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The cold case: Are rhinoviruses perfectly adapted pathogens?

S. Dreschers*, C. A. Dumitru, C. Adams and E. Gulbins

Dept. of Molecular Biology, University of Duisburg-Essen, Hufelandstr. 55 (Germany), 45122 Essen,
Fax: +49 201 7235974, e-mail: stephan.dreschers@uni-essen.de

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Abstract. Rhinoviruses, which cause common cold, belong to the Picornaviridae family, small non-enveloped viruses (diameter 15–30 nm) containing a single-stranded RNA genome (about 7 kb). Over 100 different rhinoviral serotypes have been identified thus far, establishing rhinoviruses as the most diverse group of Picornaviridae. Based on receptor binding properties, rhinoviruses are divided into two classes: the major group binding to intracellular adhesion molecule-1 and the minor group binding to the very low density lipoprotein receptors. Interactions between virus and the receptor molecules cause a conformational change in the capsid, which is a prerequisite for viral uptake. Rhinoviruses trigger a chemokine response upon infection that may lead to exacerbation of the symptoms of common cold, i.e. asthma and inflammation. The following review aims to summarize the knowledge about rhinoviral infections and discusses therapeutical approaches against this almost perfectly adapted pathogen.

Keywords. Rhinoviruses, signal transduction, endocytosis, anti-viral therapy.

Introduction

Sneezing, scratchy throat, runny nose – everyone knows the first signs of a common cold, probably the most common illness known to man and representing almost the prototype of a viral infection. From history to our time the common cold is a disease frequently described in publications. Famous ancient investigators, among them Hippocrates and Benjamin Franklin to name two, observed the relation between crowded facilities (market halls, schoolhouses etc.) and the spread of the common cold. They were the first to suggest respiratory contamination as the origin of infection. However, isolation and identification of rhinoviruses was only possible after establishing cell and tissue culture techniques in the middle of the 20th century. The progress of molecular biology allowed the isolation and *in vitro* culture of rhinoviruses as well as their ultrastructural and molecular analysis. But even today many questions about rhinoviral infection remain to be answered.

Initially, rhinoviruses were purified from nasal washings of patients suffering from common cold, and human or primate tissues were required for propagation. More recently, culture of rhinoviruses was established in HeLa epithelial cells and WI-38 fibroblasts. The characterization of rhinoviruses was started using serological methods. Polyclonal antisera that neutralized rhinoviral strains were purified using plaque assay and tested for their neutralizing potential in every freshly purified strain. Absence of cross-reactivity of polyclonal antisera with established serotypes constitutes the criterion for a new serotype [1]. Accompanied by serological characterization, the rhinoviral genome was analyzed by molecular genetic techniques. The first fundamental observation was that the rhinoviral genome consists of a single, approximately 7-kb-long RNA strand in positive orientation. An RNA sequence analysis revealed a close genetic similarity to polio- and coxsackiviruses, so that rhinoviruses were taxonomically identified as members of the Picornaviridae family.

Further analysis underlined the functional uniformity of the rhinoviral genome compared with other members of

* Corresponding author.

the Picornaviridae family. The 5′end of the RNA is joined to a small viral protein called Vpg, which is a prerequisite for the synthesis of the negative RNA strand as a template for replication. The 5′end also contains the IRES (internal ribosomal entry site) sequence and allows binding to the ribosomal subunits of the host translational machinery. Following the IRES sequence is a single long open-reading frame covering the information for structural (or capsid forming) proteins designated as the P1 unit and non-structural proteins (proteases and RNA-dependent RNA polymerase) designated as the P2/P3 unit(s) (see Fig. 1d). The protease 2A coded by the P2 unit was found to have multiple functions. It is required for the inital proteolytic separation of the P1 and P2/P3 units in the nascent rhinoviral polyprotein, but also inactivates the cellular *eLF-4F* complex, thereby inhibiting translation of the cellular messenger RNA (mRNA) and initiating the VHS (virus host shutoff – the translational machinery of the host cell produces viral proteins only). Further steps of post-translational maturation are catalyzed by the protease 3C/3CD (see below). Products of the latter proteolytic activity are Vpg protein and RNA polymerase. The RNA genome is completed by the 3′ non-translated region and a poly-A signal. The genomic organization is similar in all genera of Picornaviridae; only the genome of Aphto- and Cardioviridae (for example the foot-and-mouth-disease virus) contains an additional gene (leader) at the 5′end of the RNA. The genetical dissection of the very compact genome of Picornaviridae in general and rhinoviruses in particular elucidated the complex temporal and spatial processes leading to the synthesis of new rhinoviruses. Synthesis of rhinoviral RNA could be detected 12 h post-infection, and newly synthesized viruses were present in the host cell already 24 h post-infection. The production of viral RNA and viral particles peaks within 48–72 h and declines thereafter. The rapid propagation of viral replication and synthesis is one of the most powerful weapons to escape the immune response because relevant titers of immunglobulins are only produced 3–4 days after infection – too late to protect the host from the rhinoviral assault.

In the following sections of this review we focus on experimental results that show functional correlations between the virus, its receptor(s) and mechanisms that propagate the infection and its symptoms. Moreover, we will discuss novel therapeutical strategies.

Canyons and crevices: rhinoviral structure and its interactions with cellular receptors

Human rhinoviruses represent the serologically most diverse group of picornaviruses (over 100 serotypes have already been identified). The reason for this diversity was thought to be determined by an altered ultrastructure of viral capsids. The capsid is composed of four proteins (viral proteins (VP), VP1–VP4, see Fig. 1a–e), which arise from a single polyprotein generated from the viral RNA genome functioning as an mRNA. The very first cleavage occurs while translation is still in process. This first proteolytic maturation *in cis* is done by the protease 2A, which separates structural proteins from those necessary for replication (designated as P1 and P2/P3, see Fig. 1d, e). A series of further cleavages form VP1, VP3 and VP0; the latter is finally processed to result in VP2 and VP4 [2]. Picornaviruses undergo VP0 cleavage during virion maturation. Apparently, intact VP0 is necessary for correct assembly of the protomers, whereas processing of VP0 is necessary for final maturation of the virion. It is possible that the cleavage of VP0 is also a prerequisite for uncoating [2]. The last proteolytic steps are catalyzed by the viral protease 3C, which therefore is a target for a protease inhibitor screen (see below).

Ultrastructural analysis of rhinoviruses by X-ray crystallography [3, 4] resulted in a structural model of rhinoviruses (Fig. 1a–c). Along the fivefold icosahedric axis of the virion a 2.5-nm depression, the so-called canyon was detected. The genomic region encoding the peptide residues that form this structure is more conserved than regions encoding any other structure on the virion surface [4–6]. Studies using mutated viral strains showed that point mutations in the genomic region covering the capsid protein VP1 changed the affinity of radiolabeled mutant viruses to purified host cell membranes [7]. Alterations of amino acids at position 103 (Lys), 155 (Pro), 220 (His) or 223 (Ser), respectively, had the strongest effect regarding the binding affinity of rhinoviral capsids. The structural analysis revealed a juxtaposition of these amino acids at the ‘bottom of the canyon’. It is noteworthy that most of the substitutions reduced the binding of capsids to host cell membranes. However, the biochemical property of one amino acid (Pro 155) is of special importance because a change to glycine led to a higher binding affinity and an enhanced yield of mutated virus ([7]; see Table 1 and Fig. 2). Most publications concerning rhinoviral structure described members of the major group rhinoviruses, while only few publications dealt with the structure of minor group rhinoviruses [8, 9].

The identification of cellular receptors binding to rhinoviruses was a landmark in examining how the infection cycle works beyond the attachment, i.e. how rhinoviruses enter and release their genome into the cytoplasm of the host cell. ICAM-1 (intracellular adhesion molecule-1; CD54) was detected first as the receptor for major group rhinoviruses [10, 11] and later (V)LDL [(very)-low density lipoprotein; 12] as the receptor for minor group rhinoviruses. The receptors were isolated by using monoclonal antibodies generated for the immune mapping of the capsid surface in a screen for cross-reactivity with cellular surface factors. These antibodies, which neutralize

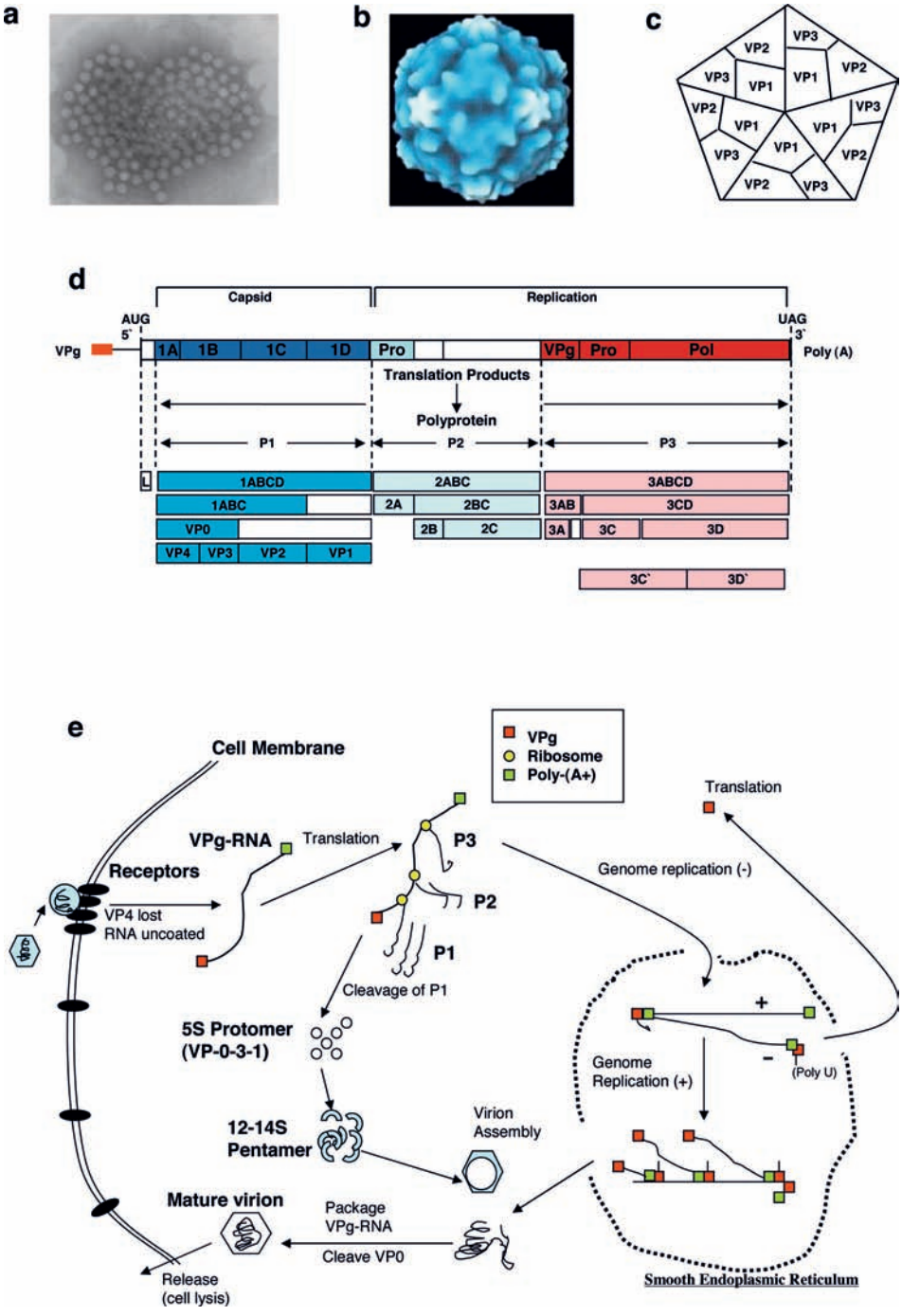

Figure 1. Electron micrograph of purified rhinoviruses/infection cycle of rhinoviruses. (*a*) Electron microscopy analysis of purified rhinoviruses; (*b*) the structural model of rhinoviruses evaluated from cryoelectron microscopic data; (*c*) the schematic drawing showing the position of the proteins VP1– P3 in the rhinoviral capsid; (*d*) the map of the rhinoviral polyprotein represents the localization of compounds forming the capsid (structural proteins P1) and nonstructural proteins exhibiting functions in protelytic maturation or replication (P2-P3), (*e*) schematic drawings summarizing the polyprotein maturation and the rhinoviral infection cycle. All pictures and drawings modified according to the lecture 'Virologie fuer Biochemiker', edited by R. Zell, University of Jena.

rhinoviruses *in vitro* and *in vivo*, permitted identification of the epitopes in the viral capsid, critically important for binding to the receptors and/or release of the RNA into the cytoplasm. It was possible to define the structural differences between HRV2 (representing minor group rhinoviruses) and HRV14 (representing major group viruses) by analyzing a special subportion of the 'canyon'. This region binds in particular to hydrophobic compounds, for example fatty acids supplemented by membranes of the host cell. The interaction of the virus with the fatty

Table 1. Mutations in the rhinoviral protein VP1 interfere with attachment and growth (modified according to [7].

Position of wt amino acids		Substitutions	Virus yield (pfu/cell)
103	Lys	–	160
		Ile	84
		Arg	54
		Asn	140
155	Pro	–	160
		Gly	1
220	His	–	160
		Ile	10
		Trp	14
223	Ser	–	160
		Ala	8
		Thr	70
		Asn	88

wt, wild-type. pfu, plague-forming units.

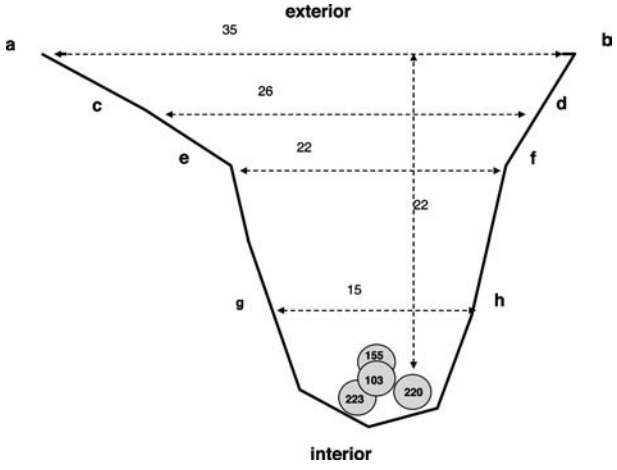

Figure 2. Mutations in the rhinoviral protein VP1 interfere with attachment and growth. The figure displays a scheme of the HRV-14 canyon. Distances are shown in angstroms and circled numbers represent the four amino acids targeted in the studies. Coordinate points used in determining distances are as follows: a = O^{81} of Asp-91, b = O^{e2} of Glu-210, c= O^{81} Asn-92, d = O of Glu-210, e = O^{e2} of Glu-95, f = N^{e2} of Gln-212, g = O^{11} of Asp-101, h = C^{81} of Ile-215. Figure modified according [7].

acid seems to stabilize the virus during its spread from cell to cell, but it must be removed prior to the uncoating process. Regarding major group viruses, the fatty acid is displaced by ICAM-1. The minor group viruses exhibit a higher affinity for the fatty acids, and binding of ICAM-1 is insufficient to replace the fatty acid in the pocket of VP1.

It is worth pointing out a common finding in this field: receptors such as ICAM-1, VCAM-1, but also the (V)LDL receptors play a predominant role in rhinoviral binding, uptake and associated signal transduction processes. However, theses data were determined from infection experiments employing poorly differentiated epithelial cells in culture. In contrast, these molecules are only infrequently expressed *in vivo* on the apical surface, questioning the significance of the *in vitro* data. In addition, the issue whether epithelial cell layers are the primary targets of rhinoviruses was discussed, since infections do not result in cytopathic effects in the ciliated epithelial cells as shown for many other respiratory viruses. Many studies focused primarily on the detection of rhinoviral RNA in rhinopharyngeal tissues by means of reverse transcriptase-polymerase chain reaction (RT-PCR) or *in situ* hybridization and showed that cells and tissues, for example the germinal layer in the adenoid tissue below the epithelial layer, are positive for rhinoviral RNA [13–16]. Rhinoviruses can be cultured in WI-38 fibroblasts, suggesting that fibroblasts in the rhinopharyngeal tissue can encounter rhinoviruses and become targets for rhinoviral infection. It is also possible that rhinoviruses attach to and invade the epithelial layer by a mechanism different from that observed in cultured epithelial cells, i.e. binding to ICAM-1 and (V)LDL receptors, respectively. Further studies are required to address these questions.

Membranes and movement: pathways of rhinoviral entry

The identification of receptors (or receptor families) to which rhinoviruses bind prompted studies investigating the entry mechanism(s) of rhinoviruses. The receptors for the minor and major group rhinoviruses are structurally non-related [17, 18], and the molecular reason why rhinoviruses as well as other members of the Picornaviridae, for instance coxsackieviruses, use different receptors is not well understood. One strain (CAV-A21) of the rhinovirus- related family of coxsackieviruses needs to engage both ICAM-1 and DAF (decay-accelerating factor; CD55), whereas other coxsackieviruses require DAF or integrins for binding [19]. Moreover, previous work underlined that environmental pH plays a significant role regarding the stability and conformation of the rhinoviral capsid and, therefore, is a crucial factor for transition of genomic RNA into the cytoplasm of the host cell [5, 20]. The requirement for pH acidification seems to be indispensable, in particular for minor group rhinoviruses. Binding to (V)LDL receptors is important for attachment and internalization, but it does not allow a conformational change of the rhinoviral capsid as binding to ICAM-1 does [10, 21–24]. (V)LDL receptors are well-described molecules, and the uptake of their natural ligands via clathrin-coated vesicles suggests that minor group rhinoviruses enter the host cell via the same pathway. Microscopical studies have detailed the uptake of the minor group rhinovirus HRV2 [25]. This viral strain was shown to cluster to membrane domains positive for clathrin, supporting the notion of an uptake mechanism

depending on clathrin-coated vesicles. In line with this finding is the blockade of HRV2 uptake by overexpression of dynamin-K44, a transdominant negative isoform of dynamin, which is required for 'pinching off' clathrin-coated vesicles from the plasma membrane. Moreover, pre-incubation with methyl-β-cyclodextrin (MBCD) interrupts the uptake of HRV2 at an early stage. Rhinoviral capsids pile up close to the plasma membrane and are not transported to the endolysosomal compartment in cells treated with MBCD [25]. MBCD depletes cholesterol from cellular membranes and leads to destabilization of membrane domains, which are thought to play a central role in signal transduction and endocytosis. Upon stimulation with cross-linking ligands, membrane domains tend to fuse to functional structures mediating the clustering of receptors, receptor-tyrosine kinases, non-receptor-tyrosine kinases, adaptor proteins and G-proteins, to name a few. Such membrane domains can be functionally differentiated depending on their lipid and protein composition. A distinct population of membrane domains are termed lipid rafts. They are very small (diameter of approximately 50 nm) and enriched with cholesterol and sphingolipids. Lipid rafts are thought to enable the attachment, uptake and secretion of enveloped viruses, for example HIV-1 (human immunodeficiency virus-1) [26–29]. Caveolae are large distinct membrane domains that are characterized by the presence of caveolin. Caveolae are suggested to function as portals of entry for pathogens into the host cell [30]. Entry through this mechanism appears to protect microbes from degradation in lysosomes, although the level at which each microbe actively participates in avoiding lysosomal fusion may vary. Other possible variations in microbial entry through caveolae or lipid rafts may include (i) the destination of trafficking after entry and (ii) how actively the microbe contributes to caveolae/lipid raft-mediated entry [31]. However, confocal microscopy analysis of HRV2-infected HeLa cells indicates that caveolae are not directly involved in the infection of at least the minor group rhinovirus HRV2. No evidence was found that HRV2 localizes in caveolin-1-positive membrane domains [25]. Recent work indicates that another subgroup of membrane domains termed ceramide-enriched membrane platforms plays a crucial role in the infection cycle of rhinoviruses. These studies demonstrate that pharmacological or genetic inhibition of ceramide-enriched platforms reduces rhinoviral titers in epithelial cells [32]. Membrane domains may not only be involved in the first steps of internalization, but also play a role in transport of vesicles and fusion of transport vesicles, endosomes and endolysosomal vesicles, finally triggering acidification of the vesicle and uncoating of rhinoviruses (Table 2).

Messengers and messages: rhinoviral induced signaling

Infection of the upper respiratory tract by HRV16 has been demonstrated to occur at localized portions of epithelia and does not cause widespread lysis of infected epithelia. This observation suggests that the pathology induced by rhinovirus may be due in part to cytokine deregulation rather than the extensive epithelial necrosis observed in other viral infections.

HRV, as well as other respiratory pathogens, have been shown to induce production of many cytokines and chemokines (see Table 3), among them interleukin IL-4, IL-6, IL-8 and granulocyte macrophage colony stimulating factor (GMCS-F) [33–36]. The induction of chemokines triggers an inflammatory response, which leads to exac-

Table 2. Chemicals interfering with the rhinoviral propagation.

Tested compound	Function in/interferes with	Reference
WIN factors	interaction with the canyon structure, viral adhesion is suppressed	58
Soluble ICAM-1	competition with ICAM-1, viral adhesion is suppressed	82
Erythromycin	reduction of ICAM-1 receptor, blocking endosomal acidification, viral adhesion and viral transport are blocked	83
Cytochalasin B	interference with microfilaments and inhibiting vesicle maturation, blocks viral transport	32
Bafilomycin	blocking endosomal acidification, release of RNA is inhibited	21
MBCD	depletes cholesterol, destroys functional membrane domains, transport of virus is blocked	25
Imipramine +	blocking ASM and inhibiting ceramide-enriched platforms	32
Amitryptilin	viral attachment, transport and/or vesicle maturation is blocked	
Nocodazole	interference with macrofilaments and inhibiting vesicle maturation	32
zVAD	anti-apoptotic agent blocking caspases and release of new viruses	24, 32, 56
AG7088	blocks protease 3C irreversibly and stops capsid maturation	71, 72, 84

Table 3. Cytokines and chemokines induced by rhinoviral infections.

Cytokine/chemokine	Induced in cell lines	Published in
Eotaxin1/2	BEAS 2B bronchial cell line	85
IL-1ß	human tracheal cells	86
IL-4	KU812 (human early basophilic leukocytes)	33, 34
IL-6	human tracheal cells	33, 34, 86
IL-8	human tracheal cells, HMC-1 (immature human mast cell line)	34–36, 41, 53, 86, 87
GMCS-F	HMC-1 (immature human mast cell line)	33, 34
RANTES	A549 (pulmonary epithelial cells), HBEC (human bronchial epithelial cells)	53, 88
TNF-α	human tracheal cell	86
MCP-1	monocytes, macrophages	55

erbations of asthma and chronic obstructive pulmonary disease (COPD) [37–40]. High levels of IL-8 and an increased number of neutrophils in sputum and nasal secretions are a common feature of asthma and COPD exacerbations after HRV infection [33, 41].

Little is known, however, about the primary signaling pathways initiated by early events of the rhinoviral infection cycle such as binding/attachment of the virus to its receptors, internalization or transition of the RNA. Because the signal transduction pathways of the LDL receptor family have been difficult to define so far, the experimental design focused on ICAM-1, the receptor for major group rhinoviruses. ICAM-1 is a type I transmembrane glycoprotein belonging to the immunoglobulin superfamily. In endothelial cells, ICAM-1 cross-linking induces phosphorylation and activation of pp60 Src [42, 44] and pp125 FAK [43]. The p85 regulatory subunit of class 1A phosphatidyl-inositol-3-kinase (PI-3-kinase) serves as a substrate for both Src [45] and FAK [46, 47], suggesting that PI-3-kinase could be a target for signal transduction by HRV ligation to ICAM-1. Similar studies with adenoviruses and respiratory syncytial virus binding to other receptors also focused on the activation of PI-3-kinase and showed that this enzyme mediates at least in part the production of IL-8. Recent studies described PI-3-kinase as the 'connecting link' between intracellular signal transduction and the chemokine response in rhinoviral infections [48]. Infection of human bronchial cells with the major group rhinoviral strain HRV39 increased the secretion of the chemokine IL-8 2 days after infection. ICAM-1-inhibiting antibodies abrogated the IL-8 production, while ultraviolet (UV)-irradiation of rhinovirus failed to alter IL-8 release. This indicates that binding and entry events induce the chemokine response rather than events related to viral replication [48]. Further studies concentrated on early phases (5–60 min post-infection) of rhinoviral infection. Active PI-3-kinase could be precipitated from lysates of infected cells, and isolated plasma membranes showed the presence of metabolic products [i.e. PI (3,4,5)P_3] of PI-3-kinase. These studies also demonstrated activation of Akt and NF-kappa-B as known downstream targets of PI-3-kinase and showed binding of NF-kappa-B to the IL-8 promoter after rhinoviral infection [48]. Other studies demonstrated binding of NF-kappa-B to GATA promoter sites of the vascular cell adhesion molecule (VCAM-1) promoting the surface expression of VCAM-1 in bronchial epithelial cell lines [49] and the upregulation of chemokine gene expression upon infection with rhinoviruses [50, 51].

Interestingly, the activation of PI-3-kinase is functionally connected to the entry of HRV39. Inhibition of PI-3-kinase reduced the uptake of radiolabeled HRV39. Furthermore, viral particles were shown to co-cluster to membrane regions positive for PI-3-kinase, providing evidence for distinct, not yet characterized membrane regions functional in rhinoviral uptake.

Additional studies investigated the role of MAP-kinases for rhinoviral infections. One member of the MAP-kinase family, the p38-kinase (p38-K), was found to be activated after infection with major group rhinoviruses [52–55]. These studies showed an early activation of p38-K, i.e. 15–30 min after infection, which declined within the next hour. Dumitru et al. detected a biphasic activation of p38-K, i.e. 30 min–2 h and 7–12 h after HRV14 infection, respectively. They identified the nuclear factor ATF-2 as a downstream element of this signal transduction pathway [52]. The second peak of phosphorylated p38-K was sensitive to UV inactivation of rhinoviruses, indicating the involvement of viral factors that are active beyond attachment and entry processes [52]. Furthermore, the onset of the second p38 activation correlates with rhinovirus-induced chemokine production [54], suggesting that late p38 phosphorylation might be involved in the chemokine response. Another effect of p38-K activation is the induction of apoptosis in cultured epithelial cells [56]. It is a matter of debate whether caspases are involved in

the onset of cell death on rhinoviral infection, because the administration of the caspase inhibitor z-VAD showed controversial results in regard to host cell survival and rhinoviral titers [32, 56, 57].

Inflammatory reactions require effector cells such as macrophages and monocytes. A recent publication addressed the question whether the production of chemokines is restricted to nasal epithelial tissues or whether chemokines are synthesized and secreted by lymphocytes. Alveolar macrophages and monocytes were subjected to rhinoviral infection [55]. After infection with HRV16 the production of the macrophage attractive protein-1 (MCP-1) was shown to be increased in monocytes and macrophages. Surprisingly, HRV16 induced the same signal transduction pathways in lymphocytes as in epithelial cells. Again, p38-K was activated and stimulated nuclear factor ATF-2 via the NF-kappa-B pathway. Signal transduction was attenuated by preincubation of the lymphocytes with soluble ICAM-1, suggesting that HRV binds to and enters lymphocytes via this adhesion molecule [55].

Finally, these results present the novel idea that cell types other than epithelial cells contribute to viral exacerbations of asthma as well as causing the symptoms of the common cold.

Poor pills: an overview of therapeutic strategies

Although the common cold is usually mild, with symptoms lasting a week or less, it is a leading cause of physician visits and of school/job absenteeism in addition to several more severe exacerbations like asthma (see above). Initial studies that employed monoclonal antibodies to define candidates of viral receptors demonstrated a surprising 'immunological resistance' of rhinoviruses. On the one hand, this is due to the fact that over 100 different rhinoviral serotypes could be isolated, and antibodies raised against one serotype after immunization are not necessarily protective against a different serotype. On the other hand, the identification of the so-called canyon allowed an additional explanation: if this canyon represents the predominant site of receptor interactions, it is difficult to block with antibodies, since the canyon is too narrow to enable interactions with the antigen [6, 7]. Both findings seem to rule out vaccination as a standard anti-rhinoviral treatment. Hence, the canyon structure dominated the development of therapeutical strategies. Researchers were able to design chemical structures designated as 'pocket factors', interacting with amino acid residues in the canyon. Because of their chemical similarity to the primarily described drug WIN51711, an isoxazole derivative [58], such chemicals are generally termed WIN compounds. WIN compounds seem to function both as competitors for binding to the receptor and in a sterical blockage interfering with the conformational change of the capsid necessary for the release of genomic RNA. While WIN compounds have a significant effect in reducing the viral titer *in vitro*, *in vivo* application was difficult. Structurally related substances like flavinoids, for example 4′-6 dichlorflavane, exhibited better feasibility regarding application, but caused only disappointing reduction of the rhinoviral titers [59, 60]. The chemokines interferon-α/β are produced after viral infection. Host cells detect the presence of double-stranded RNA via activation of the Toll-like receptor 3 (TLR-3), and the main function of interferons is the downregulation of the translation machinery, which leads to attenuation of viral replication [61]. Some groups tested the application of interferons and interferon inducers, respectively, and their interference with rhinoviral replication and production [62, 63]. Although high doses of interferons applied intranasally exhibited a prophylactic effect, these high doses of interferons required for the protective effect caused local erosions and nasal stuffiness [64].

Therefore, interest shifted to alternative methods to attenuate or even to block rhinoviral infections and their symptoms. Even though vaccination seemed to be impossible (as pointed out before), some studies dealt with the question how the human immune system responds to a rhinoviral infection. Rhinoviral inoculation induced both immunoglobulin IgG and IgA present in the serum and the airway, respectively, which neutralized the identical serotype upon reinfection. In studies using several different rhinoviral serotypes, the generation of cross-reacting antibodies could be observed. Individuals with pre-existing cross-reacting antibodies or cross-reacting antibodies derived from rabbits exhibited partial immunity when challenged with any new rhinoviral serotype [65–67]. However, complete immunity is barely to be expected, because many studies showed that anti-rhinoviral protecting IgA titers decreased only 2 months after infection. Nevertheless, a vaccination could attenuate exacerbation of the infection, i.e. reduce asthma and COPD [68, 69].

In this review we present indications that lipid domains in the plasma membrane of infected cells are functional in signal transduction events caused by rhinoviral infections. Moreover, lipid composition could influence the interaction of viral proteins with vesicular membranes during transition of RNA and replication [70]. For example, rhinoviral uptake is sensitive to treatment with MBCD, which interferes with cholesterol metabolism and disrupts lipid domain stability [25]. MBCD treatment blocked the activation of p38-K [52], thus indicating the importance of functional lipid domains for signal transduction events. The acid sphingomyelinase, which was shown to be crucial for generation of ceramide-enriched platforms, a subentity of lipid domains [32], might be an additional target to prevent HRV infections. Acid sphingomyelinase can be inactivated pharmaceutically by tricyclic antidepressants such as amitryptiline and imip-

ramine. Studies in which the acid sphingomyelinase was inhibited by these substances showed a dramatic decrease in viral reproduction, suggesting that the acid sphingomyelinase is critically involved in the propagation of rhinoviruses in human cells [32]. Drugs derived from the structure of tricyclic antidepressants might thus be effective against rhinoviral infections.

Aside from anti-viral drugs that target the attachment and entry processes, anti-rhinoviral therapy could benefit from identification of chemicals that inhibit rhinoviral maturation. As pointed out earlier, activation of protease 3C is a crucial step in the assembly of the capsid. Tripeptidyl alpha-ketoamides were identified as human rhinovirus protease 3C inhibitors [71]. The protease 3C-inhibiting drug AG7088 (ruprintrivir) showed a 100-fold reduction of rhinoviral titers and abrogated inflammatory responses as well. Clinical trials indicated that AG7088 could be administered to volunteers without adverse reactions [72]. Subsequent studies are in progress, aiming at another rhinoviral protease (i.e. 2A) as a target for anti-rhinoviral therapy [73, 74].

Concluding remarks

This review briefly summarizes the molecular structure of rhinoviruses, their interaction with cellular receptor proteins, the induction of signaling pathways and possible ways to inhibit or attenuate the propagation of this apparently optimally adapted pathogen.

As mentioned above, future experiments to understand rhinoviral infection mechanisms suffer from the lack of a valid *in vivo* and/or *ex vivo* model. Cultivation of nasal or upper respiratory tissues recovering function and morphology is not yet established. Antibodies specific for viral components, which can be utilized in biochemical and microscopical procedures, are rare. Unfortunately, they are an important tool to visualize interactions between rhinovirus and target cells. Viral RNA detecting in situ hybridization studies published thus far focused on detection of rhinoviral RNA in patients late after inoculation. Other methods, for instance real-time PCR, are powerful tools for quantification of rhinoviral replication and enable the determination of anti-viral compounds, but fail to elucidate protein-protein and protein-lipid interactions, which are centrally involved in viral propagation. Numerous projects in the field of immunity and infectious biology utilize knock-out mouse strains to investigate processes between pathogen and host. Rhinoviruses, however, show an almost strict specificity for human cells. Most studies, therefore, have to be accomplished in human volunteers or, alternatively, in primates, which necessitates special requirements regarding biosafety of the rhinoviral stock preparations and ethical questions [75, 76]. Only a few publications reported that host restriction could be overcome by using a method to select rhinoviral strains which are able to infect murine cells [77, 78]. Another possible technique to overcome this problem is by expression of human/murine chimeric rhinoviral receptors [79, 80]. Engineered ICAM-1 molecules, stably expressed in murine cell lines, bind rhinoviruses and permit the uptake in murine respiratory cells and L-cells, respectively. The latter study underlined that mutations in non-capsid proteins (genomic region P2) are necessary to adapt viral proteins to the new host cell and finally allow appropriate replication in murine cells [70]. Other researchers developed human ICAM-1 transgenic mice that were recently used in infection studies with coxsackiviruses [81], and might be also used to study rhinoviral infections.

Innovative methods now in development make it interesting to pursue new therapies to 'catch' this elusive pathogen.

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