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Review

Advances in understanding respiratory syncytial virus infection in airway epithelial cells and consequential effects on the immune response

Margarita K. Lay [a], Pablo A. González [a], Miguel A. León [a], Pablo F. Céspedes [a], Susan M. Bueno [a,c], Claudia A. Riedel [d], Alexis M. Kalergis [a,b,c,*]

[a] Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago E-8331010, Chile
[b] Departamento de Reumatología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[c] INSERM U1064, Nantes, France
[d] Millennium Institute on Immunology and Immunotherapy, Laboratorio de Biología Celular y Farmacología, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas y Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile

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Abstract

This article reviews aspects of respiratory syncytial virus (RSV) infection in airway epithelial cells (AECs), including cytopathogenesis, entry, replication and the induction of immune response to the virus, including a new role for thymic stromal lymphopoietin in RSV immunopathology.

Keywords: RSV; Airway epithelial cells; Entry; Replication cycle; Immune response; Thymic stromal lymphopoietin

1. Introduction

Respiratory syncytial virus (RSV) is the most prevalent viral etiological agent of acute respiratory tract infection (RTI) and the primary cause of hospitalization due to respiratory disease worldwide, causing major health burden for public health systems (World Health Organization, www.who.org) [1,2].

RSV causes RTI in adults and lower respiratory tract illness (LRTI), including acute bronchiolitis and pneumonia, in infants and young children [3]. Although RSV affects people of all ages, disease is more severe in infants under 6 months of age and infants at risk. The symptoms of RSV infection include labored breathing, coughing, and wheezing that resemble a "violent attack of asthma". Although exposure to RSV early in life was initially associated with increased susceptibility to suffer recurrent allergic wheezing and asthma later in life [4,5], recent studies have proposed that asthma-related genetic traits themselves could predispose to severe disease after RSV infection [6,7]. Thus, the association between severe RSV infection and asthma is reciprocal [8,9].

Despite extensive research efforts, safe and effective vaccines against RSV are currently unavailable. Nevertheless, a prophylactic strategy based on a humanized neutralizing antibody against RSV is widely used in new born at high-risk, such as preterm infants and those suffering from cardiovascular diseases and immunosuppression [10]. This strategy decreases the probability of infection in infants exposed to the virus. However, this approach is costly and unaffordable for most public health systems worldwide. Similarly, ribavirin is an antiviral drug available for treating RSV replication, but it shows debatable cost-effectiveness and it is only recommended for infants at high-risk [11]. Thus, affordable new treatments and vaccines against RSV are highly needed.

* Corresponding author. Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago E-8331010, Chile. Tel.: +56 2 686 2842; fax: +56 2 222 5515.
E-mail address: akalergis@bio.puc.cl (A.M. Kalergis).

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RSV, an enveloped virus belonging to the *Paramyxoviridae* family and the *Pneumovirus* genus, encodes eleven proteins. Early, in the course of the RSV–host interaction, the virus primarily infects AECs. Until recently, little was known about RSV entry into AECs and the resulting cytopathic effects, which include key events that modulate host innate and adaptive immune responses and generate lung disease.

This review article will discuss the interactions between RSV and AECs, examining new experimental evidences and describing RSV pathogenesis, specifically focusing on the entry and budding process, RSV replication cycle, and signaling pathway activation that stimulates innate and adaptive immune responses that could aid in understanding lung inflammation after RSV infection. Based on this information, we propose several models in which virus components and elements of the host immune response are responsible for RSV-induced pathology.

2. RSV pathogenesis in human airway tissues and AECs

Cytopathic effects of RSV in human airway tissues have been observed in histological studies of lung tissue from fatal cases of RSV infection, indicating that human bronchial, bronchiolar and alveolar epithelium are the main targets for RSV infection [12,13]. In these studies RSV infection is restricted to non-contiguous or small clumps of apical ciliated AECs, inducing sloughing of apical AECs, loss of ciliation, sporadic syncytium formation, goblet cell hyperplasia, and mucus hypersecretion, which cause thick plugs in the bronchiolar lumen. These reports also showed that RSV causes cellular infiltration of neutrophils and macrophages in the lung, causing acute pulmonary inflammation [12,13]. Therefore, RSV histopathological studies have been limited to post-mortem airway tissue samples from humans infected by RSV.

Recently, a new *ex vivo*/*in vitro* model of RSV infection, using well-differentiated primary pediatric bronchial epithelial cells (WD-PBECs) was reported [14]. To establish these cell cultures, PBECs were obtained by bronchial brushings from healthy children undergoing elective surgery and cultured on collagen-coated, 12-mm filters in transwells at an air–liquid interface [15]. The particularity of WD-PBECs is that they consist of polarized pseudostratified multilayered epithelium containing ciliated, goblet and basal cells and intact tight junctions. Thus, WD-PBEC cultures have physiological characteristics and thereby represent a relevant model system in which to study RSV/human pediatric respiratory tract interactions.

Following infection of WD-PBECs, immunofluorescence (IF) showed that the distribution of tight junction proteins and ZO-1 were intact at cell edges with no apparent cell monolayer damage, thus RSV did not cause cytopathic effects (CPE). Moreover, two different RSV strains (RSV A2 and clinical isolate BT2a) infected only the apical side of the WD-PBECs, where budding of virus progeny also occurred. These two RSV strains primarily infect ciliated epithelial cells, in a non-contiguous manner, resulting in small clumps of infected cells. A distinctive RSV syncytia CPE in cell lines, was rarely seen in RSV BT2a- or A2-infected WD-PBECs, indicating that cultured monolayer cells derived from tumors (Hep-2, A549 cells, etc.) do not reflect well what is seen in normal cells. Interestingly, RSV did not infect goblet cells. However, RSV infection of WD-PBECs enhanced Mucin-5AC detection by IF, indicating mucus secretion after 6 days. This outcome is consistent with the presence of more goblet cells in RSV-infected WD-PBECs than in controls, suggesting goblet cell hyperplasia as previously shown in the respiratory epithelium of children with fatal cases of RSV [13]. In contrast, infection of WD-PBECs with two different RSV strains induced sloughing and apoptosis of apical epithelial cells determined by TUNEL staining, a method for detecting DNA fragmentation, similar to ciliated cells. This result is consistent with the observation of caspase-3 activity in airway tissues from fatal RSV cases [12]. Thus, RSV induces cell death in ciliated AECs by apoptosis. The results of Villenave et al. [14], using the WD-PBEC model, are consistent with the histopathologic observations in fatal and severe cases of RSV. In summary, similar characteristics of RSV infection and pathogenesis are observed in AECs *in vivo* and in *ex vivo*/*in vitro* models.

3. RSV structure, genome, transcription and replication strategies in AECs

The RSV virion contains a non-segmented, negative-sense RNA genome of 15.2 kb, which is associated with viral polymerase proteins and is packaged within a helical nucleocapsid surrounded by the matrix (M) protein and an envelope derived from the host coated with viral glycoproteins (Fig. 1a). Once RSV enters into AECs, likely by the mechanism described below, the nucleocapsid and RSV polymerase are delivered to the cytoplasm, where the genome is transcribed and replicated.

Because the RSV genome is negative sense (3′–5′), mRNA transcripts encoding RSV proteins are synthesized directly from genomic RNA in the host cell cytoplasm. However, genome replication requires a 5′–3′ antigenome RNA intermediate that is synthesized from the viral genome by the viral RNA polymerase. Both, mRNA transcription and RNA replication are regulated by a unique promoter located in the leader (Le) region at the 3′-end of the viral genome [16]. At the 5′-end of the genome another extragenic sequence: trailer (Tr) is also important for the initiation of RNA synthesis. The complement of the Tr region (TrC) constitutes the 3′-end of the antigenome and contains the promoter that directs the synthesis of new genomes. Proximal to the L region is an encapsidation signal that promotes nascent antigenome RNA, but not mRNA to interact with the viral nucleoprotein (N) during genome replication [17]. This encapsidation signal is also likely to be present in the complementary trailer region (TrC) of the antigenome, although additional studies are required to convincingly demonstrate this possibility (Fig. 1b) [18]. The latter could explain how the antigenome and genome RNAs become encapsidated with N protein as they are synthesized.

Genome replication and viral gene transcription likely occur simultaneously in a regulated fashion to generate adequate amounts of each RNA form for optimal virus production in