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STATE-OF-THE-ART REVIEW

The role of goblet cells in viral pathogenesis

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Goblet cells are specialized epithelial cells that are essential to the formation of the mucus barriers in the airways and intestines. Armed with an arsenal of defenses, goblet cells can rapidly respond to infection but must balance this response with maintaining homeostasis. Whereas goblet cell defenses against bacterial and parasitic infections have been characterized, we are just beginning to understand their responses to viral infections. Here, we outline what is known about the enteric and respiratory viruses that target goblet cells, the direct and bystander effects caused by viral infection and how viral interactions with the mucus barrier can alter the course of infection. Together, these factors can play a significant role in driving viral pathogenesis and disease outcomes.

Introduction

Mucosal barrier sites throughout the body are tasked with coordinating the cellular response that distinguishes friend from foe while balancing homeostasis with a rapid response to infection [1]. Central to this balance are the epithelial cells that line these barriers, providing a plethora of heterogeneous, specialized cells that act as first responders to incoming pathogens. A common feature across mucosal sites is another layer of defense provided by mucus, a carbohydrate-rich, gel-like substance that, at its most basic level, prevents invading pathogens from reaching the underlying epithelium [2]. Goblet cells are specialized secretory cells that are the primary producers of the mucus barrier in the airways and intestines [3]. Their secretory milieu and overall function at these sites are dependent on contextual cues, including multimicrobe interactions. In fact, microbiota are key to these mucosal functions [4,5], as mucus barrier defects are well documented in germ-free [6] and antibiotic-treated mice [7,8]. Microbial sensing by goblet cells [6,9,10] and neighboring cells plays a role in shaping the mucus barrier. For example, in the presence of microbes, enterocytes in the small intestine secrete the metalloprotease, meprin β, which cleaves mucin (MUC) and enables its unfolding and full expansion [11]. However, both host responses to pathogens and the pathogens, including viruses, themselves can trigger a breakdown of this host–microbe crosstalk, with some pathogens adopting ways to modulate goblet cell numbers and functions. Whereas substantial work has been focused on the protective and disease-mediating features of goblet cells during bacterial and parasitic infections, we are just beginning to understand their functions in the context of viral infections. The purpose of this

Abbreviations
CLCA1, Calcium-activated chloride channel regulator 1; FCGBP, Fc gamma binding protein; HA, Hemagglutinin; IL, Interleukin; Math1, Mouse atonal BHLH transcription factor 1; MuAstV, Murine astrovirus; MUC, Mucin; NA, Neuraminidase; RSV, Respiratory syncytial virus; SARS-CoV, Severe acute respiratory syndrome coronavirus; SPDEF, SAM pointed domain-containing ETS transcription factor; TMEV, Theiler's murine encephalomyelitis virus; ZG16, Zymogen granule 16.

review is to highlight what is known about the direct and indirect consequences of viral infection for goblet cells, as well as the effect of mucus interactions with viruses in the intestinal and respiratory tracts.

Goblet cell development

Goblet cells were first discovered in the early to mid-nineteenth century and were characterized by their distinct morphology, which resembles a drinking goblet [12]. This cellular architecture is largely the result of the apical cytoplasm being full of secretory granules containing MUCs and other secretory factors, which causes the nucleus and other organelles to be pushed to the basal 'goblet stem'. In the intestinal crypts, promotion of *Atonal BHLH transcription factor 1* (*ATOH1, Math1* in mice) expression and Notch inhibition drives the development of secretory progenitor cells, which then receive additional signals that turn on SAM pointed domain-containing ETS transcription factor (SPDEF) to drive transcriptional programming, resulting in mature goblet cells that are fully differentiated [13,14]. In the airways, goblet cells arise from a slightly different pathway, with increasing Notch signals and expression of SPDEF, which drives full differentiation and promotes mucus secretion [15–17]. The act of secretion is thought to be similar across goblet cells at these two mucosal sites, and it is characterized by either a constitutive or regulated process to maintain homeostasis [2,3]. Regulated secretion involves vesicle secretion and also a stimulus-driven form that is mediated by compound exocytosis characterized by rapid release of secretory granules [3,18,19]. Whereas regulated secretion has been characterized for airway goblet cells [20], less is understood about the signaling cascade that drives compound exocytosis. Neither secretory pathway has been precisely defined in the gut, but reactive oxygen species generation, autophagy, and inflammasome signaling appear to play a role in goblet cell secretion in mice [9,21–23]. The details of these mechanisms have yet to be worked out in humans, but there is evidence of species-specific differences, such as regional expression of the NLPR6 inflammasome [24,25]. Secretory processes are also largely mediated by known secretagogues, or stimuli that drive secretion, including acetylcholine, carbachol, and histamine [26–28]. In addition, goblet cell differentiation and secretion are sensitive to cytokine stimulation [29], including Th2 signaling via Interleukin (IL)-4 and IL-13 [30–32]. For these reasons, goblet cells and mucus secretion can quickly mobilize as part of the innate immune response in the intestines and airways.

Intestinal goblet cells

A progressive examination along the length of the intestinal tract reveals a correlative gradient between goblet cells and the microbiota, with the highest density of both being found in the distal colon (Fig. 1). The small intestine has a single, discontinuous layer of mucus, which has not been extensively measured in humans but in mice, ranges from 500 μm in the duodenum to 200 μm in the ileum [27,33,34]. In contrast to the small intestine, the large intestine has dual layers—an adherent inner layer below a looser outer layer [2]. In the mouse colon, the attached inner layer is ~ 50 μm thick whereas the top layer is thicker in the proximal region (50 μm) than in the distal region (10 μm) [27,33,34]. In human colons, the inner mucus thickness is 200–300 μm in humans [26,35–38], whereas the outer layer is ~ 400 μm in the colon [36,39]. These mucus layers are critical for keeping microbes and other luminal contents at a safe distance from the underlying epithelium, with some commensal microbes inhabiting the outer region, creating a symbiotic environment that prevents self-digestion [2]. Goblet cells tasked with maintaining this host barrier do so primarily through regulated secretion, but a subset of cells are thought to be primed for rapid secretion to flush out bacterial, parasitic, and even fungal infections [1,40]. MUC2 is the main component of the secreted gel-forming mucus in the intestines, whereas MUC1, MUC3, MUC4, MUC12, MUC13, and MUC17 are expressed as transmembrane glycoproteins [2]. MUC undergo extensive *O*-linked glycosylation and other posttranslational modifications [2,4] (Fig. 2). Although the precise molecular mechanisms and triggers that alter these modifications remain poorly defined in the context of infection, it is intriguing to consider that goblet cells can tailor MUC structure in response to a given pathogen. Further studies in this area are greatly needed.

Besides MUC, goblet cells also secrete a multitude of other proteins that enhance the mucus barrier and have direct antimicrobial activities (Fig. 2). These proteins include calcium-activated chloride channel regulator 1 (CLCA1) [41,42], Fc gamma binding protein (FCGBP) [43], anterior gradient 2 (AGR2) [44], zymogen granule 16 (ZG16) [45], and trefoil factor 3 [46], although the antiviral functionality of these secreted proteins has yet to be elucidated. Mucus also serves as a conduit for defensins and other antimicrobial proteins and peptides secreted by Paneth cells located at the base of the crypts [47], as well as IgA, which play dual roles in modulating interactions with commensal microbes [48] and protection from pathogens [49].

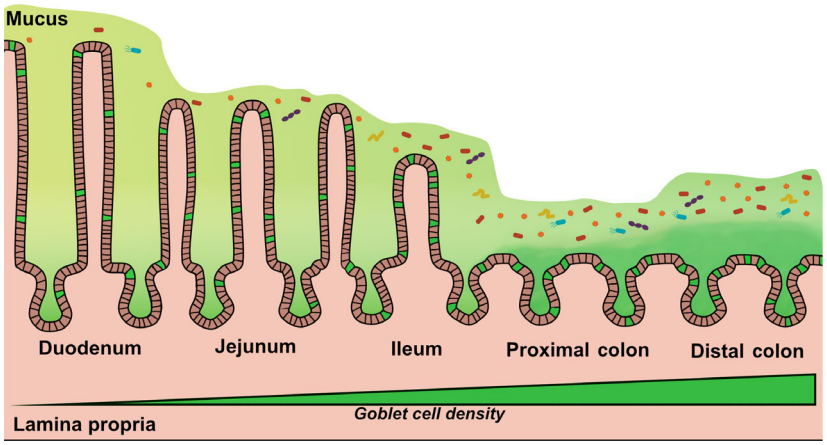

Fig 1. The epithelia that line the intestinal tract include specialized secretory cells known as goblet cells, highlighted in green, which increase in density from the proximal to the distal end of the tract. The small intestine (the duodenum, jejunum, and ileum) is coated with a single, discontinuous mucus layer (light green), whereas the large intestine (the proximal and distal colon) is coated with an inner mucus layer (dark green) and an outer layer (light green). Microbiota, shown in confetti colors, reside within the lumen and mucus layer away from the epithelium and exhibit a density gradient that mirrors that of goblet cells.

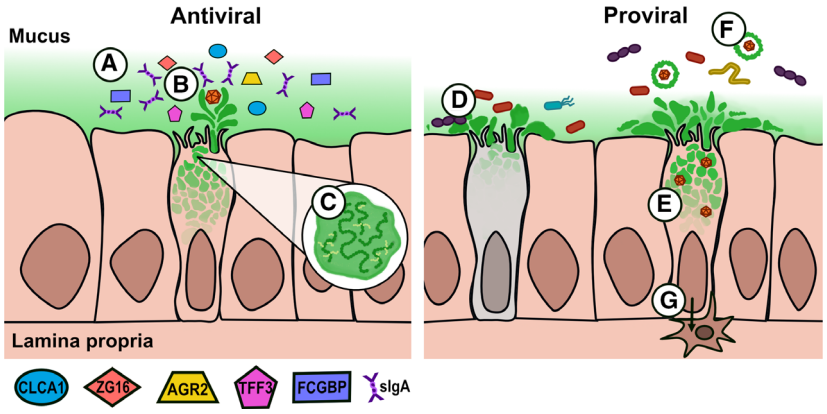

Fig 2. Goblet cells have an arsenal of defenses at their disposal. A) Mucus consists of MUC, antimicrobial proteins with unknown antiviral potential, and also secretory IgA, which modulates gut microbiota and can combat viral infection. B) Mucus can flush out viruses or bind them to block receptor interactions. C) The composition of mucus can be changed via posttranslational modification. However, viruses have found ways to co-opt goblet cells and mucus to their benefit, resulting in proviral effects. D) Increasing mucus secretion could lead to goblet cell exhaustion represented by the gray-colored goblet cell), leading to barrier defects that can result in inflammation and/or secondary bacterial infection. E) Viruses may directly infect actively secreting goblet cells, potentially facilitating viral egress and dissemination in mucus. F) Viruses may bind to mucus to enhance their stability, infection, transmission, and possibly immune evasion. G) Beyond these functions of goblet cells, viruses could serve as a trigger for goblet cell-associated pathways, which in turn could serve as mechanisms of tolerance or translocation outside the gut.

Overall, with this arsenal of defenses, goblet cells can play an active role in the response to infection and have the potential to modify their response to combat-specific pathogens.

Goblet cells and enteric viral infection

Some of the earliest reports of enteric viruses targeting goblet cells and neighboring epithelial cells in the gut relied on electron microscopy to identify virus particles. Examples include reports of 'reovirus-like' virus in an infant with nonbacterial diarrhea [50], mouse adenovirus 2 in experimentally infected mice [51], and bovine coronavirus infection in calves [52]. Most recently, a number of 'mini-gut' models have been developed using induced or embryonic pluripotent stem cells to generate organoids that contain both epithelial and mesenchymal cell populations, as well as

colonoids and enteroids, which are derived from intestinal crypt cells in the colon and small intestine, respectively, but lack a mesenchymal cell layer [53]. Together, these models can form both spherical and 2-D cell layers that reflect the tissue architecture and physiology of the intestinal villi and have increased our understanding of enteric viral infection in secretory cell types because immortalized intestinal cell lines frequently do not reflect the diverse epithelial heterogeneity found *in vivo* [53]. For example, the study of porcine epidemic diarrhea virus, a coronavirus that causes high mortality in neonatal pigs, has been limited by the lack of a robust cell culture model, but it was recently shown that multiple intestinal cell types, including goblet cells, in enteroids and colonoids are infected by this virus, which mirrors the infection *in vivo* [54].

Enteroid models have also been used extensively in the study of human enteric viruses, giving a sense of their goblet cell propensities *in vivo*. Human adenovirus species C (prototype strain 5p) was the first virus identified as preferentially infecting a subset of goblet cells in enteroids; however, this cell tropism was strain specific, and a preference for goblet cells was not observed with human adenovirus species F (prototype strain 41p) [55]. Enterovirus 71 was also shown to have a strong preference for goblet cells in 2-D epithelial monolayers derived from human fetal small intestinal crypts [56]. The infection drove a reduction in *MUC1* and *MUC2* expression, highlighting the ability of this virus to alter goblet cell function and, perhaps, combat mucus flushing from the gut [56]. The human astrovirus VA1 species has also been shown to infect goblet cells and other epithelial cell types in human enteroids [57]. Interestingly, murine astrovirus (MuAstV) appears to have an even higher propensity for goblet cells. This was first detected by in situ hybridization and electron microscopy and then confirmed by single-cell transcriptional profiling of small intestinal epithelial cells [58]. Based on the transcriptional activity in goblet cells, MuAstV was determined to target actively secreting cells specifically and to drive a further increase in the expression of *Muc2*, *Clca1*, *Fcgbp*, and *Zg16*, as well as an increase in mucus thickness between and at the tops of the villi. These data indicate that MuAstV might benefit from causing this host pathway to produce more mucus in response to infection, perhaps as a means of facilitating virus egress and/or dissemination [58]. MuAstV infection also drove a change in microbiome composition and reduced colonization by enteropathogenic *Escherichia coli*, an adherent bacterial pathogen [58]. To what extent the increase in mucus secretion is sustained after MuAstV is cleared from the gut remains to be determined, but goblet cell exhaustion has been reported after parasitic infections that similarly drive hypersecretion [59,60]. This is a critical finding, given that such exhaustion can lead to a weakened mucus barrier, which could result in inflammation or even secondary bacterial infection [61]. In fact, this increased susceptibility to bacterial infection has been proposed as a disease mechanism for porcine epidemic disease virus, which causes goblet cell depletion in neonatal pigs [61]. Therefore, the combined effects of virus-driven changes in goblet cells can alter the microbiome and potentially exacerbate gastrointestinal illness, as well as alter host susceptibility to other enteric pathogens (Fig. 2).

Even in the absence of direct infection, enteric viruses can drive substantial changes in goblet cell abundance, function, and differentiation. For example, whereas enterocytes undergo apoptosis after rotavirus infection in mice, goblet cell numbers are decreased in the duodenum and jejunum as a result of delayed intestinal repair [62]. Transmissible gastroenteritis virus, a coronavirus that causes significant mortality in neonatal pigs, also indirectly affects goblet cells by infecting Paneth cells in the crypt and causing a loss of Notch signals required for neighboring stem cells to regenerate enterocytes and mitigate villus blunting [63]. Instead, the loss of Notch signals drives an increase in goblet cell numbers and mucus production after infection [63]. Similar to the effect seen with porcine epidemic virus, these alterations in goblet cells result in increased susceptibility to enterotoxigenic *E. coli* [64]. Much like bacteria that co-opt MUC interactions to enhance colonization [65,66], the increase in mucus production caused by transmissible gastroenteritis virus is beneficial to the virus because binding the sialic acid-rich MUC helps to facilitate receptor interactions [67,68]. Indeed, a similar process has been described for the picornavirus Theiler's murine encephalomyelitis virus (TMEV), which binds to terminal sialic acid moieties on MUC to enhance infection [69]. Although the precise biological mechanism of how this binding propensity enhances infection is incompletely understood for TMEV, there are several possible explanations beyond receptor binding and entry. For example, MUC-bound virus could be protected from inactivation in a low-pH environment, as well as from host digestive enzymes. Alternatively, binding to mucus may promote longer retention of the virus in the gut, thereby enabling the virus to become peristalsis-resistant [69]. A third possibility is that the sialic acid-binding sites on TMEV correspond to neutralizing antibody sites [69], which could help the virus evade host immunity.

It is intriguing to consider whether these proviral mechanisms are more broadly applicable to other enteric viruses. A recent study noted that recovery of norovirus, rotavirus, astrovirus, sapovirus, and husavirus from sewage was enhanced by a pig-MUC capture method [70]. In separate studies, MUC has been shown to promote poliovirus infectivity [71], to enhance the thermostability of reovirus [72], and to stabilize human astrovirus serotype 1 capsid and thereby preserve the infectivity of the virus during heat treatment [73]. Given that changes in mucus production can also cause changes in microbiome composition, it is also possible that virus–bacteria interactions play a role in these proviral mechanisms, as was noted in previous comprehensive reviews [74,75].

In contrast to these benefits derived by the virus from MUC binding, there is also evidence that MUC can serve as a 'trap' for virus particles, in much the same way that it traps bacteria and parasites [1]. In multiple studies, sialic acid-dependent strains of rotavirus exhibited reduced infectivity when co-incubated with mucus [62,76–79], whereas deglycosylation or neuraminidase (NA) treatment to remove sialic acid moieties from mucus abrogated this effect and enabled cells to be infected [76,77,79]. An increase in mucolytic bacteria has also been shown to aid rotavirus in its effect to subvert mucus interference, again highlighting an important multimicrobe interaction [80]. Reovirus can also combat mucus via its σ1 protein, which exhibits glycosidase activity and, therefore, could be important for mucus penetration [81]. In short, MUC appears to serve as a 'double-edged' sword that viruses may combat or co-opt during infection (Fig. 2).

Beyond these cellular functions related to mucus barrier maintenance, goblet cells also play a critical role in the development of oral tolerance, with mucus driving tolerogenic effects via crosstalk with immune cells [82,83]. Therefore, it is intriguing to consider whether virus association with mucus could serve as an immune evasion strategy or whether viral infection of goblet cells could trigger the formation of goblet cell-associated antigen passages [84], which enable luminal contents to be sampled by the underlying antigen-presenting cells in the lamina propria. Is it possible that enteric viruses, in a similar manner to *Salmonella enteric* serovar Typhimurium [85], use goblet cell-associated antigen passages to mediate extra-gastrointestinal spread? In addition, *S.* Typhimurium is known to colonize the mouse cecum due to exposure of the epithelium in areas without a thick mucus barrier [86], which could also benefit viruses that are greatly impeded by mucus. Similarly, the overlaying mucus on the epithelial layer of Peyer's patches is known to be much thinner than the surrounding epithelium [27], indicating that viruses that gain entry or infect these sites, such as murine norovirus via microfold cells [87], may also play a role. The extent to which this and other functions of goblet cells in the gut contribute to protection against or the pathogenesis of viral infections is an exciting new avenue of research waiting to be explored.

Airway goblet cells

Distinct from the intestinal epithelium, the lung airways are lined by a pseudostratified epithelial layer that consists of basal and club progenitor cells that differentiate into ciliated and secretory cell types, including goblet cells [15,88]. The mucus barrier in the airways comprises the periciliary fluid and airway liquid surface layers (Fig. 3). In mice, the airway liquid surface layer is 10–30 μm thick whereas in humans, the periciliary fluid is ~ 4–7 μm and the top mucus layer is highly variable, ranging from 10 to 70 μm in depending on the section of the airway [89–93]. Together with ciliated cells that display motile cilia on their apical surface, mucus secretion from goblet cells and submucosal gland cells is critical for mediating mucociliary clearance, which sweeps inhaled debris and potential pathogens out of the airways [94]. Although the airways harbor 14 distinct MUC that support the mucus layers, the primary secreted MUC that are analogous to MUC2 in the intestinal tract are MUC5B and MUC5AC [15].

Respiratory viruses in goblet cells

In contrast to intestinal goblet cells, much has been uncovered about airway goblet cell interactions with viruses, particularly with respect to mucus binding (Fig. 3). Goblet cells are the direct targets of many human respiratory viruses, such as hantavirus [95], rhinovirus [96], and multiple influenza viruses, including influenza A viruses H1N1, H5N1, H7N9, H5N6, and H3N2, as well as influenza B virus [97–99]. Influenza virus is probably the most studied respiratory virus with direct MUC interactions: its hemagglutinin (HA) surface protein binds to epithelial sialic acid to mediate cell entry. Thus, mucus binding by influenza viruses is in part a side effect of their receptor usage, but mucus has been shown to both aid and hinder infection, with evidence of host-variable distinctions [100]. Previous studies demonstrated that MUC5AC overexpression in transgenic mice reduced H1N1 virus titers in the lung, which coincided with reduced immune cell infiltration [101]. Cell surface MUC can also serve as decoy

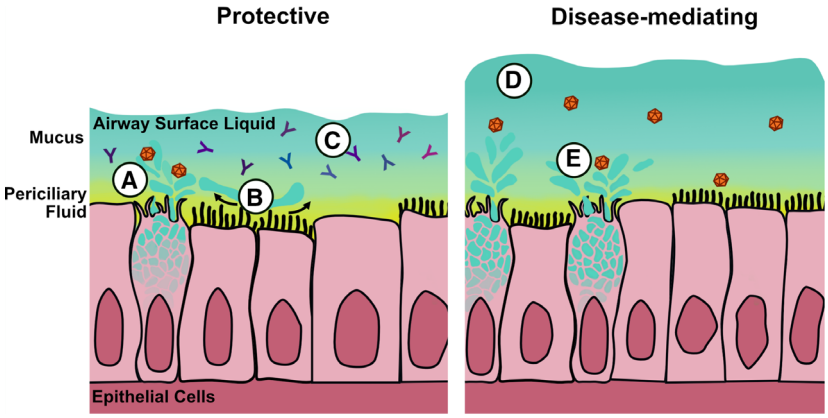

Fig 3. Goblet cells that line the airways are critical to maintaining homeostatic conditions that serve as protection against viral infection (shown at left). A) Mucus can bind the virus and sequester it from the epithelial layer. B) Mucus secretion aids mucociliary clearance of virus particles. C) Mucus-associated antibody can also help to block viral infection. D) Goblet cell-driven airway disease can be caused by overexuberant mucus responses (shown at right), leading to impaired mucociliary clearance. E) Some viruses can benefit from MUC interactions to help enhance their stability, infection, and transmission.

receptors for influenza [102,103], and *Muc1*-deficient mice exhibit more severe H1N1 virus infection [102], highlighting the important role that MUC can play in antiviral defense. However, because influenza virus also displays a NA protein on its virion surface, it can mitigate these mucus effects. Indeed, NA is important for virus release when HA molecules tether nascent virus particles to the cell surface [104,105] and also for viral entry, in which it combats mucus hindrance [106–109]. Studies have shown that influenza virus can even co-opt mucus binding as a means of traversing the mucus barrier to find its cellular receptor [110–112]. Therefore, a careful balance between HA and NA expression on the virus surface is key to optimal infection [113], and virus interactions with mucus have been shown to have a significant impact on transmission [114,115] and on cross-species restriction [116].

Much like in the gut, basal mucus production in the lung is intended to flush out pathogens and other irritants in the airways. However, this important host response can become exaggerated to the point where it contributes to acute respiratory distress syndrome (Fig. 3). Most recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was shown to infect goblet cells in human bronchial explants and was also identified by postmortem analysis [117,118], but such infection was not observed in 2-D airway organoid cultures [119], perhaps reflecting differences in the detection methodology. Interestingly, SARS-CoV-1 infection was not shown to target goblet cells *in vitro* or *in vivo* [119–121], whereas canine respiratory coronavirus, a related betacoronavirus, has been shown to infect goblet cells in canine tracheal cultures [122]. Even if goblet cells are not frequently infected by SARS-CoV-2, investigation of their role in mediating airway disease is warranted, as clinical reports have commonly identified mucus accumulation in fatal cases of COVID [123–125] and because several other viral infections drive goblet cell hyperplasia and mucus metaplasia, leading to compromised lung function. Respiratory syncytial virus (RSV) is a textbook example of this and is by far the most prevalent cause of lower respiratory disease in infants and young children [126]. Without directly infecting goblet cells, RSV induces goblet cell hyperplasia and hypersecretion via the induction of Th2 cytokines [127], but IL-4 receptor antagonism can block these cytokine signals and has been effective *in vivo* [128]. It was also shown that IL-12p40, which generally functions as a brake on Th2 hyper-responsiveness, is critical for controlling disease caused by RSV as well as that caused by human metapneumovirus, a related pneumovirus [129,130]. Rhinoviruses, which are responsible for the common cold, similarly drive an increase in MUC production *in vitro* and *in vivo* [131–133], and although this has been shown to be protective against infection [134], it can cause complications in the event that the mucus response becomes excessive. Drug treatments, including those with anticholinergic agents, corticosteroids, and anti-inflammatory drugs, have proved effective at mitigating overexuberant goblet cell responses caused by rhinovirus and RSV *in vitro* and *in vivo* [135,136]. However, it is important to note that these interventions can come at a cost to antiviral immune responses and may result in reduced viral clearance [137]. Therefore, additional study of goblet cell functions could aid

the design of appropriate interventions to mitigate aberrant host responses and promote antiviral responses that will improve respiratory virus disease outcomes.

Concluding remarks

Armed with MUC and other antimicrobial proteins that offer structural and functional interactions with microbes and host immune cells, goblet cells provide a critical link in the innate immune responses in the airways and intestines. We are only beginning to scratch the surface of the heterogeneity within goblet cell populations but improving our understanding of how goblet cell responses are shaped by host, microbe, and viral factors will be critical to developing strategies that modulate their activity. Ultimately, understanding their basic biology will help to improve host susceptibility and disease outcomes to enteric and respiratory viral infections.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

VC and SS-C came up with the idea of reviewing this topic. VC wrote the manuscript. VC and SS-C edited this manuscript.

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