Respiratory Epithelial Cells in Innate Immunity against Respiratory Viruses

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Abstract

Epithelium is considered the first barrier defense of the organism due to its contact with the environment. The respiratory epithelium is important in the innate immune response. Respiratory viruses are the leading cause of acute respiratory infections being the respiratory epithelia (RE) their primary target. These epithelial cells have the capacity to limit viral replication and to activate the immune response. RE recognize viruses through pattern recognition receptors expressed in the cell membrane or in the cytoplasm. After recognition, RE produces a variety of molecules such as defensins, nitric oxide, cytokines, chemokines, and interferons in response to viral infection. The early antiviral state by RE is crucial to control viral replication. This review analyzes the role of the RE cells as innate immunity mediator and discusses the potential use of this knowledge in the development of future mucosal therapies and vaccination.

Key words: Respiratory epithelia. Innate immunity. Respiratory viruses. Cytokines. Interferons.

Introduction

Respiratory epithelia (RE) have been recognized as the first line of defense of the respiratory tract. Upper and lower airway epithelia are morphologically similar but are different in their response to pathogens^{1,2}. Studies of different viral particles that infect human nasal and bronchial epithelial cells have demonstrated differences in susceptibility, due to innate immune system components³. The susceptibility to different bacteria and/or viruses in distinct locations of the respiratory tract is an example of the interaction of pathogens, a situation that may determine the immune response or the development of disease⁴. The upper and lower airways have different susceptibility to respiratory

pathogens. The human rhinovirus (HRV) commonly infects the upper respiratory airway, but the lower respiratory airway is more susceptible to this virus⁵.

There are known factors involved in the progression of respiratory disease due to viral infection of the RE. It has been reported that RE cells respond in different ways to infection and coinfection with the most common viral agents⁶. The controversy regarding the distinct environmental factors and physiological conditions involved in the infectious process is still unclear. Understanding the factors and conditions that lead to resolution of the infection or disease development has an immediate clinical impact, not only in treatment but also in vaccine development.

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Table 1. Respiratory epithelial cells innate immunity response to viral infection

Viral particle	Airway location	RE response to stimulus
HRV	LRT	Overexpression of RIG-1, MDA5, CXCL10, RANTES Production of IL-8, Viperin, IL-1 α , IFN- β , IP-10
IAV	LRT	Overexpression of IFN- β , OAS1, MX1, ISG56 Production of IL-8, RANTES, IL-6
RSV	LRT LRT	Expression: SOCS-1, SOCS-3 Production: IFN- β , SOCS-1, SOCS-3, IL-2, IL-12 Production: IL-8, IP-10, MCP-1, MIP-1 α , MIP-1 β , RANTES, IL-6, TNF α , IL-1 α / β , type I IFNs
hMPV	LRT URT	Expression of type I-IFNs, RIG-I, MDA-5, RANTES, IL-8, IFN- β Activation of RIG-I, STAT1 Production of IL-1 β , IL-6, IL-12, TNF α
Coronavirus	LRT	Overexpression of CXCL10, CXCL11, type I, III IFNs, IL-1 α , ISG65, IAS1, Mx 1, IL-29, CXCL10, IL-6, IL-18, RANTES Production: CCL5, FGF, CXCL10, IFN- β , IFN- λ 1/2, IL-1 α , IL-6, IL-8, PDGF

HRV: human rhinovirus, IAV: influenza A Virus, RSV: respiratory syncytial virus, hMPV: human metapneumovirus, URT: upper respiratory tract, LRT: lower respiratory tract, RIG-I: retinoic acid-inducible gene I, TLR: toll-like receptors, MDA-5: melanoma differentiation-associated gene 5, IFN: interferon, IL: interleukin, TNF: tumor necrosis factor, PDGF: platelet-derived growth factor, FGF: fibroblast growth factor

Disease Onset: from Acute to Complicated/Chronic Respiratory Disease

Acute respiratory infections (ARIs) are the most frequent acute infections worldwide; viruses are the most prevalent cause ARIs^{7,8}. The most common causes of respiratory disease are HRV, influenza virus, coronavirus (CoV), human metapneumovirus (hMPV), adenovirus (AV), bocavirus (BoV), enterovirus (EV), parainfluenza, and respiratory syncytial virus (RSV)^{6,9-12}, as summarized in **table 1**. Patients infected with these viruses show similar clinical manifestations as (1) common cold, (2) flu-like illness, or (3) severe acute respiratory syndrome^{7,13,14}. The host immune response may be a determinant of the severity of disease and not necessarily the viral load¹⁵⁻¹⁷. ARIs are not limited to one etiologic agent (monoinfection) since coinfection has frequently been detected in patients with ARIs. This phenomenon has also been reproduced in experimental models^{9,11,12}. In case of coinfection, two or more viruses have been identified in patients with ARIs¹⁸. Currently, there is controversy about which agent initiates the infection, if a virus facilitates or limits coinfection; moreover, if virus coinfections can determine clinical outcome⁶. The evidence shows that there is no correlation between the number of etiologic viruses involved and the clinical outcome, but the disease tends to be complicated and severe^{19,20}. Additional evidence suggests that patients with a coinfection develop a complicated and prolonged disease with longer hospital and intensive care unit (ICU) stays²¹⁻²³. To

date, little is known about the importance of coinfections and their clinical onset; however, external factors such as the environmental^{24,25}, microbiota²⁶, and geography²⁷ may lead to coinfection, complications, or even limit the disease onset by triggering distinct host defense mechanisms⁶. Otherwise, host factors such as age^{10,21}, location of the infection^{28,29}, and RE defense may be associated with the development and thus disease severity^{22,30}.

Coinfections and clinical manifestations

Viral coinfections are a common finding in patients with ARIs; it varies from 20% to 60% according to different studies^{20,31,32}. A study demonstrated that severe disease is associated with coinfection, being the HRV and influenza A virus (IAV) the most common viral agents coinfecting those patients; however, more studies are needed because there is an underdiagnose of coinfections²⁶.

In the United States of America, respiratory viruses' cause 52% of respiratory infections, 21% of this are by IAV H1N1, and 20% of cases are coinfecting with HRV²⁷. The IAV and HRV are among the most common respiratory viruses to cause disease; the coinfection among these viruses is the most common reported finding. A factor that may promote coinfection is overexpression of the ICAM-1 receptor in IAV infection³³.

The respiratory viruses have a tropism to the upper or lower airway^{13,34}. The clinical manifestations and disease severity are determined by the response of RE and the immune system^{7,35}. Complications of coinfection may

Figure 1. Schematic representation that summarizes the interaction between respiratory epithelium and respiratory viruses. **(A)** Molecules involved in virus entry. **(B)** TLRs, MDA5, RIG-I, and NLRP3 interact with viral genome. **(C)** Signaling pathway induced by respiratory viruses in epithelial cell. **(D)** Interferon, cytokines, chemokines, and other molecules that mediate the antiviral response

include stay in ICU³⁶, respiratory failure³⁷, “cytokine storm”³⁸, and even death³⁰. Allergies³⁹, asthma⁴⁰, genetics, lifestyle, diet, age, and smoking are risk factors associated with severe disease, as demonstrated in patients infected with HRV³², RSV⁴¹, and IAV¹³ infections.

Viral agents, entry receptors

The respiratory viruses have a genome composed of ssRNA, except for AV and BoV, which have a dsDNA

genome⁴²⁻⁴⁴. These viral particles express proteins on their surface that interacts with molecules present in RE (Fig. 1A). The viruses enter the epithelial cell and initiate their replication cycle. RE membrane has ICAM-1⁴⁵, sialic acid (α 2,3-galactose and α 2,6-galactose)^{13,46,47}, and toll-like receptors (TLRs) are involved in viral entry. It is unclear if, after the first viral entry, a restriction or promotion of another viral agents invasion will be present^{33,48}. The RE response may be modified after infection as demonstrated *in vitro* studies^{6,49}.

Entry receptors in RE cells may vary among viruses; HRV uses ICAM-1, the very low-density lipoprotein receptor, and the low-density lipoprotein receptor^{34,39,40}. IAV interacts with sialic acid at the surface of epithelial cells, through the hemagglutinin protein⁴⁶. The RSV F protein interacts with the heparin sulfate present in the cell membrane⁵⁰. hMPV entry mechanism is still unclear. However, there is evidence that protein F is important for the virus to attach to the host cell⁵¹. In human cells, CoV (HCoV-NL63 strain) uses angiotensin-converting enzyme 2 to enter into the cell⁵². MERS-CoV and SARS-CoV use dipeptidyl-peptidase 4 as an entry receptor in RE. CoV-HKU1 and HCoV-OC43 bind to O-acetylated sialic acid and N-Acetyl-9-O-acetylneuraminic acid, respectively^{53,54}. BoV entry receptors are unknown, and the mechanism used to infect host cell has not been described yet^{42,55}. In **figure 1A**, we summarize the viruses and the receptors used for entry.

The RE Immune Response to Viral Infections

RE sensing and activation

RE has three major molecules to detect viral nucleic acids: TLR, retinoic acid-inducible gene I (RIG-I), and melanoma differentiation-associated gene 5 (MDA-5)^{56,57}. The detection of viral agents is critical to establish an antiviral state mediated by TLR3, TLR7, or TLR9 in the endosome. This recognition activates nuclear factor-kappa B (NF- κ B), IRF3, and IRF7 pathways to promote inflammatory and interferon (IFN) response (**Fig. 1B and C**). This response to the viral genome has been demonstrated for IAV, RSV, and HRV^{41,58,59}.

The RIG-I-like receptor detects the 5' triphosphate of the viral RNA, particularly sensing a ssRNA of negative polarity^{34,60}. RIG-I has been shown to be involved in the antiviral response to influenza^{13,47}, hMPV⁶¹, and RSV⁶². MDA-5 shares the signaling pathway with RIG-I, both pattern recognition receptors (PRRs) discriminate between ligands, allowing RE to sense many respiratory viruses and activate the innate immune response to these agents².

The interaction of the caspase recruitment domain (CARD) with the adaptor protein IPS-1, MAVS, VISA, or Cardif will activate protein kinases that phosphorylate the transcription factor IRF-3, leading to the synthesis of type I and type III IFN^{29,63,64}. Activation of RIG-I and MDA-5 by the respiratory virus genome leads to IFN and inflammatory cytokines production^{35,60,65}.

Cytokines and Others Molecules Produced by RE in Response to a Viral Infection

Respiratory mucus contains IgA, lysozyme, cytokines, type I IFN, lactoferrin, human beta-defensins (hBDs), nitric oxide (NO), and other peptides with antimicrobial activity². There is evidence that HRV⁶⁶, IAV⁶⁷, and RSV⁶⁸ induce the production of mucus by activation of proinflammatory signaling pathways. Lactoferrin has antiviral activity against HRV through two mechanisms: (1) blocking cellular receptors⁶⁹ and (2) binding to the viral particles⁷⁰. HBD-2 protects against HRV⁷¹, IAV⁷², and RSV⁷³ infection by neutralizing viral particles and acting synergistically with other cytokines.

NO is necessary for the defense mechanism of RE and for immune system cells to eliminate pathogens and inhibit viral replication^{74,75}. Nitric oxide synthase 2 is constitutively expressed and increases after infection with HRV, IAV, and RSV^{67,76,77}. It has been demonstrated that NO inhibits HRV replication⁷⁸ and in IAV inhibits neuraminidase function NO⁷⁹.

The RE produces type I-IFN and type III-IFN, these induce the expression of interferon-stimulated genes (ISGs) in cells^{29,80}.

Type I-IFN receptors are expressed by all cells and type III-IFN receptors are expressed in the RE⁸¹. The signaling pathways are common; both use JAK-kinase and the phosphorylation of STAT1 and STAT2. The type III receptor is more specific and uses JAK1, JAK2, and TYK2. The activation of IRF1 and IRF7 is necessary for type I-IFN production. In the other hand, IRF1, IRF7, and NF- κ B activation are necessary for type III-IFN production (87, 90, 139). For this reason, type III-IFN is delayed and has a later production and secretion.

The antiviral activation in RE occurs by the expression of ISGs, leading to antiviral protein production⁸²⁻⁸⁴. The difference among the activation of ISGs is that type I-IFNs activate numerous ISGs, but type III-IFNs activates only a selected number of ISGs^{81,85-87} (**Fig. 1D**).

The most important antiviral proteins (ISGs) induced by these IFNs are MxA, MxB, CH23H, IFITIM, and TRIM that affect viral entry. In the other hand, ISG15, UBE1L6, HERC5, HERC6, UBE1LA, TRIM25, and USP18 affect the viral translation and replication. Other ISGs proteins as viperin and tetherin interfere with the egress of the virus^{88,89}. The "ISGylation" is an antiviral mechanism that consists of a covalent union to a viral protein, modifying its electrical charge, leading to inactivation of the virus^{88,89}. Another ISG antiviral

mechanism is due to the decrease of the pH in the lysosome, resulting in viral inactivation⁸¹. It has been demonstrated that ISG15 has IAV antiviral effect after binding with the NS1 protein⁹⁰. However, this effect has not been observed with HRV⁹¹.

It has been demonstrated *in vitro* that the presence of type III-IFN reduces viral titers and the proinflammatory cytokines, but the same expression of antiviral ISGs occurs compared to the stimulus induced by type I-IFN⁹²⁻⁹⁴. Type III-IFN induces a more limited expression of proinflammatory cytokines, avoiding the cytokine storm, preventing tissue damage, and severe complications^{95,96}.

The importance of type III-IFN is such that it is being probed as a potential therapeutic target for influenza infection. Type III-IFN has been tested as an adjuvant in influenza vaccine with promising results since anti-IAV immunity is prolonged^{97,98}. Type III-IFN is predominant in IAV infection⁹⁹; its clinical relevance demands more studies in other respiratory viruses and coinfections.

The inflammasome is a signaling complex composed of Nod-like receptor (NLR), an adaptor apoptotic speck containing protein with a CARD (ASC) and procaspase-1^{100,101}. This signaling complex processes pro-IL1 β and IL-18 into their secreted forms¹⁰². Activation of NLRP3 and production of IL-1 β has been demonstrated in RE in response to an IAV infection^{103,104}; however, there is controversy whether NLRP3 acts directly as a PRR for viral RNA, or if it is activated by another PRR signaling pathway¹⁰⁰.

RE as a Therapeutic Target in Respiratory Viral Infections

RE is a potential therapeutic target to prime the immune system even to induce systemic protection through mucosal vaccination against viral agents¹⁰⁵⁻¹⁰⁷. The use of type III-IFN is a potential therapy for influenza infection when IAV has developed antiviral drug resistance¹⁰⁸⁻¹¹⁰. RE, dendritic, and macrophages cells have been used as a target for respiratory immunization and proven to be effective and less invasive^{105,106,111,112}. An example of mucosal vaccination against RSV, IAV, as well as EV has been published^{107,113-115}.

Respiratory Viruses Exacerbate Chronic Respiratory Conditions

Viral infections exacerbate chronic respiratory conditions such as asthma (38) and chronic obstructive

pulmonary disease (COPD)¹¹⁶. These chronic conditions are characterized by mucus overproduction induced by TH2 and proinflammatory cytokines¹¹⁷⁻¹²⁰. The type II immune response tends to diminish mucus production (88), but in patients with chronic respiratory conditions stimulates overproduction of mucus (42).

There are many proteins and antimicrobial molecules present in the airway¹. The most remarkable is mucus, which creates a semipermeable barrier that traps viral particles and other agents preventing the infection¹²⁰. Respiratory viruses' infection that alters mucus clearance also induces mucus stasis, contributing to inflammation, and exacerbation of the underlying conditions¹²¹⁻¹²³.

MUC5AC and MUC5B are proteins present in human sputum and related to chronic inflammatory conditions^{66,124,125}. It has been demonstrated that HRV16 and RSV increase MUC5AC production in human RE and experimental models, respectively^{68,126}, exacerbating the chronic inflammatory condition.

Respiratory Virome and Its Interactions with the Immune System

The interaction between mammalian and microbiota defines the physiological mechanisms of homeostasis^{127,128}. Microbiota includes virome where the presences of numerous viral particles have been described in different tissues, life stages, host, and environmental conditions^{129,130}. The virome changes through human lifetime^{129,131}, environmental^{130,132}, and internal conditions¹³³. The virome modulates the immune response, promoting the production of cytokines that promote inflammation, anti-inflammation, or regulation^{127,134}. The RE virome has been little studied^{135,136}. HRV and IAV were found in nasal lavages as part of the virome in healthy subjects^{135,137}. This finding proves that the immune response to viral particles depends on the equilibrium between microbiota/virome, internal, and environmental conditions^{127,134}. HRV is present in URT after disease resolution, as demonstrated in a study performed in a published study¹³⁷. The presence of certain viral particles modulates the immune response and leads to different clinical manifestations in chronic diseases¹³⁸⁻¹⁴⁰.

Conclusion

We conclude that RE is an active barrier of the innate immune system with the capacity to sense and initiate an immune response against infections by respiratory viruses. The study of the different responses of the RE

to respiratory viruses is of interest due to the clinical impact of such diseases. The viral coinfection may determine the severity of the disease. Type III-IFN is a potential therapy in respiratory viral infections and may lead to a better outcome of severe disease. The cytokine microenvironment is important because it determines which viruses the individual is more susceptible to. The virome may also determine the immune response to different respiratory virus infections.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Conflict of interest

The authors declare that they have no competing interests.

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