DO WE KNOW RHINOVIRUSES AND THEIR CLINICAL IMPACT?

(Mini review)

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ABSTRACT

Acute respiratory infections cause significant morbidity and mortality even before the COVID-19 pandemic. Pandemic restrictions decreased circulation of many respiratory viruses but some less troubling infections such as common cold are still circulating.

One of the most frequent causative agents of common cold are rhinoviruses. The fact that these pathogens have been able to slip through anti-COVID preventive measures raises the question of whether we really know this group of viruses and whether these viruses cause only common cold. The clinical impact of rhinoviruses seems to be underestimated.

In searching of an answer how rhinoviruses have slipped through the anti-COVID precautions we referred to the work of infectious disease specialists, virologists and epidemiologists -much of it conducted decades before the current pandemic. A non-systematic search of the literature is performed. Some of the latest findings on rhinoviruses along with basic knowledge on their biology and clinical impact are summarized in this review.

Keywords: *rhinovirus, common cold, asthma, bronchiolitis*

INTRODUCTION

Rhinoviruses (RVs) are the causative agent for more than a half of the upper respiratory tract infections (1). RVs are widespread and affect all age groups with the highest incidence documented in early childhood (2). Although rhinovirus infections are considered as benign, self-limited and generally mild human diseases, being so common, they have significant economic impact on the health systems and the quality of life (3). The upper respiratory tract is the most common site of the rhinovirus infection, but RVs have been associated with some lower respiratory tract diseases such as bronchitis, bronchiolitis and pneumonia (4).

Rhinovirus infections on top of chronic obstructive pulmonary disease (COPD), asthma, or cystic fibrosis might even become a life-threatening condition (5, 6, 7, 8, 9, 10). Furthermore, RVs have been recognized as a common cause of wheezing in early childhood. Children who experience wheezing during a rhinovirus infection are at a higher risk of asthma development later in life (11, 12). The lack of a specific treatment or vaccines for RVs results in underestimation of their clinical impact. Most often rhinovirus infections are left unobserved and underdiagnosed and hence, the uses of diverse over-the-counter medications or inappropriate and unnecessary antibiotic prescriptions are a common occurrence (13).

BIOLOGY OF RHINOVIRUSES

1. Classification

RVs are extremely heterogeneous group of viruses - members of the *Enterovirus* genus within the *Picornaviridae* family. RVs were discovered in the 1950s and initially were classified into two groups, designated as A and B (RV-A and RV-B) based on their antigenic characteristics and other physical characteristics of the virions (e.g. pH lability). Molecular and genetic characterization of RVs reveals a much greater diversity. More than one-third of the rhinovirus infections are caused by a third group of RVs (RV-C), which do not grow in cells culture and therefore were left undetected until 2006 (14, 15, 16, 17, 18, 19).

To date, 169 RV types have been described. RVs are now assigned to the species *Rhinovirus A* (n=80), *Rhinovirus B* (n=32) and *Rhinovirus C* (n=57): (http://ictv.global/report/picornaviridae/enterovirus/).

Recommendations on the nomenclature of enteroviruses and RVs have recently been published (20). There is a molecular typing system, originally proposed for enteroviruses but then adapted for RVs.

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The accepted threshold for type assignment on the basis of the divergence of the VP1 nucleotide sequences is at least 13% (for RV-A), 12% (RV-B), or 13% (RV-C) nucleotide divergence from all other RV types (17).

Some aspects of the previously used biological classification of RVs are still accepted as far as the groups are partly associated with their genetic relationships. On the basis of the cellular binding sites, RVs are grouped into "major" and "minor" receptor groups.

2. Structure

RVs are small, non-enveloped RNA viruses (21). The virus particle is about 30 nm in size, icosahedral with a pseudo T = 3 (P = 3) type of symmetry. The capsid consists of 60 copies of all four structural proteins referred to as VP1, VP2, VP3 and VP4. On the virion surface there is a centrally located depression called a "canyon" which surrounds the fivefold axis of symmetry of the icosahedron. At the basis of the canyon there is a hydrophobic "pocket", formed by VP1 (22). The canyon frequently serves as the receptor binding site (23). This structure is the target for some antiviral agents (24, 25).

The structural features are common for most of the enteroviruses. Despite the similarities, the capsid of RV-C contains protrusions on its surface which are smoother and spherical. Canyons of RV-C particles are shallower and narrower, and there is no hydrophobic pocket at their floor (26).

3. Genome

The rhinovirus genome is a single positive-stranded RNA of about 7.2 to 7.5 kb in size. The genetic information is coded in a single open reading frame flanked by two untranslated regions (UTRs). Although positive-stranded RNA can serve directly as a messenger RNA for translation, it lacks the typical *cap*-structure at the 5'-end. Instead, there is a small viral protein (VPg) covalently bound to the 5'- end of the genome. The 5' -UTR contains also an internal ribosomal entry site (IRES) allowing translation via *cap*-independent mechanism (27, 28).

4. Replication cycle

RVs utilize several types of cellular receptors: The RVs from the major group, which accounts for about 90% of *Rhinovirus A* and *Rhinovirus B*, utilize intercellular adhesion molecule 1 (ICAM-1) for cell entry (29, 30). The minor receptor group alternatively binds low density lipoprotein receptor (LDLR). RV-C binds a different receptor molecule - human cadherin-related family member 3 (CDHR3) (18, 19). The canyon is the receptor binding site for most of the RVs. For some members of the minor receptor group, however, despite the presence of such a structure in the virion, attachment to the receptor occurs with a star-shaped plateau, located on the fivefold axis of symmetry, which is surrounded by the canyon (31). For some viruses from the major receptor group, heparin sulfate serves as an additional receptor (32).

Viral RNA genome is released into the cytoplasm of the infected cell, where the host-cell translation machinery directly translates positive-sense RNA (33, 34). Translation is initiated by a *cap*-independent mechanism (35), resulting in a single large polyprotein which is further proteolytically processed into ten proteins and several functional intermediates. The replication of the genome takes place on virus-induced membrane structures (36). The process is carried out by a virus specific RNA- dependent RNA polymerase via semi-conservative mechanism (35).

Mature rhinovirus virions exit the host cells without destroying the cell. By analogy with other enteroviruses, a possible spread from cell to cell by microvesicles carrying the virus can be assumed (37, 38).

5. Evolution and genetic diversity

One of the major characteristics of RVs is their vast genetic diversity. Like other RNA viruses this feature arises mostly from the error-prone nature of the viral RNA- dependent RNA polymerase. The frequency of misincorporated nucleotides is 10^{-3} to 10^{-5} per nucleotide site. Fast replication cycle and high mutation frequency result in the existence of mixtures of related, but non-identical viral variants or quasispecies (39). Many of these mutations lead to a variety of amino acids sequences of the capsid proteins, which can explain the existence of many antigenically distinct RV variants (40).

Another possible mechanism for genetic diversity is recombination, and for non-RV enteroviruses recombination has been extensively studied and documented (41, 42, 43). Surprisingly, recombination events in rhinoviruses seem to be rare and are probably limited to ancient events. Evidence for such ancient evolutionary events have been identified as a result of interspecies recombination between RV-A and RV-C in the 5'UTR and 2A sequences (44). Contemporary recombination events among RV circulating strains are believed to occur mainly between the same species and thus would give

rise to recombinants highly related to the parental strains. Such intraspecies recombinations within the coding region have been documented for RV-A (45), but not for RV-B and –C (17, 45).

PATHOGENESIS

1. Transmission

The transmission of rhinoviruses from infected to susceptible individuals occurs via inhalation of viral particles – direct contact, or through a fomite, with self-inoculation into eye or nose in the absence of adequate hand hygiene. RVs are able to survive on hands for several hours, which allow an easy human-to-human transmission through this route, particularly when viral load is higher and secretions are plentiful and difficult to control (46). RVs spread most efficiently within families, school groups, students, and on military bases (47, 48).

2. Target tissues and receptors

The primary site of rhinovirus infection is the nasal mucosa and the airway epithelium. Rhinovirus receptors can be found both in ciliated and non-ciliated epithelium cells of the nasopharynx. Until recently, rhinovirus infection was thought to be restricted to the upper respiratory tract due to temperature sensitivity of the viruses. This was supported by early observations of reduced RV replication at higher temperatures (37°C or 39°C compared to 33°C). Recent studies suggest that rhinovirus replication is reduced by host-defense systems, and particularly interferon-response, because IFN induction is increased at 37°C, compared to 33°C (49). The RV replication is not only effective in lower airway epithelium, but also the difference in replication capacity at lower temperatures is minimal and may be RV type-specific (50, 51).

It was generally accepted that RVs are unable to spread by viremia and to infect other organs. But currently there are multiple studies reporting detection of RV RNA in sinuses (52) or in the middle ear (53). However, infection of these sites is presumed to happen by local extension. The detection of RVs in blood and stools, as well as the great number of different RV types add an extra complexity to the understanding of rhinovirus pathogenesis. It remains unclear if detection of rhinovirus RNA in plasma or stools represents systemic infection (54, 55, 56, 57, 58).

3. Pathogenesis

RVs do not cause epithelial cell destruction by themselves, but as a result of rhinovirus replication the tight junctions between cells are dissociated and hence, the barrier function of the epithelium is compromised. This may increase paracellular permeability and would promote the translocation of the virus and other pathogens like bacteria across the polarized airway epithelial cells, which can result in a complicated disease (59). Some authors suggest similar mechanism for airway inflammation and allergic sensitization and the rhinovirus associated development of asthma (60, 61).

4. Host response

Once rhinovirus infection occurs, the host responds with an impetuous inflammatory response including a variety of antiviral factors, proinflammatory cytokines and chemokines. The concentration of these inflammatory mediators correlates with the severity of symptoms, hereof it is generally accepted that the majority of symptoms are due to the host inflammatory response. There are some indicators that neurogenic reflexes also play a role in the pathogenesis of the infection with parasympathetic nerves controlling the flow of secretions from the nasal seromucous glands (62).

An antibody response to RV infection also occurs with the development of serotype-specific neutralizing serum antibodies and secretory antibodies (IgA) in the airways, detectable usually after one- or two-weeks post inoculation and maintained for at least one year. However, there is large number of RV types, which means repeated infections are common. Moreover, antibody production in natural RV infections occurs on an average only in 50% of patients (63). The resolution of the symptoms and clearance of virus (usually within 7 days) occur before the induction of the antibodies suggesting that clearance involve other mechanism like cellular immune response (64). Taken together immune response against RVs as well as the recovery from rhinovirus infection is still incompletely understood.

5. Clinical syndromes and complications

Human susceptibility to rhinovirus infection is high and depends on age, immune status, and ambient temperature. Risk factors like stress, lack of sleep, tobacco smoke and other air pollutants increase the body susceptibility to RVs (2).

In children, rhinovirus detection in asymptomatic patients ranges from 12% to 40% (65, 66, 67, 68, 69), but only 2% for adults (70). Children are most often the target for rhinovirus infections

and experience up to 12 infections per year. The susceptibility to RV infections decreases with age and an immunocompetent adult may be infected two to three times per year (71) and is more likely to be symptomatic.

The incubation period varies from 1-2 days to 6 days. When symptomatic, the infection has an acute beginning with symptoms peak at 48-72 hours after infection. The duration of the illness is about 7 days on average, but in some cases may be up to 2 weeks (72, 32, 73). The most frequent clinical manifestation is the common cold or acute upper respiratory infection. Symptoms include sore throat, cough, sneezing, nasal congestion, rhinorrhea with clear, muco-watery secretion flows, which later become mucoid or purulent. Low-grade fever, malaise and headache may also be presented. In imunocompetent individuals symptoms spontaneously resolve whithin a week although viral shedding in nasal secretion may continue up to three weeks (73).

Rhinovirus infections are considered as benign, self-limited and generally mild human diseases, but complications are not uncommon.

In children acute otitis media (AOM) is a frequent complication (74) with an abnormal middle ear pressure, swelling and obstruction of the Eustachian tube. AOM may be due to direct viral infection of the middle ear fluid or bacterial co-infection (75).

In adults the frequent complication is acute sinusitis, possibly through the increased pressure during nose blowing, sneezing, and coughing (21). Rhinovirus infection may trigger exacerbation of pre-existing chronic rhinosinusitis, especially in combination with cigarette smoking (76).

It is not uncommon to develop some longer-lasting olfactory disorders after a rhinovirus infection. This complication affects adults, mainly women in a percentage varying from 11% to 40%. In some cases complete recovery may take up to two years (77).

RVs have the ability to infect lower airways and are linked to laryngotracheobronchitis, bronchiolitis and pneumonia. In fact, RVs are the second most common viral causative agent (after respiratory syncytial virus) for children hospitalization due to bronchiolitis and pneumonia (4, 78).

In immunocompromised individuals rhinovirus infections are associated with severe lower respiratory tract disease and fatal pneumonia (79).

The linkage of rhinovirus infection and asthma development and exacerbation has been extensively studied in last decades. Several studies suggest that rhinovirus-induced wheezing in early childhood may be associated with increased risk for recurrent wheezing and subsequent childhood asthma development (11, 12). It is characterized by reversible airflow obstruction, bronchial hyper-responsiveness, and underlying inflammation leading to clinical symptoms (80, 81). RV infection may cause an acute loss of symptoms control or exacerbation. While the association is clear, the mechanisms behind RV-induced asthma exacerbations remain uncertain and many authors suggest that aberrant immune response to RV infection as a possible reason for exacerbation of asthma (reviewed by *Hammond et al.* (81) *and Stone et al.* (82). In addition to asthma, RVs have been associated with more than 40% of acute exacerbations of COPD (21).

DIAGNOSIS

The symptoms of a rhinovirus infection are indistinguishable from those of other viral respiratory pathogens. For that reason etiologic diagnosis rely on laboratory conformation. RVs can be found at the highest titers in nasal secretions, hence nasal secretions and nasal lavage fluids, nasopharyngeal swabs, and combined nose and throat swabs are the most suitable specimen types for diagnostic purposes. Considering the ability of RVs to infect lower respiratory tract, sputum and bronchoalveolar lavage samples also can be used (9). Excreted RVs are at their highest titers during the first days after onset of symptoms (83).

The specific virologic diagnosis is usually done by a molecular assay applying RT-PCR. Although virus isolation is considered as a “golden standard” for identification of viruses, it is a very time-consuming method and hence, is not appropriate for diagnostic purposes. The cytopathic effect, produced by RVs and enteroviruses is quite similar and it cannot be relied on for differentiation. Furthermore, not all RVs grow in cell cultures, like RV-C, in particular. Consequently laboratory confirmation is rarely performed by viral culture methods.

Rapidly advancing molecular methods have led to a better understanding of the burden of diseases associated with RV infection and RT-PCR is proven to be efficient, sensitive and specific for detection of RVs. RT-PCR assays use primers that target a conserved

region in the 5'-UTR of the rhinovirus genome, but still there is the problem of differentiation between rhinoviruses and enteroviruses and rhinovirus typing can be done only by sequencing (62).

TREATMENT

Picornaviruses are one of the most studied virus group and there are plenty of compounds tested for antiviral activity against them. Many compounds alone or in combination exhibit anti-rhinoviral activity *in vitro* (84, 85, 86). However, currently there is no specific antiviral therapeutic agent that is licensed for treatment of rhinovirus infections. A few agents showed modest results in decreasing either symptom severity or viral activity, in clinical trials (62, 21). Currently, the therapy is supportive with the use of over-the-counter products aimed at symptoms relief. These include nonsteroidal anti-inflammatory drugs, antihistamines, decongestants, and anticholinergic nasal solutions. The use of antihistamines is a subject of debates, because the beneficial effect on severity of symptoms is limited and short-term, and they are often associated with side effects like sedation (62, 87).

PREVENTION

Since the transmission of RVs occurs via a direct contact, adequate hand hygiene is the most appropriate and effective preventive strategy. It should be noted that the lack of lipid envelope in the virion makes RVs resistant to ether, chloroform, ethanol and other organic solvents so that ethanol-containing hand rubs should be avoided as a substitute for hand washing with soap and water (88). In the presence of contaminated surfaces, handwashing could be insufficient to prevent transmission. Effective disinfection of environmental surfaces could be applied with the use of bleach, phenol-based and ammonium-based environmental surface disinfectants (89).

The development of vaccines for specific prevention is labored due to the large number of RV types, the lack of common group antigen and large genetic variability in antigenic regions. Moreover, unlike influenza where usually one strain dominates a given flu season and the vaccine can be tailored to match, RVs do not have such pattern and several strains co-circulate simultaneously in a given population at any given time (90). RVs replicate only in higher primates and the lack of suitable small animal model to test vaccine candidate effectiveness add an extra complexity to developing of cross-serotype rhinovirus vaccine. For that reason RV vaccine research was abandoned for more than 20 years. Recent progress in molecular techniques and sequencing of RV genomes (91), including RV-C (19), as well as developments of mouse models may speed-up the process and maybe a vaccine against all rhinovirus serotypes could be possible (92).

EPIDEMIOLOGY

Not much is known about the circulation pattern of rhinoviruses. This is mostly because RV infection is considered as mild and often is not diagnosed. Understanding of RV distribution is also hampered by diagnostic methods used until recently. Molecular epidemiological studies as well as whole-genome sequencing of circulating viruses may contribute to clearly understand the virus's circulation patterns. The seasonality of RV is still not clearly understood as well. In the temperate zone, respiratory infections are traditionally associated with the colder part of the year. Growing number of studies reported RV detections in all seasons with slightly higher incidence rate in the autumn and spring (93, 94). It is only in winter that other infective agents predominate (95). In many parts of the world, including many European countries and Bulgaria rhinovirus infections are left unobserved and therefore, not much is known about their circulation patterns and seasonality.

CONCLUSIONS

Even with the advances of today's medicine and health-care systems RVs constitute a significant burden with associated sociological and economic impact. It is more concerning that in the ongoing COVID-19 pandemic and all the precautions, rhinoviruses are still there. To date, 169 RV types have been described. What we know is that RVs are characterized with vast genetic diversity due to high mutation frequency and recombinantions. This can explain the existence of many antigenically distinct RV variants, but present knowledge still does not provide a strategy for controlling them. Human susceptibility to rhinovirus infection is high and symptoms of a RV infection are indistinguishable from those of other viral respiratory pathogens. The lack of lipid envelope in the virion makes RVs resistant to ethanol-containing hand sanitizers, which are widely

recommended as a precaution against other viruses. In the light of current COVID-19 pandemic, it should be kept in mind that olfactory disorders are not uncommon after a rhinovirus infection.

Although upper respiratory tract is the most common site of the rhinovirus infection, it remains unclear whether RVs are able to cause systemic infection. Moreover, linkage of RV with complicated lower respiratory tract diseases like bronchiolitis and pneumonia underlines the fact that RVs are not such a benign cause of the ordinary common cold. For people living with COPD or asthma mere rhinovirus infections might become a life-threatening condition. The development of vaccines for specific prevention is labored due to the large number of RV types, the lack of common group antigen and the large genetic variability in antigenic regions.

There are still many aspects of rhinovirus pathogenesis, immune response, as well as the recovery from infection that are not fully understood. Design of effective preventive and therapeutic strategies to control RVs will be supported by improved knowledge of their pathogenesis, immune response and transmission.

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