://www.nist.gov/srm) and volcanic ash from the Eyjafjallajökull eruption [8]. Urban PM originated as atmospheric PM, containing polycyclic aromatic hydrocarbons, nitrosubstituted polycyclic aromatic hydrocarbons, polychlorinated biphenyl congeners, and chlorinated pesticides, common in urban air pollution. IAP PM was collected from residences. Finally, we tested Icelandic volcanic ash as a relatively inert PM control condition. To measure the electrokinetic potential in PM colloidal dispersions, we assessed the zeta potential of PM and ASL in triplicate (Zetasizer Nano ZS; Malvern Panalytical), as described elsewhere [9].

To assess the effect of ASL and PM on viral infection, PM was added to vehicle or ASL for 20 minutes, and then virus was added and the mixture applied to permissive cells for 1 hour [10]. After 3–7 days, cells were washed, and viruses were quantified [7, 11]. All experiments were performed in triplicate with vehicle and ASL controls.

Our PM dose was based on a healthy adult resting minute ventilation of 6 L/min (8.64 m^3/24 h) and the US Environmental Protection Agency (EPA) $PM_{2.5}$ standard (35 μg/m^3), totaling 302.40 μg/24 h of exposure. Accounting for the airway tree's internal lung surface area (approximately 2471 cm^2 [12]) and the ASL lung volume (approximately 1 μL/cm^2 in vivo [13]), the airway tree contains approximately 2.47 mL ASL. Applying the 24-hour EPA standard $PM_{2.5}$ exposure (302.40 μg/2.47 mL ASL), the normalized EPA standard $PM_{2.5}$ concentration is 122.4 μg/mL. Therefore, respectively, 50- and 500-μg/mL PM are approximately 40% and 400% the EPA standard normalized to lung surface area and ASL volume. Because urban PM is the most associated with COVID-19 infection and severity, we tested its effects at both doses. Exposure to PM varies widely worldwide [9], so we selected doses representative of different regions.

RESULTS

ASL Inactivation of CoV-229E and SARS-CoV-2

We determined the effect of primary hAEC donor ASL on CoV-229E or SARS-CoV-2 infectivity of epithelial cells by mixing virus with ASL or vehicle before their application onto MRC-5 or Vero E6 cells and then quantifying the virus. ASL reduced CoV-229E and SARS-CoV-2 infection by 28% and 41%, respectively Figure 1A ($P < .001$ and $P = .004$, respectively).

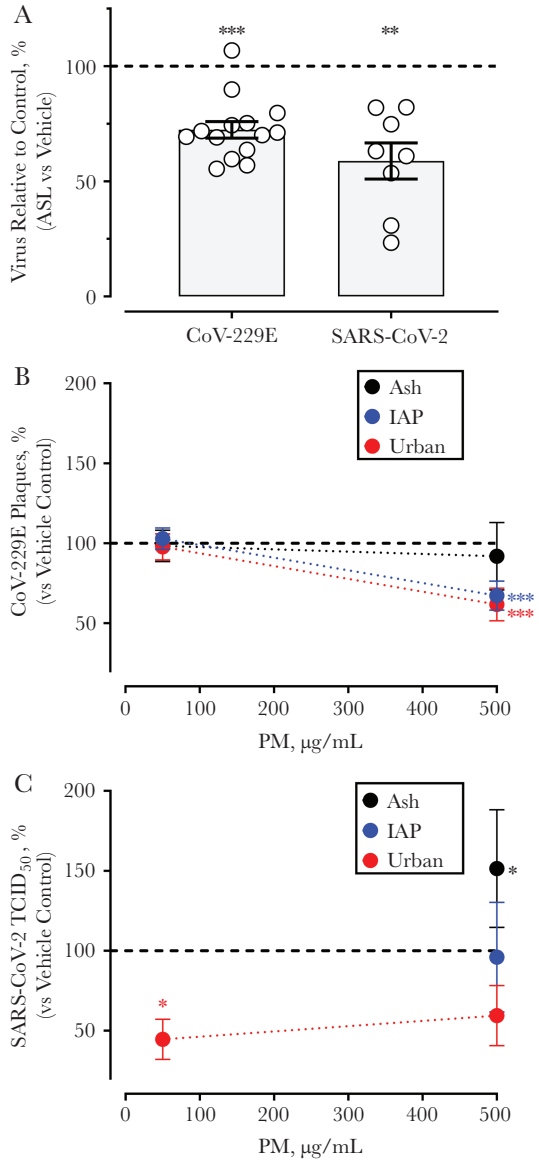

Figure 1. *A,* Percentage of viral human alphacoronavirus 229E (CoV-229E) plaques (*left*) or median tissue culture infectious dose ($TCID_{50}$) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) (*right*) with airway surface liquid (ASL) compared with viruses with vehicle. Dots represent experiments performed on 1 cell passage in triplicate, using freshly obtained ASL from >4 donors; significance was determined using Wilcoxon matched-pairs signed rank test with untransformed data. *B,* CoV-229E plaque formation with particulate matter (PM), compared with CoV-229E plaques with vehicle; significance was determined by comparing triplicate data from 4 experiments per condition, using a 1-sample *t* test with a theoretical mean of 100. *C,* SARS-CoV-2 $TCID_{50}$ with PM versus vehicle; significance was determined by comparing triplicate data from 4 experiments per condition, using a 1-sample *t* test with a theoretical mean of 100. Error bars represent standard errors of the mean. *$P < .05$; **$P < .01$; ***$P < .001$. Abbreviation: IAP, indoor air pollution PM.

Effect of PM on CoV-229E

We then tested whether 50- and 500 μg/mL PM affected CoV-229E infection of MRC-5 cells. At 50 μg/mL, PM had no effect, while 500 μg/mL of IAP PM and urban PM resulted in less CoV-229E infection ($P = .04$ and .03, respectively) and ash had no effect ($P = .73$) Figure 1B. Because PM impaired viral replication, we tested whether it affected MRC-5 and Vero E6 cell viability (Supplementary Methods) and found no effects (Supplementary Figure 2*A* and 2*B*).

Effect of PM on SARS-CoV-2

Urban PM inactivated SARS-CoV-2 ($P = .02$ for 50 and $P = .06$ for 500 μg/mL) (Figure 1C). Contrary to results with CoV-229E, 500-μg/mL ash increased SARS-CoV-2 infection, while IAP PM had no effect ($P = .03$ and $P = .54$, respectively) (Figure 1C).

Effect of PM on ASL Inactivation of CoV-229E

We tested whether PM affected ASL anti–CoV-229E activity by applying 50- and 500-μg/mL PM or vehicle to ASL for 20 minutes and then incubating the mixture with virus and quantifying infectious virus by means of plaque assay. Compared with baseline ASL viral inactivation, 500-μg/mL urban PM resulted in significantly more CoV-229E plaques ($P < .001$), while IAP PM and ash had no significant effect (Figure 2A). We then assessed whether PM physically interfered with the ability of the virus to infect cells by quantifying CoV-229E binding and entry in MRC-5 cells (Supplementary Methods). Including PM in media did not lead to significant differences in viral binding or entry compared with vehicle control (Supplementary Figures 3 and 4*A*–4*C*).

Effect of PM on ASL Inactivation of SARS-CoV-2

Urban PM affected both viral viability and impaired ASL CoV-229E inactivation. We therefore probed whether it affected ASL inactivation of SARS-CoV-2 at both doses, and we tested IAP PM and ash at 500 μg/mL. In the presence of both PM and ASL, there was increased SARS-CoV-2 infection compared with either alone; however, only ash (500 μg/mL) significantly increased SARS-CoV-2 infection (Figure 2B).

Finally, we tested whether PM's electrostatic state affected its affinity to AMPs within the ASL and found that the zeta potential of each PM was significantly more negatively charged than the ASL ($P < .001$; −17, −15, and −13 mV, respectively). Nevertheless, there were no significant differences by particle type (Supplementary Figure 5); thus, charge alone is unlikely to explain the antiviral effect of PM or its inhibitory effect on ASL.

DISCUSSION

It is established that ASL can be antimicrobial, and this is often mediated by AMPs [1, 2, 4]. Our findings are the first to demonstrate that primary human airway ASL significantly inactivates CoV-229E and SARS-CoV-2 in vitro (Figure 1A). Therefore, ASL likely contributes to innate immune protection against coronavirus infection.

Epidemiological studies demonstrate an association between urban PM air pollution and the incidence of viral respiratory infection (influenza and influenzalike illness), hospitalizations (respiratory syncytial virus and pneumonia), and mortality rates (SARS), including increased risk of COVID-19 incidence and severity [5, 14]. After establishing that ASL was antiviral against both coronaviruses, we asked 2 distinct research questions. First, in isolation, does PM affect CoV-229E and SARS-CoV-2 infection of epithelial cells? Second, does PM impair ASL-mediated viral inactivation of CoV-229E and SARS-CoV-2?

Urban PM independently reduced CoV-229E and SARS-CoV-2 infection of epithelial cells. IAP PM (500 μg/mL) also decreased CoV-229E infection, but not SARS-CoV-2 infection.

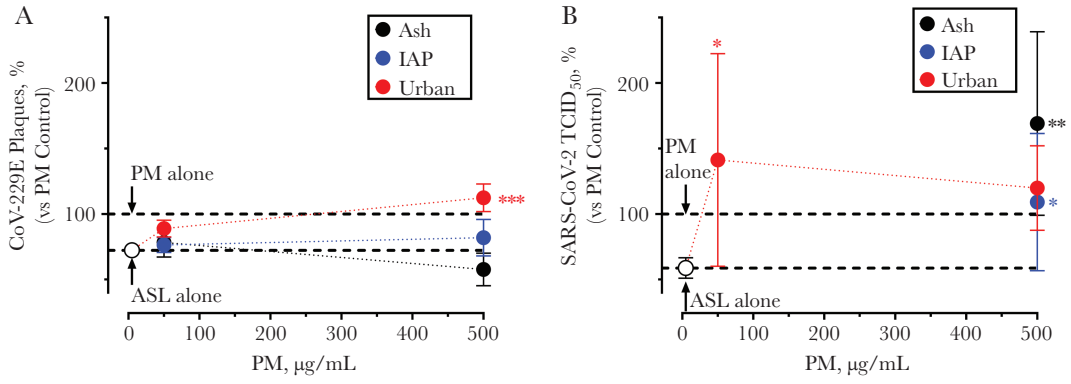

Figure 2. *A,* Percentage viral human alphacoronavirus 229E (CoV-229E) plaque formation with airway surface liquid (ASL) combined with 50- or 500-μg/mL particulate matter (PM), normalized to PM control; significance was determined by comparing triplicate data (per condition from 3 experiments) with ASL control data, using 1-way analysis of variance (ANOVA). *B,* Percentage viral severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) median tissue culture infectious dose ($TCID_{50}$) with ASL combined with 50-or 500-μg/mL PM, normalized to PM control; significance was determined by comparing triplicate data (per condition from 3 experiments) with ASL control data, using 1-way ANOVA when 2 doses were tested or unpaired *t* test for single doses (IAP, ash). Error bars represent standard errors of the mean. *$P < .05$; **$P < .01$; ***$P < .001$. Abbreviation: IAP, indoor air pollution PM.

Distinctly, volcanic ash had no effect on CoV-229E epithelial cell infection but increased SARS-CoV-2 infectivity (500 μg/mL) (Figure 1B and 1C), potentially owing to "viral hitchhiking" on ash PM. These findings demonstrate that different sources of PM differentially affect viral infection.

The addition of urban PM to ASL led to significantly more CoV-229E (at 500 μg/mL) and SARS-CoV-2 (at 50 μg/mL) infections, relative to untreated ASL (Figure 2A and 2B). Adding IAP PM and ash to ASL did not significantly affect CoV-229E infection, but each impaired anti–SARS-CoV-2 ASL activity (500 μg/mL) (Figure 2A and 2B). These data demonstrate that PM decreases the efficacy of ASL against coronaviruses. They also reveal that while both PM and ASL are antiviral, effects are not additive. Delineating the percentage of viral inactivation contributed by each component is not feasible. as it would require quantification of all ASL proteins and free of bound PM for any given assay.

We expected the PM charge state to affect viral viability. While we did not find differences between charge state in the PM tested (Supplementary Figure 5), viral stability and adsorption are affected by many factors, including pH, humidity, temperature, and surface conditions, and the role of each of these factors in the context of ambient PM is underexplored in SARS-CoV-2 [15]. The complexity of PM, including metal content, charge, size, and conformation, may explain the differences we observed.

Strengths of the current study include the use of primary human donor hAEC-derived ASL to examine airway immune function with a reductionist approach, the use of common air pollutants (urban/indoor PM), and similarity between ASL findings despite the use of 2 discrete human coronaviruses propagated in distinct epithelial cell lines (lung and kidney) from 2 organisms (human and monkey). Study limitations include the narrow mechanism tested (infection) and the limited PM sources. For example, we cannot speculate on PM composition from rural environments, which may influence the severity of COVID-19.

Although we were not able to measure outcomes such as infection severity, other studies have reported increased viral circulation, infections, length of hospital stay, illness severity, and mortality rates for influenza, H1N1, SARS, and rhinovirus, owing to PM and its associated air pollution exposures [14]. Furthermore, we strictly examined ASL viral inactivation and the effect of PM without examining the role of macrophages or neutrophils or the effect of PM on intracellular antiviral pathways. Nevertheless, our in vitro findings provide a rationale to further examine the effects of air pollution PM on viral replication in vivo. Future directions include translational research to validate in vitro results using an in vivo model and identification of specific physicochemical characteristics in PM responsible for ASL antiviral impairment. Our in vitro data provide new insights into how PM exposures may influence respiratory viral infections and thus may inform future PM exposure policy discussions.

Our data suggest that PM influences coronavirus infectivity via at least 2 distinct mechanisms. PM decreases viral viability and impairs ASL viral inactivation. Urban PM consistently inactivated both coronaviruses, thereby decreasing ambient viral titers before inhalation. Once urban PM was introduced to ASL, it consistently inhibited the antiviral effect of ASL, while IAP PM and ash had differential effects, depending on the virus studied. These results support epidemiological observations concerning urban air pollution and viral infection. In addition, IAP PM and ash results indicate that air pollution from other sources may affect the risk of viral infection. To our knowledge, this is the first report of viral inactivation by primary human ASL and the first to report its inhibition by PM in vitro.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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