AN UPDATE ON THE PATHOPHYSIOLOGY OF RHINOVIRUS UPPER RESPIRATORY TRACT INFECTIONS

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Summary

Upper respiratory tract infections are one of the most common infectious diseases in man and are characterized by relatively mild symptoms. However, complications of bacterial superinfection or asthma exacerbations are not seldomly seen. Most upper respiratory tract infections are caused by rhinoviruses. The rhinovirus is a non-enveloped 30 nm RNA-virus with over 100 serotypes that belongs to the Picornaviridae family and only replicates in primates. It is characterized by a single positive stranded genome acting not only as a template for RNA synthesis, but also encoding for a single polypeptide necessary for viral replication. The viral capsid has an icosahedral symmetry and demonstrates deep canyons, with a receptor-binding domain. Rhinoviruses are transmitted mainly via direct or indirect contact with infected secretions and invade their host by binding to the ICAM-1 receptor on the nasal epithelium. Typical for rhinovirus upper respiratory tract infections are isolated scattered foci of infected epithelium, not showing any striking damage or cytopathic alterations, between large areas of normal epithelium.

Today there is still little detailed knowledge on the pathophysiology of common cold, especially on the aspect of cellular migration and defense. A better understanding in mechanisms underlying this cellular response would not only have therapeutical consequences, but may also explain the relationship between viral infectious rhinitis and asthma or atopy.

During a rhinovirus infection, a selective neutrophil and monocyte recruitment is observed. In vitro and in vivo data have demonstrated a time-limited, rhinovirus-induced increase in bradykinin, cytokine, chemokine and sICAM-1 concentrations. Epithelial derived proinflammatory cytokines initiate an adhesion cascade and activate T lymphocytes that create a TH1-type cytokine environment within the infected tissue, necessary to eradicate the viral infection. The selective recruitment of neutrophils seems linked to increased concentrations of the chemokine IL-8 and common cold symptoms. It is doubtful that the cytokine-regulated-production of specific neutralising immunoglobulins is necessary for recovery from viral illnesses and presumably only contributes to a late and temporary protection against rhinovirus reinfection.

These observations confirm the crucial role that cytokines and mediators play in the pathogenesis of a rhinovirus infection by mediating chemotaxis, transmigration and activation of inflammatory-and immunocompetent cells.

Introduction

An upper respiratory tract infection is characterized by rhinorrhea, nasal congestion and sneezing, sometimes accompanied by fever, a sore throat and malaise. It is one of the most frequent infectious
diseases in man\(^1\). Throughout our lifetime, we spend between 1 to 2 years suffering from a cold\(^2\). Especially preschool children often, approximately 6 up to 12 times a year, suffer from an upper respiratory infection while the incidence decreases with age and averages 2 to 3 common colds a year in adolescents and adults\(^3\). Although these infections are generally mild and self-limited, they account for a big economical burden due to an enormous loss in productivity and high medical costs. In the United States, the morbidity of an upper respiratory tract infection accounts for approximately 26 million days of school absence and 23 million days of work absence annually\(^3\). Each year, U.S. physicians are consulted for an upper respiratory tract infection about 27 million times and almost $2 billion are spent on over the counter medications for treatment of common cold symptoms\(^4\).

A bacterial invasion may follow the initial infection, and it is these invaders that may produce complications like otitis media, acute sinusitis or even pneumonia with sometimes lethal impact\(^5\). In the elderly, for example, a Staphylococcus aureus co-infection in an influenza upper respiratory tract infection may result in a pneumonia with a mortality rate up to 42%\(^6\). In addition, several epidemiological studies have demonstrated that an upper respiratory tract infection is a potent trigger for exacerbations of asthma\(^6\). Not only does it increase the nonspecific airway responsiveness, but it also changes the immediate and late responses to allergen challenge\(^7\).

However, despite its high prevalence and its severe complications, there is still little detailed knowledge on the pathogenesis of viral rhinitis, especially on the aspect of cellular migration and immune response.

In the present article, we intend to review the pathophysiology of viral rhinitis with special attention to the role of cytokines in initiating the anti-infectious response.

**Classification**

Viruses cause about 80% of the nasal infections\(^8\). It is generally accepted that approximately one third to half of the viral upper respiratory tract infections in adults are caused by rhinoviruses while coronaviruses account for about 10%\(^9\). However, using the sensitive reverse transcription polymerase chain reaction technique (RT–PCR), the relative importance of rhinoviruses has recently increased and they are now held responsible for up to 80% of the common colds in adults in autumn\(^10\). Other viruses like influenza, parainfluenza, adenoviruses and respiratory syncytial virus (RSV) cause less frequently upper respiratory tract infections in adults\(^11\). However, a strict prediction of the causing virus is impossible since the causing agent varies according to population, age and season. For example, outbreaks throughout the year of adenovirus infections are frequently seen at children's summer camp (swimming pool pharyngo-conjunctivitis) but also among military recruits (acute respiratory disease)\(^5\). A true epidemic virus is RSV, appearing only in wintertime, especially January, while a rhinovirus infection is typically an endemic disease with a peak incidence in spring and autumn\(^10\).

**Rhinovirus**

Since upper respiratory tract infections are resulting for the greater part from rhinoviruses, we will focus on this virus in the present article.

The rhinovirus is a non-enveloped 30 nm RNA-virus with over 100 serotypes and only replicates in primates\(^9\). It belongs to the Picornaviridae family, consisting of small RNA-viruses (‘Pico RNA Viruses’) also including enteroviruses, aphtho-and cardioviruses and hepatitis A virus\(^9\). It is characterized by a single positive stranded genome encoding for a single polypeptide encapsidated in a protein shell. The capsid has an icosahedral symmetry and consists of 60 copies of three proteins VP1, VP2, VP3 (Figure 1.). Deep canyons, with a receptor-binding domain, encircle each vertex formed by five VP1 proteins\(^2,12,13\). While the rest of the virus surface constantly mutates thereby escaping the host immune system, these canyons maintain their antigenic specificity. However, this immunogenic stable receptor binding domain escapes possible host neutralization, as the canyons are physically too narrow for the Fab antibody portion, but large enough to admit the host cell receptor\(^2,11\).

The major ligand binding site for rhinoviruses is ICAM-1 (intercellular adhesion receptor molecule-1),
a receptor that belongs to the immunoglobulin superfamily and is constitutively expressed on basal respiratory epithelial cells. Approximately 90% of the rhinoviruses infect their host by binding to this receptor, mainly located in the ICAM-1 rich area of the adenoid. After intracellular invasion and replication, the rhinovirus infection spreads intranasally and to the pharynx. Typical for rhinovirus infections are the isolated scattered foci of infected epithelium between large areas of normal epithelium. In contrast to other common cold viruses like influenza and adenoviruses, the epithelium does not show any striking damage or cytopathic alterations during a rhinovirus infection.

A rhinovirus is spread from person to person by virus contaminated respiratory secretions, partially through inhalation of small- or large-particle aerosols, but mainly via direct- or indirect contact with infected secretions. In 40% up to 90% of common cold patients, a rhinovirus could be detected on the hands presumably due to frequent contact with the virus-shedding nose. Hendley and Gwaltney supported the importance of hand-to-hand transmission by demonstrating that treatment of the hands with a virucidal compound significantly reduced transmission of rhinovirus infection. Since rhinoviruses retain their virulence up to 3 days on plastic surfaces, transmission is very easy. Once the hands are contaminated, introduction of a finger in the eyes or nose will do to efficiently initiate infection.

**The host response**

Once the epithelium is invaded and viral replication has started, inflammatory- and immune responses are evoked by the host. Vasodilatation and an increased vascular permeability, a cellular infiltration and the release of various mediators characterize this response. Besides, an increased mucus production by seromucous glands and irritation of the sensible nerve receptors in the subepithelium and epithelium is observed. Finally, two to three weeks after infection, specific neutralising antibodies can be detected in serum as well as in nasal secretions.

1. **Cellular immune response**

Several studies have demonstrated increased neutrophil counts in the nasal mucosa, nasal secretions as well as peripheral blood of symptomatic subjects during both natural- and experimental common colds. A nasal biopsy study found increased neutrophil numbers on the first and second day of infection while others failed to show an alteration in neutrophil number in biopsies performed on day 4 of illness. Naclerio and coworkers also observed an early but transient increase in neutrophil count in nasal washes within 24 h after rhinovirus inoculation.

Some days after the inflammatory response has begun, the recruitment of monocytes that cross the endothelium to become tissue macrophages was observed.

In contrast to the clear increase in neutrophils, the involvement of lymphocytes is still a matter of debate. Winther et al studied lymphocyte populations in the nasal mucosa during an experimentally induced viral rhinitis by immunohistochemistry and found no striking alterations in the number nor subset distribution.
of T-and B-lymphocytes in comparison to normal nasal mucosa\textsuperscript{28}. Nevertheless, Lewandowski et al demonstrated an increased number of T-lymphocytes in nasal secretions, a reduced number of circulating lymphocytes and a decreased serum T-helper/T-suppressor ratio during an experimentally induced rhinovirus infection\textsuperscript{29}. They assumed that the reduced number of circulating T-lymphocytes was related to the recruitment of T-lymphocytes in the nasal mucosa.

Despite the discrepant findings on lymphocyte count, unanimity exists on the T-helper cell polarisation (see below).

2. Mediators and cytokines

The observed migration of immune effector cells and their subsequent activation is orchestrated by mediators and cytokines. These proteins regulate chemotaxis, cellular differentiation and activation by the induction of adhesion molecule expression and the release of additive, synergistic or antagonistic cytokines. A rhinovirus infection is supposed to trigger the synthesis and release of these mediators and cytokines resulting in a cascade of inflammatory reactions, which are held responsible for common cold symptoms (Figure 2).

\textit{In vitro experiments}

Indeed, \textit{in vitro} studies of cell cultures have demonstrated the production of IL-8 in response to rhinoviruses stimulation in both fibroblasts as well as respiratory epithelial cells\textsuperscript{30}\textsuperscript{32}. Not only IL-8, but also IL-6 were found to be released in human rhinovirus infected epithelial cell lines\textsuperscript{33}. Similar cytokines were produced after RSV challenge, indicating the virustype nonspecific induction of cytokine production\textsuperscript{34}\textsuperscript{35}.

Especially IL-8 is of great importance in rhinovirus colds, as it is a strong chemoattractant for neutrophils\textsuperscript{36}. It belongs to the C–X–C family of chemokines and induces the expression of $\beta_2$-integrins, namely LFA-1 and Mac-1 on neutrophils, which bind to the endothelium via adhesion receptor molecules that mediate their transendothelial migration\textsuperscript{36}\textsuperscript{37}. Besides, IL-8 is capable of activating the recruited neutrophils, resulting in the release of their cytotoxic granule content\textsuperscript{38}\textsuperscript{39}.

Another cytokine found to be released is IL-6. Zhu et al demonstrated in an \textit{in vitro} model significantly elevated IL-6 protein production 24–72 hours after infection with major, ICAM-1 mediated, as well as minor receptor group rhinoviruses, indicating that rhinoviruses influence IL-6 production at a post receptor level\textsuperscript{33}. IL-6 is a pleiotropic cytokine. It has

![Figure 2](image-url)
activating and proliferative effects on lymphocytes. Namely, under the influence of IL-6, B-lymphocytes mature, differentiate into plasma cells and the subsequent production of immunoglobulins is stimulated. In addition, IL-6 mediates T-cell activation, growth and differentiation. The critical role of IL-6 in initiating humoral responses is confirmed by IL-6 knockout mice with deficient immunoglobulin responses after viral challenge. Furthermore, IL-6 plays an important role in the host response to trauma by inducing acute phase proteins such as C-reactive protein. Finally, it could participate in common cold symptoms, as it induces pyrexia. In fact, a direct correlation between IL-6 and symptom severity has been shown in experimental induced rhinovirus colds.

Not only induce rhinoviruses the local production of these and other cytokines, they also influence per se the expression of their receptor molecule, ICAM-1, on epithelial cells.

In vitro studies have shown that rhinovirus infected nasal epithelial cells display a significant upregulation of ICAM-1 expression compared to preinoculation receptor numbers. This direct effect of rhinovirus infection on ICAM-1 expression facilitates further viral spread by increasing cell attachment and entry. Besides, rhinoviruses also exert indirect ICAM-1 upregulating effects via the induction of cytokine production. For instance, in vitro pre-treatment of uninfected nasal epithelial cells with IL-8 increases ICAM-1 expression, whilst IL-8 pre-treatment followed by rhinovirus inoculation additionally upregulates epithelial ICAM-1 expression. However, it has been shown that epithelial ICAM-1 expression depends on the kind of mediator released, as also ICAM-1 downregulating cytokines have been found.

Studies in experimentally induced common cold

Besides in in vitro experiments, mediator and cytokine production was also studied in experimentally induced rhinovirus upper respiratory tract infection in humans.

Largely contributing to our current ‘cold’ understanding was the work of Naclerio and coworkers, who challenged healthy volunteers with two strains of rhinoviruses (T39 and HH) and performed nasal washes every four hours around the clock for five days. They found no alterations in histamine concentrations, but significantly increased levels of kinins in nasal lavage fluid of infected symptomatic subjects compared to baseline values. This observation, together with nasal biopsy studies showing no change neither in mast cell number nor mast cell degranulation, suggests that mast cells and basophils do not participate in the pathophysiology of a rhinovirus infection.

The observed 10-fold increase in kinin concentrations of infected-ill subjects compared to pre-chal-
lengle levels contrasted the relative unchanged levels in infected non-ill and control subjects. Besides, these increased kinin concentrations correlated with the severity of common cold symptoms.

Bradykinin is a known, potent inflammatory mediator, which causes vasodilatation, increased vascular permeability and stimulates pain and glandular secretion via neuronal reflexes. When administered intranasally in normal subjects, symptoms of nasal obstruction, rhinorrhea and a sore throat are evoked, suggesting that this mediator might contribute to common cold symptoms. However, a possible causal role of kinins in the development of common cold symptoms can only be established when kinin-antagonists are available to block their action.

Recently, Proud et al reported increased IL-1β concentrations in nasal wash fluids of infected symptomatic subjects compared to infected asymptomatic and noninfected volunteers during an experimental rhinovirus cold.

IL-1β has important pro-inflammatory properties. It enhances the expression of adhesion molecules such as E-selectin and ICAM-1 on endothelial cells. These endothelial receptor molecules induce the recruitment and migration of granulocytes and lymphocytes to the site of inflammation by interacting with their cognate receptor. However, IL-1β also upregulates ICAM-1 expression on epithelial cells.

Therefore, by upregulating ICAM-1 expression, IL-1β possibly exerts dual effects on rhinovirus infections. First, it initiates the host response to infection by enhancing the recruitment of immune effector cells into the inflammatory site, and second, it could increase rhinoviral spread via upregulation of its receptor. Besides, IL-1β also exerts important activating and proliferative effects on lymphocytes via IL-2 receptor induction, thus stimulating the specific cellular host response as well. In addition, IL-1β increases vascular permeability and induces the release of other pro-inflammatory mediators like platelet-activating factor (PAF), as well as the cytokine IL-8 from epithelial cells.

As viruses, in contrast to bacteria, remain intracellular a cell-mediated immune response is essential to eradicate a rhinovirus infection. The most important cytokine for macrophage activation and thereby for cell mediated immunity is IFN-γ. It stimulates macrophage accumulation, activation and cytokine production. In addition, it stimulates NK cell function and antigen specific B-cell proliferation. In fact, in experimentally induced upper respiratory tract infections increased concentrations of IFN-γ were observed in nasal secretions of symptomatic subjects. As IFN-γ is mainly produced by T-helper-1 cells, this observation demonstrates a polarization of the T-lymphocyte response towards a Th1 response necessary to overcome the rhinovirus infection. Interestingly, in vitro experiments have shown an additional antiviral function of IFN-γ, as it persistently downregulates ICAM-1 expression on rhinovirus infected epithelial cells.

Studies in naturally acquired common cold

Studies in naturally acquired upper respiratory tract infections have confirmed the increased concentrations of IL-1β, IL-6, IL-8 and IFN-γ in nasal washes of symptomatic subjects compared to baseline values. Noah et al also observed significantly elevated TNF-α levels, which gradually returned to normal values in nasal lavage fluids of children during acute upper respiratory tract infections. Like IL-1β, TNF-α has strong proinflammatory properties, as it co-stimulates T- and B-lymphocytes and activates endothelial cells to express adhesion molecules and to release further cytokines. In addition, it is a potent activator of neutrophils, mediating adherence, chemotaxis and degranulation. Furthermore, it induces vascular leakage and stimulates MHC class I or II expression on various cells. Finally, it activates macrophages and augments their capacity to release inflammatory mediators and cytokines such as IL-6 and IL-8.

Recently, we have focussed on the time course of release of proinflammatory cytokines, chemokines and MPO (myeloperoxidase) in nasal lavages of subjects during a naturally acquired upper respiratory tract infection. Compared to baseline, we found transient, significantly elevated concentrations of IL-1β, IL-6, TNF-α, IL-8, MPO, IFN-γ and MCP-1 (monocyte chemotactic protein-1) in nasal secretions.
which all returned to baseline levels three weeks after the symptomatic cold. In addition we measured the concentrations of IL-1ra, the naturally occurring IL-1 receptor antagonist, which levels have been found unaltered during the overall study period. Since IL-1ra binds to the IL-1 receptors without effecting a biological response, constant IL-1ra concentrations and increased levels of IL-1β would result in favoring the proinflammatory process.

Interestingly, we observed a rapid increase in IL-8 that is held responsible for the early neutrophil recruitment observed in nasal biopsy studies. Furthermore, a strong correlation was found between IL-8 and neutrophil MPO levels, a cytoplasmic granule constituent, which is set free by activated neutrophils. This observation demonstrates the major role of IL-8 in neutrophil recruitment and activation with subsequent degranulation in rhinovirus colds. MPO and other neutrophilic enzymes like lysozyme and elastase have been shown to cause severe tissue damage. In fact, Teran and coworkers found a direct relation between MPO concentrations and the severity of common cold symptoms in children with virus induced asthma. These correlations between IL-8, MPO and common cold symptoms were very recently confirmed by Turner et al, who observed a direct relation between the magnitude of the rise in IL-8 and the severity of common cold symptoms in volunteers after rhinovirus type 23 inoculation.

Similar to the elevated IL-8 levels in nasal lavage fluids, we found significantly increased concentrations of MCP-1 compared to baseline during the study period. MCP-1 is a member of the C-C chemokine family, which preferentially attracts and stimulates monocytes. The increase in MCP-1 levels is in accordance with the observed monocyte mucosal infiltration in other studies.

Finally, besides cytokines and chemokine, we also focussed on the soluble form of the adhesion molecule ICAM-1, sICAM-1, which was found to be elevated in nasal secretions of naturally acquired common cold patients. This increase is likely to be secondary to an upregulated epithelial ICAM-1 expression by cytokines, followed by shedding of the molecule from the cell surface into nasal secretions. In contrast to cell-bound ICAM-1, the exact function of sICAM-1 is not clarified yet. It could inhibit rhinovirus infection, block the recruitment of inflammatory cells or serve just as a marker of infection.

Summarizing, we could state that a rhinovirus infection induces an increased release of epithelial derived proinflammatory cytokines, which initiate an adhesion cascade and activate the recruited inflammatory cells. These cells produce themselves cytokines, resulting in an amplification of the inflammatory process. The typical selective neutrophil recruitment seems linked to the increased concentrations of IL-8 and common cold symptoms.

In addition, T lymphocytes are activated to create a TH1-type cytokine environment, necessary to eradicate the virus infection. This entire process seems very subtle regulated by the type and time kinetics of the inflammatory cytokines in the microenvironment.

3. Humoral immune response

During a rhinovirus infection, an increase in IgG concentrations can be observed in nasal secretions in the early course of a rhinovirus illness. IgG is partially produced locally, but predominantly serum derived. However, as it is not virus specific it only amplifies the inflammatory process by activation of the classical complement pathway and contributes to the host immune response by antigen opsonisation.

Local and systemic neutralising antibodies usually are not detectable until 2 to 3 weeks after rhinovirus infection, indicating that alternate, previously discussed, cell mediated defence mechanisms combat the acute infection. Therefore, the role of neutralising antibodies in rhinovirus infections seems limited to rhinovirus serotype specific protection.

In an earlier study, we investigated the protective serum antibody level in subjects experimentally infected by rhinovirus type 2. A seroconversion, defined as a fourfold increase of specific neutralising antibodies, could be observed in 41 out of 50 volunteers (82%) three weeks after inoculation. Subjects with an initial antibody titre greater than 16 did not show seroconversion, indicating the protective serum level of specific neutralising antibodies. Six months after inoculation, only 40.9% of the subjects...
still demonstrated a seroconversion. After 9 months, this percentage dropped to 10% indicating an only transient increase in specific neutralising antibodies. This results in susceptibility for a rhinovirus infection even with the same serotype as early as nine months after primary infection. Furthermore, it has been suggested recently that the protection against upper respiratory tract infection rather results from a transient production of slgA (secretory IgA) by mucosal plasma cells than increased serum neutralising antibodies indicating that even high serum level of specific neutralising antibodies do not always protect against reinfection.

**Conclusion**

A rhinovirus infection represents a neutrophilic inflammatory reaction with relatively mild symptoms. In vitro and in vivo data have demonstrated a time-limited, rhinovirus-induced, increase in bradykinin, cytokine, chemokine and sICAM-1 concentrations. Epithelial derived proinflammatory cytokines initiate an adhesion cascade and activate T-lymphocytes that create a TH1-type cytokine environment within the infected tissue, necessary to eradicate the virus infection. The selective recruitment of neutrophils seems linked to the increased concentration of the chemokine IL-8 and common cold symptoms. The cytokine-regulated-production of specific neutralising immunoglobulins only contributes to a temporary protection against rhinovirus reinfection. These observations confirm the crucial role that cytokines and mediators play in the pathogenesis of a rhinovirus infection.

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**Table 1** Seroconversion of neutralising antibodies after intranasal challenge of 50 volunteers with rhinovirus type 2 in relation to the pre-challenge serum antibody titre.

<table>
<thead>
<tr>
<th>Initial antibody titre</th>
<th>Seroconversion (n)</th>
<th>No-seroconversion (n)</th>
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<tr>
<td>&lt;2</td>
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</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>9</td>
</tr>
</tbody>
</table>

n = number of subjects
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