MARCH 23, 2021

Tweet

expert reaction to lab study looking at respiratory cells infected with SARS-CoV-2 in the presence or absence of rhinovirus infection

A lab study published in the *Journal of Infectious Diseases* suggests that infection with human rhinovirus, the virus that causes the common cold, might provide some level of protection against COVID-19.

Prof Lawrence Young, Professor of Molecular Oncology, Warwick Medical School, said:

"This laboratory study clearly shows that infection with a human common cold virus (rhinovirus or HRVs) can block the growth of the SARS-CoV-2 virus, at least in cells in the lab. As HRVs are the most frequent cause of the common cold and are highly transmissible, this study suggests that this common infection could impact the burden of COVID-19 and influence the spread of SARS-CoV-2 particularly over the autumn and winter months when seasonal colds are more frequent.

"The study uses human bronchial cells infected with the viruses in tissue culture and shows that HRV infection significantly inhibits the replication of SARS-CoV-2. This effect was observed irrespective of whether the viruses are used as simultaneous co-infections or infections were staggered e.g. SARS-CoV-2 infection followed 24 hours later by HRV infection. The study also showed that this inhibitory effect was due to HRV inducing robust activation of the interferon-mediated innate immune response. The interferon response induced by SARS-CoV-2 infection is much lower and weaker.

"Finally, the study modelled the possible impact of this effect on COVID-19 cases in the population concluding that the number of new SARS-CoV-2 infections would decrease as the number of HRV infections increase – an effect that could restrict the spread of SARS-CoV-2.

"This is an interesting study that highlights the need for us to understand more about the biology of SARS-CoV-2 infections and how co-infections with other respiratory viruses (e.g. flu) might affect COVID-19. It also stresses the importance of the interferon response in controlling SARS-CoV-2 replication, supporting current clinical trials that are exploring the therapeutic benefits of interferon treatment in COVID0-19 patients."

said:

"This is a good in vitro study using human airway epithelial cells in a culture system designed to mimic in vivo conditions. The authors show that rhinovirus infection can limit the infection of SARS-CoV-2 and suggest the mechanism is through induction by rhinovirus of innate mediators. Although the proof of this mechanism is somewhat limited.

"The major limitation of the study is that it is performed with just one strain of rhinovirus, there are at least 160, and there are no guarantees that each rhinovirus strain would have the same effect on SARS-CoV-2 infections. They also do not fully prove the induction by interferon is responsible here. Lastly, translating this to the situation in real life is very tricky. Although it is likely that a common cold virus such as rhinovirus would induce a strong innate immune response that could block SARS-CoV-2 infections, it would still require both infections to occur at a similar time. In addition, with all the non pharmaceutical interventions that have been in play over the past year it is not only limiting SARS-CoV-2 transmissions but any respiratory virus. Therefore, with reduced frequencies of circulating common colds in the community, as we know has happened, there would likely be less effect and chance of this innate protection inhibiting SARS-CoV-2."

Prof Ian Jones, Professor of Virology, University of Reading, said:

"The seasonality of respiratory viruses is well known but even in the winter months of temperate climates, like the UK, different viruses peak at different times suggesting they jostle with each other for their slot in the human respiratory tract. This study formally shows that infection of human respiratory cells by the common cold virus releases interferon and sets up an antiviral state which then resists infection by SARS-CoV-2. Effectively, one virus pushes out another. The data support the limited trial data available for interferon nasal sprays which have shown some ability to improve Covid outcomes. However, the great problem for any prophylactic is when to give it and how long to give it for and although interesting the practical application of the data described is hard to see."

Dr Julian Tang, Honorary Associate Professor/Clinical Virologist, University of Leicester, said:

"Interesting paper – which potentially touches on lots of phenomena.

"Virologists already know about the 'viral interference' between rhinoviruses and seasonal influenza – innate host immune responses (including interferon) induced by rhinoviruses can reduce the successful infection of influenza in humans.

"The fact that rhinoviruses may also 'interfere' with SARS-COV-2 infection is intriguing and needs further confirmation.

"One weakness of the study is that their in vitro viral culture system does not include the presence of cross-reactive, potentially cross-protective antibodies from other common cold coronaviruses (OC43, 229E, NL63, HKU1) – that may also inhibit successful SARS-COV-2 infection in humans.

smc

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3553067/ – and it is not clear from this study alone if all these different rhinovirus serotypes have the same degree of 'viral interference' effect on SARS-COV-2 infection.

"Some data on the dominance of rhinovirus amongst the seasonal respiratory viruses during the COVID-19 pandemic has been published already from Australia and New Zealand: https://www.nature.com/articles/s41467-021-21157-9 and https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.47.2001847?crawler=true and the UK PHE surveillance data shows something similar: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/9712…19_report_w11_v2.pdf

"Intriguingly, if you look at Figures 13 (showing SARS-COV-2 %positivity) and 14 (showing rhinovirus %positivity) over the same time period (weeks 35, 2020 to week 10, 2021), you might see a lower incidence of SARS-COV-2 at the peak rhinovirus incidence, which then increases during the Christmas/New Year surge, as the rhinovirus incidence starts falling. But a counter argument to this could also be that if rhinovirus was truly interfering with SARS-COV-2 infections, then there should not be a rise in SARS-COV-2 %positivity during weeks 39-43 when rhinovirus %positivity was also peaking.

"Further study is needed here as in real life situations the rhinovirus interference effect may have a more complex and multiphasic time-dependent impact on human (rather than in vitro) SARS-COV-2 infections.

"Finally, many of these seasonal coronaviruses and rhinoviruses will start to infect children from birth and may induce this innate immune 'interference' effect – to reduce/modify the SARS-COV-2 infection rates and severity of COVID-19 symptoms that we are seeing in children of different ages."

'Human rhinovirus infection blocks SARS-CoV-2 replication within the respiratory epithelium: implications for COVID-19 epidemiology' by Kieran Dee *et al.* was published in the *Journal of Infectious Diseases* at 00:01 UK time on Tuesday 23 March 2021.

Declared interests

Prof Gary McLean: "No conflict of interest to declare."

Prof Ian Jones: "No conflicts."

None others received.

Human rhinovirus infection blocks SARS-CoV-2 replication within the respiratory epithelium: implications for COVID-19 epidemiology

Summary: Human rhinovirus triggers an innate immune response that blocks SARS-CoV-2 replication within the human respiratory epithelium. Given the high prevalence of human rhinovirus, this interference effect might cause a population-wide reduction in the number of new COVID-19 infections.

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Abstract: Virus-virus interactions influence the epidemiology of respiratory infections. However, the impact of viruses causing upper respiratory infections on SARS-CoV-2 replication and transmission is currently unknown. Human rhinoviruses cause the common cold and are the most prevalent respiratory viruses of humans. Interactions between rhinoviruses and co-circulating respiratory viruses have been shown to shape virus epidemiology at the individual host and population level. Here, we examined the replication kinetics of SARS-CoV-2 in the human respiratory epithelium in the presence or absence of rhinovirus. We show that human rhinovirus triggers an interferon response that blocks SARS-CoV-2 replication. Mathematical simulations show that this virus-virus interaction is likely to have a population-wide effect as an increasing prevalence of rhinovirus will reduce the number of new COVID-19 cases.

Keywords: SARS-CoV-2; Rhinovirus; Virus-virus interactions.

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Footnote page

PRM owns shares of Astra Zeneca. The rest of the authors declare no conflicts of interest.

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Background

The rapid spread of COVID-19 and its impact on global health highlights the importance of viral respiratory diseases. The human respiratory tract hosts a community of viruses that includes members of the *Orthomyxoviridae* (e.g., influenza virus A and B), *Pneumoviridae* (e.g., respiratory syncytial virus), *Picornaviridae* (e.g., rhinovirus), *Coronaviridae* (e.g., severe acute respiratory syndrome coronavirus 2) and others [1, 2]. We and others showed that interactions between co-circulating, taxonomically different respiratory viruses, can influence patterns of infection [3, 4]. We showed that human rhinoviruses (HRVs) and influenza A viruses (IAVs) interact negatively at the individual patient and population level. Additionally, it has been postulated that the circulation of HRV delayed the spread of pandemic H1N1 influenza virus in France in 2009 [5]. Viral interference interactions at the host level are considered important in influencing observed population dynamics. Wu et al. demonstrated that HRV induces an interferon (IFN) response that protects against subsequent IAV infection in differentiated airway cultures [4], whereas Gonzalez et al. showed that RV attenuates influenza severity in a mouse model [6].

Non-pharmacological interventions have hampered our ability to determine the impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on the epidemiology of respiratory viruses. However, it is possible that the emergence of SARS-CoV-2 will affect their ecology. Co-infection studies using air-liquid interface cultures of differentiated respiratory epithelial cells can shed light on the nature of SARS-CoV-2 interactions with other viruses and their effect on virus replication. Here, we examined the replication kinetics of SARS-CoV-2 in the presence of HRV in the human respiratory epithelium. HRV was selected due to i) its high prevalence in the human population [7]; ii) its negative interaction with IAV at the host and population level [3, 4]; iii) its ability to induce a strong IFN response [4]; and iv) the sensitivity of SARS-CoV-2 to IFN [8]. We used our experimental results as a proxy of within-host coinfection dynamics to simulate the impact of HRV circulation on the epidemiology of SARS-CoV-2 under different scenarios of HRV prevalence.

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Methods

Cells

Primary human bronchial epithelial cells (HBEC) were sourced from Epithelix Sarl (Geneva, Switzerland). Cells were maintained and seeded on transwells (Cell culture inserts, Falcon® Cat. No.: 734-0036) using Epithelix hAEC media (Epithelix, EP09AM) and incubated at 37oC with 5% CO2. An air-liquid interface (ALI) was initiated once they reached confluency, when the maintenance media was switched to Pneumacult^{TM}-ALI media (Cat. No.: 05001, STEMCELL Technologies). Vero E6 F5 cells were subcloned from Vero E6 cells, which were a gift from Prof. Michele Bouloy. A bulk population of VeroE6 cells was diluted in DMEM supplemented with 10% (v/v) foetal calf serum to 1 cell per 100 ul and plated into a 96 well format and incubated at 37C in a 5% CO2, humidified incubator. Wells were assessed for cell number with 0 and 3 cells/well observed. Once the population had expanded, each clonal population was further seeded into a single well in a 96 well-plate. The next day, the plate was infected with 8400 pfu/well of SARS-CoV2 and left for 72 hours. The plates were fixed in 8% (w/v) formaldehyde in PBS and stained with Coomassie brilliant blue (0.1% [w/v] Coomassie Brilliant Blue R-250; 45% [v/v] methanol; 10% [v/v] glacial acetic acid) and assessed for cytopathic effect. Plates were scanned using a using the Celigo platform (Nexcelcom). Infection of 3 of 288 clones resulted in the clearance of the monolayer (2H6, 5F3 and 6F5). These clones were further assessed for changes in plaque morphology, and whether the well-clearance assay generated representative titers. They were further assessed for growth characteristics. Two of the three clones were discarded due and underestimate of virus titer (2H6) and longer mean generation time of the cells (5F2) in comparison the bulk population of VeroE6. HeLa Ohio cells were a gift from Dr. Toby Tuthill (The Pirbright Institute). Both cell lines were grown in Dulbecco's Minimum Essential Media (DMEM), high glucose, GlutaMAX supplemented with 10% fetal bovine serum (FBS) and 1% non-essential amino acids (NEAA).

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Viruses

SARS-CoV-2 strain HCoV-19/England/02/2020 was sourced from Public Health England (GISAID accession: EPI_ISL_407073) originating from a clinical isolate and was passaged twice in VeroE6 cells. HRV-A16 was sourced from the American Type Culture Collection (ATCC) (ATCC VR-283).

Infection of HBEC cultures

Infection of HBECs. HBEC cultures were infected at ≥ 35 days post ALI initiation. The apical surface of the cultures was washed twice with serum free DMEM before infection (24 hours prior to infection and immediately before infection). Cells were inoculated with 10^4 PFU of either SARS-CoV-2 or HRV-A16, or a mixture containing 10^4 PFU of each virus and incubated at 37°C for 120 minutes. Previous experiments showed that inoculation of ALI cultures with 10,000 plaque forming units (PFU) resulted in consistent replication of HRV and SARS-CoV-2 [9]. The inoculum was removed, and cultures were washed once. This wash was titrated by 50% tissue culture infectious dose ($TCID_{50}$) assay and served as the 0-hour time point for growth curves. Cells were incubated at 37°C with 5% CO_2. At each time point, serum free DMEM was added apically to each culture and incubated for 30 minutes at 37°C. This was removed, aliquoted and stored at -80°C prior to subsequent titration. Each infection was carried out in two independent experiments and each experiment consisted of at least three technical replicates. Titrations of SARS-CoV-2 and HRV-A16 were performed on Vero E6 6F5 and HeLa OH cells, respectively. Virus samples were titrated in ten-fold serial dilutions in DMEM with 2% FBS and 1% NEAA on confluent monolayers of cells. Each sample was titrated in triplicate. SARS-CoV-2 $TCID_{50}$ plates were incubated at 37°C and HRV-A16 plates were incubated at 33°C. Plates were incubated for approximately 72 hours and fixed in 8% formaldehyde and stained with 0.1% Coomassie Brilliant Blue. Cytopathic effect was recorded, and a TCID50/ml titre determined as calculated by the Spearman and Kärber algorithm [10]. For BX795 experiments, ALI cultures were transferred to

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Pneumacult™-ALI media containing 6 uM of BX795 (or DMSO) 18 hours prior to infection and media were changed daily. All experimental infections were carried out under Biosafety Level 3 conditions.

Tissue processing and immunostaining

After fixation in 8% formaldehyde for 16-24 hours, HBEC cultures were processed overnight for paraffin-embedding, sectioned to 2-3 µm-thick sections and mounted on glass slides. Two sections for each condition were sectioned and processed using pH 8 EDTA antigen retrieval and permeabilized with 1% triton. DAPI (Thermo Fisher Scientific, cat# P36392) was included in the mounting medium, and slides were stained with primary sheep anti-N (nucleocapsid) IgG antibody (DA114, mrcppu-covid.bio, 1: 1000 dilution), primary mouse anti-MxA antibody [11], primary mouse anti-VP2 antibody (QED Bioscience Ltd. – 18758), or a primary rabbit-anti-hACE2 (Cell Signalling Technology) antibody. For immunofluorescence, primary antibodies were detected using an AlexaFluor 555-conjugated donkey anti-sheep antibody (A11015, Thermo Fisher Scientific, 1:1000 dilution) and an AlexaFluor 488-conjugated goat anti-mouse (Sigma SAB4600056, 1:1000 dilution). For immunohistochemistry, anti-hACE2 was detected using EnVision+ anti-rabbit HRP (Agilent K4003). IF sections were imaged using a Zeiss LSM880 confocal microscope and IHC sections were imaged with an Olympus BX51 microscope.

Statistical analysis and data visualisation

Statistical analysis and data visualisation were carried out in R 3.5.1 [12]. Multivariable logistic regression models were used to investigate significance among the different conditions. Those models accounted for biological replicates as this parameter was uneven, as well as treatment, and time post-infection. When biological replicate was not a significant parameter, this latter was removed to simplify the model. Models were run using the lme4 package [13]. Data visualisation and figures were generated using ggplot2 package [14].

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Results

To determine if SARS-CoV-2 and HRV interact within the human respiratory epithelium, we infected air-liquid interface (ALI)-cultures of human bronchial epithelial cells (HBECs) with either SARS-CoV-2, HRV or with both viruses simultaneously. To assess the impact of coinfections on the replication kinetics of each virus, HRV and SARS-CoV-2 titers were determined at different times post infection from apical washes of coinfected cells and compared to their respective titers from single virus infections. SARS-CoV-2 exhibited highly contrasting replication kinetics in single and coinfections (p= 0.03928, Figure 1A). SARS-CoV-2 titers increased slowly from 24 hours post-infection (hpi) onwards and up to 96 hpi in single infections, whereas in coinfections with HRV, SARS-CoV-2 titers decreased rapidly and were undetectable at 48 hpi (Figure 1A). In contrast, HRV titers displayed the same kinetics in single and coinfections: they increased rapidly during the first 24 hours, followed by a gradual and sustained decline (Figure 1B). As simultaneous coinfections might not occur frequently during natural infection, we performed staggered coinfections of ALI-cultures of HBECs as follows: cells were infected with HRV, and 24 hours later they were infected with SARS-CoV-2. This experiment was also repeated in the reverse order (i.e., SARS-CoV-2 first, followed by HRV). As observed in simultaneous coinfections, SARS-CoV-2 growth was severely impaired in both staggered coinfections: when SARS-CoV-2 inoculation was followed by HRV infection (p= 0.0260) SARS-CoV-2 replication increased between 24 and 48 hpi as seen in SARS-CoV-2 single infection, but a subsequent sharp decrease was observed between 48 and 96 hpi (Fig, 1C). When HRV inoculation was followed by SARS-CoV-2 infection, SARS-CoV-2 replication did not exceed the inoculum titer and viral titers quickly declined (p= 0.0063) (Figure 1D). In contrast, the growth of HRV was unaffected by SARS-CoV-2 (p= 0.2027) regardless of the sequence order of infections (Figure 1C and 1D). When SARS-CoV-2 was inoculated first, the growth curve of HRV shifted and peaked at 72 hpi (Figure 1C), reflecting the delay in HRV inoculation. We tested if the observed reduction of SARS-CoV-2 titers was due to a block in virus entry due to HRV-

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induced downregulation of the SARS-CoV-2 receptor, ACE2 [15]. To this end, we used immunohistochemistry to detect ACE2 in HRV or SARS-CoV-2 single infected and coinfected epithelial cells. We observed high levels of ACE2 expression on the apical surface of the epithelium regardless of the infection status of the cells (Figure S1) suggesting that HRV blocks SARS-CoV-2 infection via mechanisms that are independent of virus entry.

SARS-CoV-2 is susceptible to IFN and encodes multiple genes that alter signaling pathways upstream and downstream of IFN production [8]. As HRV induces an interferon-mediated innate immune response that blocks IAV in ALI-cultures [4] we hypothesized that the observed block in SARS-CoV-2 replication was due to an HRV-triggered IFN response. To test this, we used fluorescence microscopy to examine the IFN-mediated innate immune activation induced by each virus. Specifically, we compared the *in situ* expression of MxA, a protein encoded by an IFN-stimulated gene that is highly upregulated upon IFN production [11]. Figure 2 shows that ALI-cultures of HBECs infected with HRV express high levels of MxA, contrasting with the low levels of MxA observed in SARS-CoV-2 infected cultures. Coinfected cultures exhibited high levels of MxA expression, similar to those exhibited in single infections with HRV (Figure 2). We further performed immunofluorescence using antibodies directed against the nucleocapsid (N) of SARS-CoV-2 and observed that N expression is clearly detected mainly on the apical area of epithelial cells subject to single SARS-CoV-2 infection, but undetectable in co-infected cells (Figure 3). Overall, our combined experiments confirmed i) that SARS-CoV-2 replication within the ALI-cultures of HBECs does not progress in the presence of HRV and ii) that HRV triggers a faster and likely stronger IFN response compared to SARS-CoV-2. We therefore hypothesized that the block observed in SARS-CoV-2 replication was due to an innate immune response triggered by HRV. To test this, we performed HRV/SARS-CoV-2 coinfections in the presence of BX795, an inhibitor of TANK-binding kinase 1 that has been shown to block the IFN-mediated innate immune response in differentiated cultures of respiratory epithelium [4]. In the presence of BX795, the ability of SARS-CoV-2 to replicate in the respiratory epithelium is

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restored to comparable levels to SARS-CoV-2 single infection, despite the presence of HRV (Figure 4A). This confirms that the observed block in SARS-CoV-2 replication in coinfections with HRV was the result of negative interactions driven by the innate immune response induced by HRV. Interestingly, HRV replication was also increased in the presence of BX795 and titers plateau between 48 and 96 hpi, rather than declining as observed in the DMSO control coinfection and HRV single infection (Fig 4B). This indicates that virus-induced innate immune signaling also hampers HRV replication in HBECs.

Given the high prevalence of HRV, we wanted to test if the observed within-host interference could have an impact on the number of new COVID-19 cases in the population. We performed mathematical simulations using the moment generating function equation [16] to derive the change in the growth rate of SARS-CoV-2 infections as a result from having a fraction of the population refractory to COVID-19 due to an episode of HRV infection (Data analysis S1 in Supplementary Material). Our results show that the number of new SARS-CoV-2 infections decreases as the number of HRV infections increase, and this reduction increases with higher HRV prevalences and longer duration of the interference effect (Figure 5). When SARS-CoV-2 growth rates are low, HRV circulation can lead to SARS-CoV-2 infections not spreading, whereas exponential growth is expected in the absence of HRV.

Discussion

Respiratory explants and ALI-cultures of human airway epithelium provide a highly controlled cellular environment that mimics to a considerable extent the natural site of infection and thus enables us to model the impact of virus tropism and innate immune responses on within-host infection dynamics [17]. Here we showed that HRV infection impairs SARS-CoV-2 replication and spread within the human respiratory epithelium. Our study shows that HRV exerts an indirect negative interaction, with a dominant inhibitory phenotype against SARS-CoV-2. Specifically, we showed that HRV triggers an IFN response that makes most cells nonpermissive to SARS-Cov-2 infection, while HRV is unaffected by the presence of SARS-

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CoV-2. The susceptibility of SARS-CoV-2 to the IFN response is illustrated by the number of genes present in its genome that are devoted to overcome the innate immune response (reviewed in [18]). We also showed that HRV hampers SARS-CoV-2 replication even when the former was inoculated 24 hours after SARS-CoV-2. Overall, our results demonstrate that viral interference interactions induced by HRV infection can inhibit SARS-CoV-2 replication in the respiratory epithelium and builds on previous epidemiological, modelling, and experimental work on virus-virus interactions [3-5, 19]. Future studies to elucidate the molecular mechanisms of viral interference could enable us to wield virus-virus interactions to our advantage and use them as control strategies or therapeutic measures. For example, screening for HRV-induced genes with anti-SARS-CoV-2 activity might constitute a future research avenue to develop antiviral therapies against coronaviruses.

Recently, Wu et al. [4] showed that the IFN response triggered by HRV also interferes with IAV replication. Our combined studies suggest that viruses that stimulate an IFN response in the respiratory epithelium might interfere with SARS-CoV-2 and IAVs. These findings have important implications, as they suggest that immune-mediated effects induced by mild, common cold virus infections, including HRV, might confer some level of protection against SARS-CoV-2, potentially attenuating the severity of COVID-19. Given the high transmissibility and prevalence of HRV, this effect might have an impact on the disease burden caused by COVID-19 at the population scale, with expected heterogeneities depending on HRV prevalence among different age groups. For example, this interference effect can contribute to differences in SARS-CoV-2 transmission between school-aged children (with high prevalence of HRV) and adult populations (with comparatively lower HRV prevalence).

Viruses are obligate intracellular pathogens that can only infect a restricted number of cell types within the body (a property known as tropism). Virus-virus interactions are likely to occur not only in the respiratory tract but also in other tissues that support multi-virus environments, such as the gastrointestinal tract, where enteric infections are modulated by

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the gut virome [20] and also affect the immunogenicity of the live attenuated polio vaccine [21]. The nature of such interactions (i.e., positive, negative, or neutral) is largely unknown and likely to be influenced by the specific viruses involved, the timing of each infection and the interplay between the host's response to each virus.

There is a vast body of knowledge on the impact of evolution on virus-host interactions [22-25]. Many studies have focused on the evolutionary arms race between viruses and hosts, where the host's immune system evolves antiviral mechanisms to stop viral replication and viruses evolve to evade antiviral proteins. We propose that virus-virus interactions influence this arms race and contribute to shaping their molecular interplay. For example, it is feasible to think that HRV infections in humans might be mutually beneficial: from a HRV perspective, humans evolved a tightly regulated immune response that allows HRV to replicate and transmit while it blocks other potentially competing viruses. From a host's perspective, HRV infections, which are usually associated with mild disease, stimulate an antiviral response that prevents infections by more severe (and sometimes lethal) viruses such as SARS-CoV-2 and IAV. Future studies using co-infections are needed to shed light on the role of ecology and evolution on virus-virus interactions and their impact on virus host range, transmission and disease.

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Figure legends

Figure 1. Replication kinetics of SARS-CoV-2 and HRV in ALI-cultures of HBECs. (A) SARS-CoV-2 titers in single SARS-CoV-2 infections (solid red line) and simultaneous SARS-CoV-2/HRV coinfections (dashed red line). (B) HRV titers in single HRV infections (solid cyan line) and simultaneous SARS-CoV-2/HRV coinfections (dashed cyan line). (C & D) SARS-CoV-2 (red) and HRV (cyan) titers in single infections (solid lines) and staggered SARS-CoV-2/HRV coinfections (dashed lines). The order of infections is described below each graph. SARS-CoV-2 is shown in red and HRV is shown in cyan.

Figure 2. MxA expression in ALI-cultures of HBECs. Representative images of MxA expression by fluorescence microscopy at various times post infection. ALI-cultures were mock infected, infected with SARS-CoV-2 only, HRV only, and coinfected with SARS-CoV-2 and HRV. Nuclei are colored in blue and MxA is colored in magenta. The scale bar indicates 50 μm.

Figure 3. Detection of SARS-CoV-2 in ALI-cultures of HBECs. Representative images of SARS-CoV-2 N detection by immunofluorescence in cells infected with SARS-CoV-2 (A); co-infected with SARS-CoV-2 and HRV (B); or mock infected (C). Nuclei are colored in blue and SARS-CoV-2 N protein is colored in red. The scale bar indicates 50 μm.

Figure 4. Replication kinetics of SARS-CoV-2 and HRV in ALI-cultures of HBECs coinfected simultaneously with SARS-CoV-2 and HRV in the presence or absence of BX795. (A) SARS-CoV-2 titers. (B) HRV titers. SARS-CoV-2 is shown in red and HRV is shown in cyan. Solid and dotted lines show infections in the presence of absence of BX795, respectively.

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Figure 5. Reduction in COVID-19 growth rate for varying prevalence of rhinovirus infections in a given population and different assumptions for the duration of the refractory period. The growth rate in the absence of rhinovirus is assumed to be a 5% increase/day. Colors show the reduction in growth rate expressed as percentage.

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Figure 1

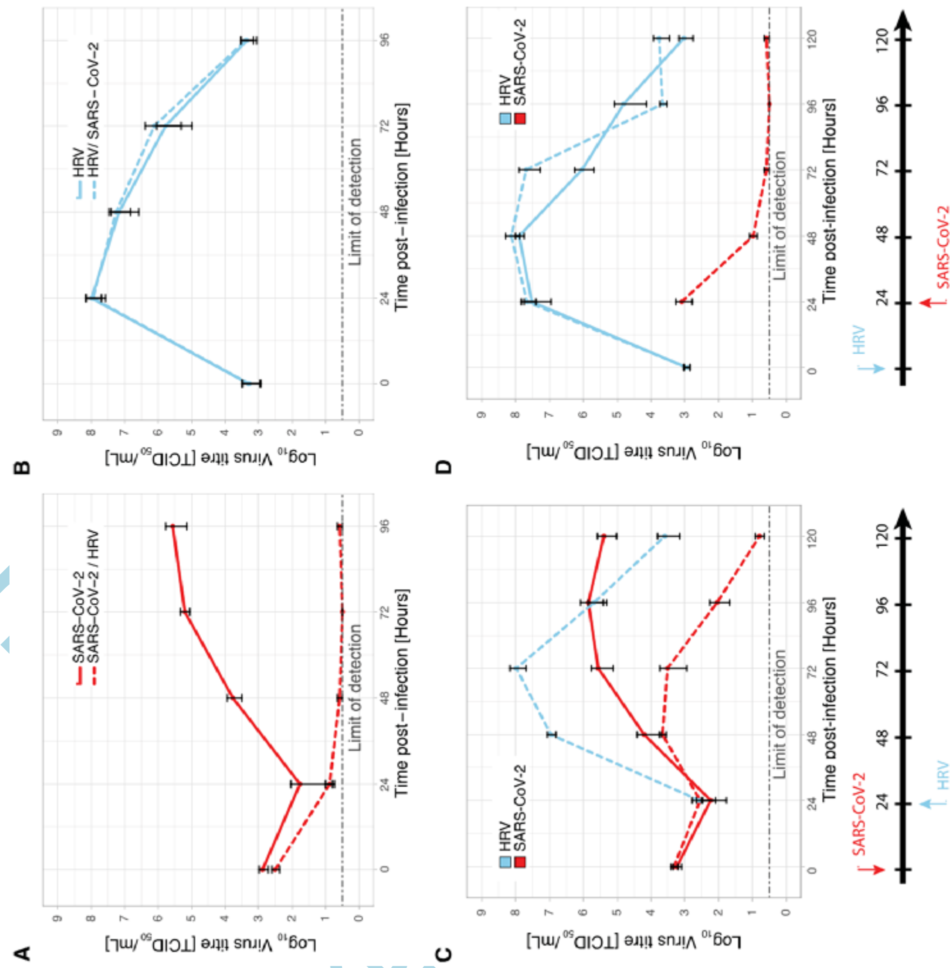

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Figure 2

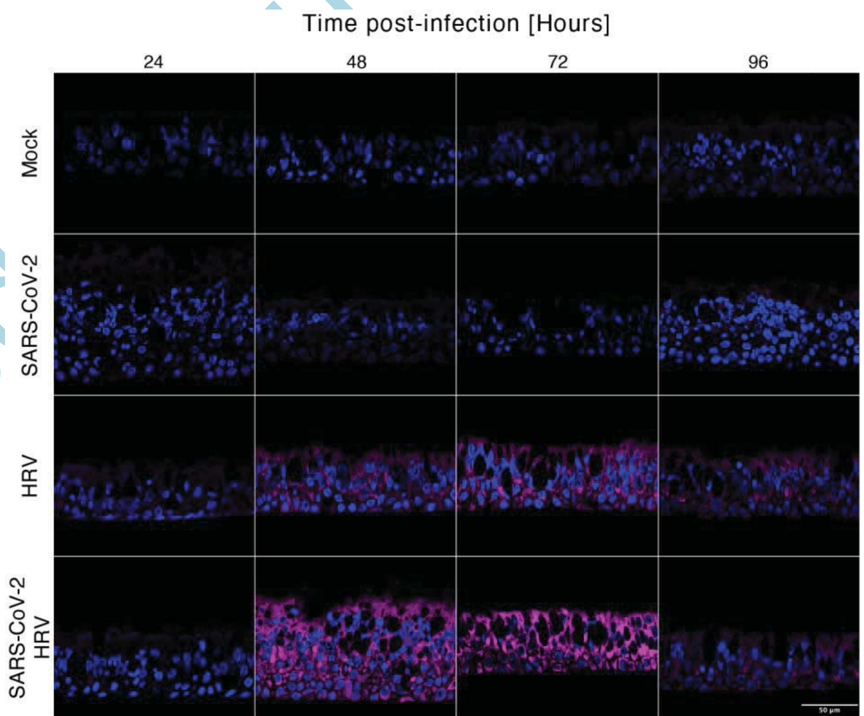

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Figure 3

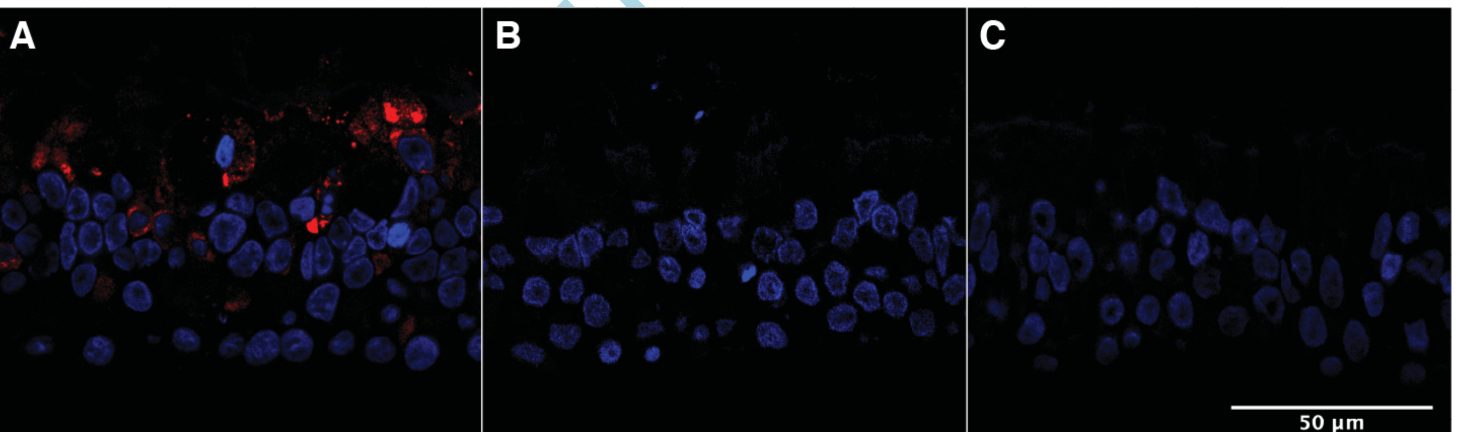

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Figure 4

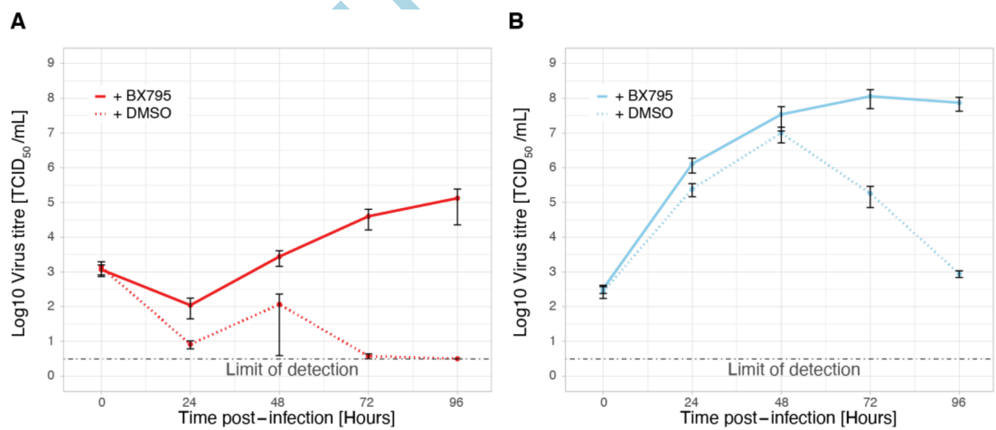

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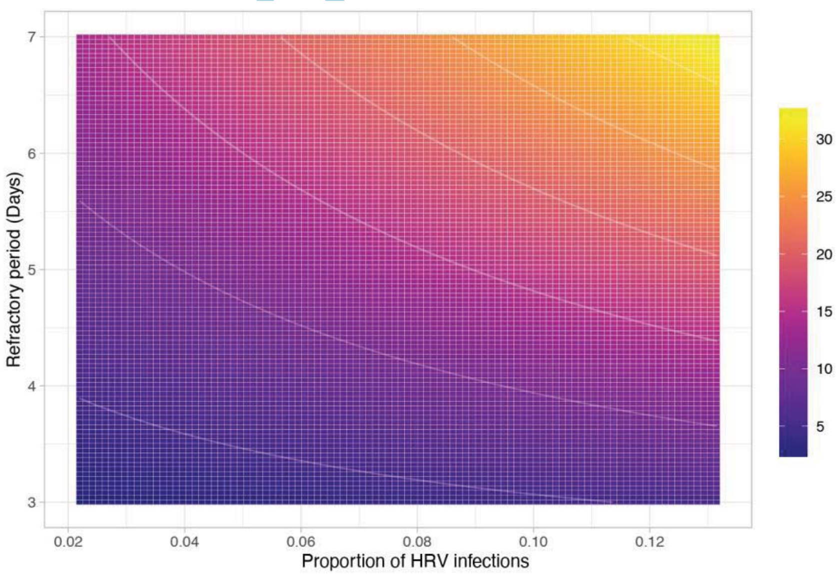
Figure 5



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CONCISE COMMUNICATION

Rhinovirus and Coronavirus Infection–Associated Hospitalizations among Older Adults

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Rhinoviruses and coronaviruses are recognized as the major causes of the common cold syndrome. The role of these viruses in more serious respiratory illnesses resulting in hospitalization is less well defined. During a winter when influenza A infection was prevalent, 100 elderly adults hospitalized because of cardiopulmonary illnesses were evaluated for rhinovirus and coronavirus infection. Patients who tested negative for influenza or respiratory syncytial virus had nasal swab samples tested for rhinovirus, coronavirus OC43, and coronavirus 229E by reverse-transcription polymerase chain reaction and for coronaviruses by serologic testing. Twelve percent of patients had rhinovirus or coronavirus identified (rhinovirus, 4 patients; coronavirus 229E, 4 patients; coronavirus OC43, 3 patients; and mixed rhinovirus/coronavirus 229E infection, 1 patient). All patients had significant underlying diseases. Although all patients recovered, the mean length of stay was 8 days; 4 persons had pneumonia, and 1 required ventilator support. These data suggest that rhinoviruses and coronaviruses may be associated with serious respiratory illnesses in frail older adults.

Influenza virus and respiratory syncytial virus (RSV) have been recognized as important causes of hospitalization in elderly adults during the winter months. However, the role of other respiratory viruses has been less well defined. Rhinoviruses and coronaviruses cause the majority of common cold syndromes [1, 2]. With the exception of severely immunocompromised hosts, these viruses are uncommonly associated with severe illnesses resulting in hospitalizations [3, 4]. This phenomenon may be due to the limited virulence of these pathogens and the pathogenesis of their infections, but it may also be due to the lack of detection, either because of the failure to perform appropriate tests or the difficulty in identifying these organisms using standard viral culture and serologic techniques. Several reports describe the effects of coronavirus and rhinovirus infection in elderly persons in day care or long-term care [5, 6]. Although lower respiratory signs and symptoms were common, serious sequelae were not often observed. Rhinoviruses and coronaviruses also have been implicated in exacerbations of chronic obstructive pulmonary disease (COPD) [7, 8]. However, most studies used viral culture or serologic testing to diagnose infections and did not comment on hospitalization rates. A recent large study of patients of all ages hospitalized with respiratory illnesses demonstrated that rhinoviruses and coronaviruses accounted for 5.1% of illnesses [9]. Because culture and serologic testing, rather than new molecular techniques, were used for diagnosis, the authors postulated that the incidence of disease may have been significantly underestimated. Reverse-transcription (RT) polymerase chain reaction (PCR) is a very sensitive and specific method of diagnosis that has been used with success to accurately define the true burden of disease due to these common viruses [10, 11]. The purpose of this study was to determine whether rhinoviruses and coronaviruses, as diagnosed by RT-PCR and serologic testing, are associated with hospitalization in elderly adults during the winter months and to describe the presenting signs and symptoms of these illnesses.

Patients and Methods

Study protocol. Active surveillance for respiratory infections was performed at Rochester General Hospital (Rochester, New York) from 15 November 1999 to 15 April 2000 as part of an ongoing study to identify influenza and RSV infections. All patients >65 years old and those with underlying heart or lung conditions admitted with a clinical diagnosis of acute respiratory infection, bronchitis, exacerbation of COPD, congestive heart failure (CHF), influenza,

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The study was approved by the Rochester General Hospital institutional review board, and informed consent was obtained from all patients or their legal guardians.

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The authors have no associations that pose a conflict of interest.

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or pneumonia were invited to participate. Demographic and clinical information was collected during patient interviews and from medical records. Combined nasal and pharyngeal swab specimens were tested for influenza virus and RSV by culture and RT-PCR. Acute and convalescent blood samples (at ~4 weeks) were obtained for serologic testing in as many cases as possible. During the 5 months of surveillance, 332 illnesses in 316 hospitalized persons were evaluated. Sixty-one cases of influenza A infection and 22 cases of RSV infection were identified and excluded from subsequent analysis. Of the 249 remaining cases, 100 samples were randomly selected for rhinovirus and coronavirus testing.

Laboratory methods for rhinovirus and coronavirus RT-PCR. RT-PCR for human rhinovirus (HRV) and human coronaviruses (HCV) was done by methods described elsewhere, with minor modifications [12, 13]. Total RNA was extracted from 200 μL of sample by matrix affinity chromatography (QIAamp DNA blood kit; Qiagen). The eluted RNA was transcribed into cDNA with murine reverse transcriptase (MMLV-RT; Gibco BRL) and virus specific oligonucleotide primer, for 1 h at 37°C. After MMLV-RT denaturation at 95°C, virus-specific 5′ biotinylated oligonucleotide primer and Taq polymerase (Applied Biosystems) were added, and 35 cycles of PCR were run, consisting of denaturation (1 min at 95°C for HRV; 2 min at 95°C for HCV), annealing (1.5 min at 48.2°C for HRV; 1 min at 60°C for HCV), and DNA extension for 1 min at 72°C. The pairs of primers for coronaviruses OC43 and 229E were used in the same reaction in a multiplex format. Excess primers, dNTPs, Taq DNA polymerase, and salts were removed by adsorbing the amplified product to the QIAquick silica-gel membrane (QIAquick PCR purification kit; Qiagen). The presence of the PCR product was detected by microplate hybridization with digoxigenin-labeled virus-specific probes, as described elsewhere [13]. Positive and negative controls were previously tested nasal washings either containing or lacking specific HRV or HCV RNA.

Laboratory methods for coronavirus serologic testing. Serologic evidence of coronavirus infection was defined as a ⩾3-fold increase in coronavirus-specific IgG, as measured by EIA. Coronavirus 229E antigens were prepared from infected WI-38 cell lysates and OC43 antigens in suckling mouse brains. EIA plates were coated with either 229E or OC43 antigens to Nunc flat-bottom plates in bicarbonate buffer. Control plates were prepared by using uninfected WI-38 cell lysates or mouse brain suspensions. Serum samples obtained during acute and convalescent illness (acute and convalescent serum samples) were added to wells in duplicate in serial 2-fold dilutions from 1:400 to 102:400. Serum IgG was detected with alkaline phosphatase–conjugated goat anti–human antibody, followed by substrate. The end-point titer was defined as the highest dilution with an optical density ⩾0.100 that was at least twice that of the control plate [6].

Statistical methods. Means were compared with Student's *t* test, and proportions were compared using χ^2 and Fisher's exact tests, as appropriate.

Results

Of the 100 cases evaluated, rhinovirus and/or coronavirus were identified as a pathogen in 12 of them. Four nasal specimens were RT-PCR positive for rhinovirus, 4 were positive for coronavirus 229E, 1 was positive for coronavirus OC43, and 1 was positive for both rhinovirus and coronavirus 229E. Of the 100 cases, acute and convalescent serum samples were available for 88 of them, from which 2 additional OC43 infections were identified. The 6 patients who tested positive by PCR for coronaviruses were seronegative for coronavirus.

Rhinovirus infections occurred sporadically throughout the winter, although coronavirus OC43 infections occurred primarily in December and January and coronavirus 229E infections occurred primarily in March and April. The mean (±SD) age of patients was 74 ± 11 years, with similar numbers of men and women in both groups (table 1). All patients had significant underlying chronic medical conditions, primarily CHF and COPD, and 1 patient had chronic lymphocytic leukemia (CLL). Consistent with the high rate of lung disease, 92% were active or previous smokers, 33% took corticosteroids daily, and 50% received oxygen at home. Seventy-five percent had exposure

Table 1. Demographic and clinical data among elderly patients hospitalized for cardiopulmonary illness.

Demographic or clinical data	Rhinovirus or coronavirus (*n* = 12)	Influenza virus (*n* = 61)	No viral diagnosis (*n* = 88)
Age, mean years (SD)	74 (11)	79 (10)	76 (12)
Underlying medical condition			
Lung	10 (83)[a]	24 (39)	53 (60)
Cardiac	3 (25)	27 (44)	42 (48)
Previous or active smoker	11 (92)	34 (63)	76 (86)
Long-term oral steroid use	4 (33)	5 (8)	20 (23)
Home oxygen use	6 (50)[b]	7 (11)	28 (32)
Clinical data			
Nasal congestion	8 (67)	33 (54)	42 (48)
Sore throat	2 (17)	20 (32)	23 (26)
Hoarseness	3 (25)	19 (31)	14 (16)
Cough	10 (83)	59 (97)	81 (92)
Sputum	7 (58)	37 (60)	64 (73)
Dyspnea	11 (92)	46 (75)	82 (93)
Constitutional	4 (33)	33 (54)	45 (51)
Days ill prior to admission, mean ± SD	5.2 ± 5.5	3.8 ± 3.2	6.2 ± 6.4
Physical findings			
Rhinorrhea	3 (25)	10 (17)	9 (10)
Wheezing	9 (75)	29 (70)	51 (59)
Rales	9 (75)	42 (69)	65 (74)
Temperature, mean °C ± SD	37.4 ± 1.0[b]	38.4 ± 1.1	37.7 ± 1.0
CXR infiltrate	4 (33)	21 (34)	30 (34)
Outcomes			
Steroid use	8 (67)	32 (52)	53 (60)
Bronchodilator use	10 (83)	48 (79)	65 (74)
Antibiotic treatment	10 (83)	56 (92)	81 (92)
Intensive care	1 (8)	5 (8)	11 (13)
Mechanical ventilation	1 (8)	5 (8)	9 (10)
Death	0	5 (8)	2 (2)

NOTE. Data are no. (%) of patients, unless otherwise indicated. CXR, chest x-ray.
[a] $P = .009$, rhinovirus or coronavirus vs. influenza virus.
[b] $P = .005$, rhinovirus or coronavirus vs. influenza virus.

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to young children. Compared with patients infected with influenza during the same period, the rhinovirus/coronavirus group had significantly more lung disease. Nasal congestion and cough were common symptoms (67% and 78%, respectively), as were lower respiratory tract symptoms of dyspnea (83%) and sputum production (58%). Constitutional symptoms, such as fatigue and myalgias, occurred in only 33% of patients with HRV or HCV, compared with 54% in influenza virus–infected patients. Fever was significantly less common in the HRV and HCV group than in the influenza group (mean temperature, 37.4°C ± 1.0°C vs. 38.4°C ± 1.1°C; $P = .005$). Patients were quite ill at admission, with 75% demonstrating wheezing and rales by auscultation. Supplemental oxygen was required for all but 1 individual. Most patients received steroids (75%), bronchodilators (83%), and antibiotics (83%). All patients eventually recovered and were discharged after a mean length of stay of 8 days. There were no distinct features of rhinovirus or coronavirus infection.

Four patients had radiographically proven pneumonia (2 with coronavirus and 2 with rhinovirus infection). One 89-year-old woman with rhinovirus infection had a history of COPD and presented with a temperature of 39.3°C, an SaO_2 level of 57%, and an infiltrate on chest *X*-ray. No blood or sputum cultures were obtained. The other rhinovirus-positive patient with pneumonia had CLL, a temperature of 39.0°C, and blood and sputum cultures that grew no pathogens. Both patients with coronavirus infection and pneumonia were elderly women with CHF and presented with nasal congestion, cough, and dyspnea and had visible rhinorrhea at examination. Blood cultures were sterile, and 1 patient tested positive for *Pseudomonas aeruginosa* in an inadequate sputum sample. Two individuals without pneumonia had evidence of concurrent bacterial infection: 1 patient with positive coronavirus serologic test results had *Streptococcus pneumoniae* bacteremia, and *Staphylococcus aureus* was isolated from the sputum of 1 person with coronavirus 229E.

Discussion

During a winter season when influenza was prevalent and accounted for a substantial number of hospitalizations, rhinoviruses and coronaviruses were diagnosed by RT-PCR and serologic testing in 12% of patients without influenza or with RSV. These data provide more evidence that not all serious respiratory illnesses that occur during the winter months are always “the flu.” Our results are consistent with a recent study by El-Sahly et al. [9], documenting coronavirus/rhinovirus infections in 26 (4.7%) of 546 persons >35 years old who were hospitalized with cardiopulmonary illnesses, and with a 4-year study by Glezen et al. [4], in which viruses were found to be a common trigger for acute decompensation leading to hospitalization in persons with preexisting lung diseases. In the latter study of 417 adults ⩾45 years old who were hospitalized for respiratory infections, influenza viruses were detected most frequently in 8% of patients, whereas rhinoviruses were detected in 2% and coronaviruses in 3% of patients. However, this study did not use RT-PCR to detect viruses, and rates likely would have been higher if more-sensitive diagnostic tools were available. In a study of inner-city adults with asthma, rhinoviruses and coronaviruses were more common than influenza in patients presenting to an emergency room [10]. Sixty percent of rhinovirus and 71% of coronavirus infections were detectable only by RT-PCR.

The higher rate of infection in our study likely reflects the use of RT-PCR, rather than culture, for diagnosis, as well as the months studied and the elderly population. A limitation of this study is the lack of a control group of hospitalized elderly patients without respiratory illnesses. Previous studies have shown that rhinoviruses may be detected in 3% of asymptomatic elderly adults [11]. The use of a method of diagnosis that detects minute quantities of RNA raises the question of the role of the virus in causality of the illness. Unfortunately, samples from a control group were not available. It was not unexpected that there were patients with positive coronavirus PCR results but negative serologic test results, since antibody responses may be impaired in frail elderly persons.

Of note, persons hospitalized because of rhinovirus/coronavirus infections were more frail than those with influenza, with all persons having either significant cardiopulmonary disease or cancer. This finding is consistent with a study by Nicholson et al. [11], in which the presence of chronic medical conditions or smoking increased the risk of lower respiratory tract disease by ~40% in elderly adults with rhinovirus infection. In the study by El-Sahly et al. [9], asthma, COPD, or CHF was present in 73% of patients ⩾35 years old who were hospitalized because of rhinovirus or coronavirus infections. In addition, Wald et al. [5] found that rhinovirus infections produced more-protracted lower respiratory tract symptoms in nursing home residents with COPD or a history of smoking.

Illnesses were significant, with the mean length of stay >1 week, and nearly all patients received supplemental oxygen, steroids, and antibiotics. One patient required intensive care, but all survived. Of interest, 2 patients with rhinovirus infections had high fevers and radiographic evidence of pneumonia. Rhinoviruses are rarely implicated as a cause of pneumonia, which is believed to reflect their biologic characteristics [14]. However, recent data from Papadopoulos et al. [15] suggest that infection of the lower airways is possible and that rhinoviruses may have direct effects on the lower respiratory tract epithelium. The high frequency of lower respiratory tract symptoms in ambulatory elderly persons with rhinovirus infections and the recovery of rhinovirus from expectorated sputum samples are additional evidence for viral invasion of the lower airways [5, 11]. It is unknown how often bacterial suprainfection in the lower respiratory tract follows HRV or HCV infection. Despite these uncertainties, mixed viral-bacterial infection in our patients seems likely, although one person had CLL, thus making a viral pneumonia a possibility.

In summary, infection with agents that typically cause colds in young adults may lead to hospitalization in frail elderly persons

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with underlying heart and lung problems. It does not appear that healthy elderly persons are at high risk from these agents. These viruses, although not as common as influenza and RSV among hospitalized adults, also circulate during the winter months, producing similar clinical syndromes. The use of RT-PCR improves the ability to identify these previously difficult to detect viruses, but further studies with control groups are needed to fully define the impact of HCV and HRV in hospitalized elderly adults.

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