

COMMENTARY



Vaccine strategies to induce broadly protective immunity to rhinoviruses

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ABSTRACT

Rhinoviruses are ubiquitous human pathogens of the upper respiratory tract and are the major cause of acute exacerbations of asthma and chronic obstructive pulmonary disease. At least 160 antigenically distinct serotypes or strains have been identified and protective immunity is largely serotype specific. Attempts to produce vaccines that induce broad immunity have met with limited success which is due in part to this antigenic diversity and a lack of information regarding the ideal protective immune responses. Recent approaches identifying conserved rhinovirus epitopes and better definitions of the immune correlates of protection have raised hope. Here, these newer findings are outlined and the prospects for such a universal rhinovirus vaccine are discussed.

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After years of neglect, recently there has been renewed interest in a vaccine for human rhinoviruses (RVs).¹ RVs are ubiquitous human pathogens of the upper respiratory tract² and of the lower respiratory tract in certain pathological situations.³ They are a highly diverse group of viruses with approximately 160 antigenically and serologically distinct strains or serotypes known to exist.⁴ Knowledge of what constitutes protective immunity or immunological correlate of protection is not completely understood although antibodies (Abs) and in particular secretory IgA, are known to protect against re-infection with the same serotype.⁵ Vaccines that will generate durable humoral immunity against antigenically diverse pathogens such as RVs therefore should probably elicit Abs that recognize conserved epitopes. Herein lies the extreme challenge with the development of a vaccine for RVs. Conserved epitopes are often buried within the capsid structure and are therefore not available to neutralizing Abs which tend to be directed toward surface-exposed regions that are hypervariable and serotype/strain specific. A successful vaccine must circumvent these challenges.

- (1) Numerous and evolving groups of antigenically diverse strains
- (2) The immune correlate of protection is not fully understood
- (3) RV structure shields the conserved epitopes from antibodies
- (4) Neutralizing antibodies are often serotype specific
- (5) Animal models for preclinical evaluation have limitations

Challenges associated with the development of RV vaccines

This commentary will evaluate the historical studies and clinical trials of RV vaccines, the current vaccine strategies that

have been investigated more recently in animal models, the viral features that should be considered to allow the generation of broadly protective Abs to RVs.

There are four broad types or groups of infectious disease vaccines that are licensed for use in humans. These include; live-attenuated vaccines; inactivated vaccines; subunit, recombinant, polysaccharide or conjugate vaccines; toxoid vaccines.⁶ For RVs, vaccine strategies are limited to live-attenuated, inactivated and subunit/recombinant due to the nature of the pathogen. i.e. there is no polysaccharide or toxin. In addition, more experimental vaccine approaches such as nucleic acid (DNA or RNA) or recombinant vector vaccines may be possible but have not been evaluated for RVs or approved for human use as yet.

The earliest clinical trials for RV vaccines were performed 40–50 years ago using live or formalin-inactivated RV serotypes (reviewed in⁷) and found that long lasting but only serotype-specific immunity was generated to the inoculum. Studies by Perkins^{8,9} demonstrated that intranasal immunization rather than intramuscular administration was required to generate nasal secretory Abs which were defined as the correlate of protection. However, the failure of these approaches to establish broad cross-serotype protection necessitated the testing of vaccines containing 10 distinct serotypes (decavalent) that unfortunately also provided limited breadth of protection.¹⁰ Recent studies immunizing macaques expanded the number of serotypes within these formulations to 50, but again these could not provide further breadth of protective responses other than to the RV serotypes found within the vaccine.¹¹ This multivalent vaccine approach therefore has several deficiencies that can explain their limited potential in this setting. The use of formalin-inactivated RVs is prevalent and is an approach that might be unfavorable for the generation of significant immune responses due to the loss of protective epitopes. Such inactivated virus formulations will also

require the use of adjuvants to enhance the vaccine efficacy¹² and many of the early studies did not or could not make use of these. It is likely that an appropriate adjuvant that can tune the desired immunity for RVs will be required for this approach to be more successful.¹³ Another issue is that knowledge of exactly what type of immunity is necessary for protection to RVs is limited despite vast progress in recent years.¹⁴ Although the induction of nasal secretory Abs is thought to be necessary, vaccine approaches that generate this immunity are imperfect. Lastly, the manufacturing process required to produce the desired vaccine variability is extremely complicated, especially when considering the increased viral titer that would be needed to maintain a small enough dose containing the serotype diversity for human administration. It is therefore unlikely that such multivalent vaccine preparations will gain clinical relevance.

A vaccine approach that might have the best chance of success for RVs is to identify highly conserved regions and to use these as vaccine antigens in an appropriate formulation. This would likely take the form of polypeptides in combination with an effective adjuvant. Herein lies the difficulty and challenge associated with RVs. Neutralizing Abs are thought to interact with exposed external regions of the capsid subunits VP1, VP2 and VP3 and to neutralize by a variety of mechanisms (reviewed in¹⁵). In fact neutralizing sites have been identified in capsid proteins VP1 (NIm-IA, NIm-IB), VP2 (NIm-II) and VP3 (NIm-III) for serotypes RV14 and RV2.^{16,17} These are often discontinuous epitopes that form protrusions from the capsid surface and are highly diverse sequences, often differing significantly between serotypes. This feature of the known RV neutralizing epitopes can therefore explain the serotype specificity of neutralizing Abs. A vaccine with broad RV neutralizing potential will need to induce a different spectrum of Abs that target regions of the capsid shared by numerous serotypes and where binding is still capable of neutralization. The identification of such a unique site is still under investigation but several studies have identified potentially exciting candidates.

Early studies by McCray and Werner¹⁸ found that immunizing with peptides corresponding to conserved structural regions of VP1 and VP3 from RV14 could induce Abs capable of neutralizing more than 20 distinct RV serotypes. It is unknown why this approach was not pursued further but presumably the use of peptide immunogens displayed limitations, as was described later by Barnett et al.¹⁹ when analyzing monoclonal Abs obtained after immunizing with a short VP2 peptide of RV2. It is unlikely that short peptides will form the correct 3D structure similar to complete capsid subunits and therefore the induction of Abs capable of binding the intact virion for neutralization will not occur. Nevertheless, a conserved region of VP4 has been identified that shows some promise. The N-terminus of VP4 is not surface exposed on the RV capsid but is transiently displayed by a process known as capsid breathing and peptide immunogens corresponding to this region can induce cross-serotype neutralizing Abs.²⁰ However, the physiological role of such Abs in humans has not been investigated and the cross-serotype neutralization induced by VP4 peptide immunization was limited to just two serotypes which are some way from the recognized number of RV serotypes discovered.

In recent years, immunization with recombinant capsid proteins of RVs has generated cross-serotype immunity. Edlymayr et al.²¹ produced recombinant VP1 of two distinct RV serotypes and demonstrated the production of neutralizing Abs for additional RV serotypes. However, polyclonal antisera were not particularly potent requiring dilutions of less than 1:8 for efficacy in most cases. Furthermore, neutralization activity versus a full spectrum of RV serotypes was not evaluated and effects were only seen with four or five strains. Additionally, bacterial production of the recombinant capsid proteins is unlikely to produce native folded material and the Abs induced will again mostly target linear epitopes over the better suited discontinuous epitopes. In the most novel RV vaccine approach attempted to date, Glanville et al.²² identified a conserved region of the RV polyprotein encompassing VP4 and VP2 (known as VP0), generated RV16 VP0 and immunized mice that were subsequently challenged with live RV to study protective immune responses *in vivo*. This was the first animal model that allowed for a challenge to evaluate RV vaccine candidates. Whilst VP0 was immunogenic when combined with a strong adjuvant, the generation of neutralizing Abs required live RV challenge and was restricted to the infecting serotype. Again, suggesting that native capsid configurations are needed to induce neutralizing Abs. Interestingly, significant increases in cross-serotype binding Abs were observed with this approach which suggests that a modified prime-boost immunization regimen might have future potential. The most recent study investigating VP0 immunization has determined the immunodominant epitope for Abs corresponds to the previously identified NIm-II region of VP2²³ which helps explain the serotype-specific neutralization of this approach. One could envisage that modified recombinant capsid variants lacking specific domains of the capsid proteins could find utility as inducers of more broadly reactive Abs by removing the immunodominance of useful but serotype-specific Ab responses.

Another difficulty associated with the development of a vaccine for RVs is the suitability of a small animal model to evaluate candidates and their protective abilities *in vivo*. RVs can infect mice²⁴ and cotton rats²⁵ and these have been used effectively to evaluate immune responses, pathophysiology of infection and vaccine protective responses,^{24–27} however despite similarities to that of humans in many inflammatory parameters, the important marker of viral replication is meager in these models. Until a preclinical model that faithfully recapitulates the outcomes of RV infection observed in humans is developed, RV vaccine development will continue its relatively slow trajectory.

In conclusion, investigations of vaccines to generate broad Abs responses to RVs are hampered by features of the viral structure, viral mutation and evolution, the lack of in-depth knowledge of immune correlates of protection, limited availability of preclinical models, and the relative priority placed on this family of viruses in the context of human disease. Nevertheless, a universal RV vaccine that induces strong protective nasal Abs to conserved epitopes could find utility – however discovery of such an epitope shared by 160 viral strains awaits.

Disclosure of potential conflicts of interest

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