

METHODS IN MOLECULAR BIOLOGY

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Rhinoviruses

Methods and Protocols

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Cover image: Infection with rhinovirus leads to reduced localisation of Sam68 in nuclear bodies. HeLa cells infected with human rhinovirus 16 were fixed and probed for viral 3C proteinase (green) and cellular RNA-binding protein Sam68 (red), followed by confocal laser scanning microscopy.

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Preface

Human rhinoviruses (HRV) are the major cause of common colds as well as being recognized more recently as the major viral cause of asthma and chronic obstructive pulmonary disease (COPD) exacerbations. The advent of molecular diagnostic techniques and confirmation of HRV as the main cause of asthma exacerbations has led to a heightened interest in HRV biology, with a significant increase in our understanding of mechanisms of pathogenesis. Seminal discoveries of the past decade include the demonstration that HRV infection is not limited to the epithelium, but can infect subepithelial layers of the airway wall, the discovery of a third HRV genotype that with special properties in terms of disease severity, and the description of disrupted nuclear transport in HRV infected cells through action of the HRV proteases on the host cell nuclear pore. New and improved molecular, cellular, protocols developed specifically to studying HRV have played a key role in enabling all of these discoveries. Important technical advances include the development of a validated mouse model for HRV infection, the establishment of HRV infection in primary airway cells, the generation of a reverse genetics system for HRV, and application of spectroscopic and microscopic analysis to understanding HRV biology. Given the increasing global incidence of asthma, the importance of HRV in asthma and COPD exacerbations, together with the rapid advancements in methodology, this is a more than opportune time to bring together current state-of-the-art methodologies to study HRV biology.

HRV are the viruses most commonly isolated from persons experiencing mild upper respiratory illnesses (common colds). Although infections are chiefly limited to the upper respiratory tract, HRV may also cause otitis media and sinusitis. Importantly, HRVs may also exacerbate asthma, COPD, cystic fibrosis, chronic bronchitis, and serious lower respiratory tract illness in infants, elderly persons, and immunocompromised persons. Although infections occur all year round, the incidence is highest in the fall and the spring. 70–80 % of persons exposed to the virus have symptomatic disease, which in most cases is mild and self-limited.

Isolation of HRV was first accomplished by Pelon et al. and Price using Rhesus monkey kidney tissue cultures and nasopharyngeal washings from persons with colds. In 1960, Tyrrell and Parsons at Salisbury, isolated strain HGP by reducing the incubation temperature of cultures to 33 °C, lowering the pH of tissue culture media to around 7.0, and gently rotating cultures during incubation. A profusion of HRV serotypes was subsequently detected when the sensitive strains of human embryonic fibroblast cell cultures described by Hayflick and Moorehead and the conditions described by the Salisbury group were used routinely. The name “rhinovirus” was originally suggested by Andrews, and a description of the group was proposed by Tyrrell and Chanock in 1963. In 1967, a collaborative program assigned numbers 1A through 55 to the known HRV types. In 1971, a second phase added types 56 through 89, and a third phase increased the number to 100.

These multiple serotypes have also been grouped on the basis of cellular receptor specificity (major, utilizing intercellular adhesion molecule 1 (ICAM-1) and minor, low-density lipoprotein receptor (LDLR) groups) and sensitivity to antiviral capsid-binding compounds (A and B groups). Full-length and partial (mainly VP4/VP2 and VP1 coding regions) genome sequencing of prototype strains and clinical isolates has revealed two major HRV

genotypes or clades. This phylogenetic classification correlated better with antiviral drug sensitivity than with the receptor grouping, and suggested a fundamental division of HRVs into two species, HRV-A and HRV-B. The development of highly sensitive molecular techniques for detecting HRV genome in a variety of clinical specimens led to discovery of novel rhinoviruses designated HRV-C (aka HRV-A2 or HRV-X), that were subsequently shown to meet the species sequence demarcation criteria (less than 70 % amino acid identity in the P1, 2C and 3CD regions) in the genus Enterovirus (Chapter 1).

Clinical, diagnostic laboratories by and large utilize PCR- and sequencing-based techniques for isolating, identifying and genotyping HRV from clinical samples (Chapters 2–4). Virus culture has been the “gold standard” for laboratory diagnosis of respiratory virus infections and viruses are routinely propagated in susceptible cell lines for use in research laboratories where large quantities of high-titer virus suspensions are needed for investigations (Chapter 5). Additionally, cell culture-based assays are still required to determine concentrations of infectious virus and are used routinely in research laboratories (Chapter 7). However, current assays do not allow infectivity measurements of the HRV-C group, isolates of which do not grow in standard cell culture (e.g. HeLa or embryonic lung fibroblasts). This feature has most likely prevented their discovery until recent molecular diagnostic approaches that do not require virus isolation or passaging. Sequencing of the complete reference set of HRV-A and HRV-B at the full-genome level and comparative analysis with available complete genomes of HRV-Cs has confirmed clustering of all strains into three phylogenetically distinct groups. Recent advances in tissue culture, wherein the structure of the airway wall is able to be replicated in culture systems using primary human tissue have allowed the culture of HRV-C which should lead to future advances in our understanding of the pathology of this group of HRVs (Chapter 6).

The huge advancements in technical applications of biophysics and improvements in high-end imaging techniques have also had implications for HRV research among other infectious diseases. These high-resolution techniques have allowed scientists to dissect individual events in the attachment, infection, replication, assembly, and release of HRV, with resultant increases in our understanding of the intracellular mechanisms of this important virus (Chapters 8 and 9). HRV encoded proteases, like those of their picornavirus counterparts, play crucial roles in virus replication through their cleavage of the HRV polyprotein; inactivation of either protease results in inability to rescue infectious virus. The role of HRV proteases as mediators of virus pathogenesis has been known for some time; however recent increases in the known cellular targets has highlighted the fact that our knowledge of cellular targets of HRV proteases is still incomplete (Chapter 10). The importance of proteases as anti-HRV targets is shown by the effectiveness of the Pfizer antiviral Rupintrivir (a 3C protease inhibitor) in experimental HRV infections. *In vitro* functional assays are important to understand protein functions and one such assay is described in Chapter 11 to enable in depth study of cellular targets of HRV proteases.

A great advance in HRV research has been the availability of reverse genetics systems for specific minor and major group HRV (Chapter 12). These systems have facilitated the study of individual viral proteins in the context of the infected cell, allowing specific mutations to be inserted in the full length genome (Chapter 13). Although HRV infectious clones have only recently started to be used widely, they provide an invaluable tool to study HRV biology and will be crucial in any future drug or vaccine strategies.

The study of the host response to HRV was stalled for many years due to the lack of a suitable animal model; all *in vivo* investigations thus had to rely on examination of human clinical samples, which limited the investigation of HRV in the context of asthma.

The development of both minor group (wild type Balb/c) and major group (transgenic Balb/c) mouse infection models has opened up the field for *in vivo* studies and has already resulted in several publications on the host immune response to HRV infection (Chapter 14). This advance in HRV research has been supported by the development of techniques for HRV culture in suspension, which, aligned with classical purification techniques based on sucrose gradients has resulted in suspensions of high concentration of virus required for animal studies. Importantly, high-titer virus and validated mouse models will help to clarify the roles of specific host response pathways in HRV-induced asthma and COPD exacerbations in the future.

In short, HRV research has come a very long way from the virus's initial identification as the causative agent for common colds, with numerous molecular, cellular and *in vivo* tools now available, as this volume highlights. The approaches described have led to increased understanding of the clinical disease caused by HRV, including functions of its individual proteins and its replication. Much still remains to be investigated, however, as exemplified by the recent discovery of an important group of HRV that is now known to cause severe disease. Future research in the HRV area, driven by technical advances such as those showcased here, should ultimately enable the development of therapeutic approaches to combat HRV, and most importantly, its pathogenic effects in asthma exacerbation.

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