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Challenges in developing a cross-serotype rhinovirus vaccine

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A great burden of disease is attributable to human rhinovirus (HRV) infections which are the major cause of the common cold, exacerbations of both asthma and chronic obstructive pulmonary disease (COPD), and are associated with asthma development. Despite this there is currently no vaccine for HRV. The first vaccine studies showed some promise in terms of serotype-specific protection against cold symptoms, but antigenic heterogeneity amongst the >150 HRVs has been regarded as a major barrier to effective vaccine development and has resulted in little progress over 50 years. Here we review those vaccine studies conducted to date, discuss the difficulties posed by antigenic heterogeneity and describe some recent advances in generating cross-reactive antibodies and T cell responses using peptide immunogens.

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Introduction

HRVs are small, non-enveloped RNA viruses belonging to the picornaviridae family. HRVs comprise 3 species, A–C, and are commonly referred to by numbered serotype. HRVs have an icosahedral capsid comprising 60 copies of each of four proteins, VP1–VP4, of which VP1–3 are surface exposed and represent the major targets for antibody responses.

HRV infections are suffered by everyone. During childhood this is at a rate of 5–12 infections per year [1] and importantly, infections continue throughout life suggesting that effective immunity is either not developed or not maintained. Infections in otherwise healthy adults cause symptoms of the common cold. They also however cause much more severe disease. Respiratory virus infections, the majority of which are HRVs, have been associated with 60–80% and 40–50% of exacerbations of asthma and COPD, respectively [2,3]. Recent reports suggest that HRV C infection in particular is associated with severe respiratory illness in children [4] and a large scale birth cohort study has found that infections in early life are associated with development of asthma later in childhood [5]. Given that the only available therapies are non-prescription cold remedies or standard corticosteroid and bronchodilator therapy in asthma and COPD, which is regarded as inadequate during exacerbations, the medical need for a vaccine is clear. We review those HRV vaccine studies conducted to date, highlighting advances made in the last 3 years and discuss the challenges posed by antigenic heterogeneity amongst HRVs.

Antibodies can protect against infection

It was established soon after their discovery, in the early1960s, that higher HRV-specific serum neutralising antibody levels were associated with protection against infection with a particular HRV type [6–8]. It was also shown that nasal HRV-binding immunoglobulin (Ig) A level is additionally associated with protection from secondary infection and/or symptoms [9]. Examination of the protective effect of antibody has recently been extended in a new cotton rat model of infection where both passive transfer of immune serum and maternal antibody transfer were shown to be capable of reducing lung virus titres upon subsequent challenge [10]. These human studies pre-dated the discovery of the HRV C species, but serum HRV C-binding IgG1 was recently measured in two studies and shown to be of significantly lower titre than A and B species, which the authors suggest may indicate a less efficacious humoral immune response to these viruses [11,12•]. To determine if this translates to less protection against disease C species challenge studies are now needed. This is however dependent on advances in culturing C species HRVs, such as the recent development of a differentiated sinus epithelial cell system [13] in order to create an effective inoculum, because those C species viruses tested to date do not infect conventional cell lines used for virus propagation [14].

Early vaccine studies showed efficacy

A small number of human vaccine studies were conducted in the few decades following the initial characterisation of HRVs and showed some efficacy. Mitchison *et al.* showed that an intramuscular prime-boost strategy

Table 1

Summary of human vaccine trials and of animal studies involving virus challenge. I.M.: intramuscular, I.N.: intranasal, S.C.: subcutaneous.

Immunogen	Delivery route	Challenge	Main findings	Citation
Human trials				
Whole HRV2 (formalin inactivated)	I.M.	Yes	Homologous virus infection: Reduced proportion vaccinated subjects with cold symptoms versus unvaccinated controls, but similar proportion with nasal virus isolation. Heterologous virus infection: No protection from symptoms or reduction in virus recovery.	[15]
Whole HRV13 (formalin inactivated)	I.N.	Yes	Reduced proportion of subjects with upper respiratory tract symptoms versus unvaccinated controls (homologous virus challenge). No difference nasal virus recovery.	[16]
Whole HRV13 (formalin inactivated)	I.N.	Yes	Protection from symptoms, reduced nasal virus shedding and reduced duration of virus shedding versus unvaccinated controls (homologous virus challenge) at 5 months post-immunisation. No rise in cross-serotype reactive nasal neutralising antibody.	[17]
Whole decavalent (formalin inactivated)	I.M.	No	Limited and variable rises in neutralising antibody titres to vaccine serotypes. Few increases in heterologous virus antibody.	[27]
HRV2 (Live, heat killed or formalin inactivated)	I.N., I.M. or oral	No	I.M. vaccination gives similar or higher antibody titres as I.N. challenge.	[28]
Animal studies				
HRV16 VP0 peptide	S.C. (Mouse)	Yes	Enhanced lung T cell and serum neutralising antibody responses to challenge with heterologous viruses.	[33••]
Whole HRV16 or HRV1B (live)	I.M. (Rat)	Yes	Reduced lung and nasal tissue virus load with homologous but not heterologous virus infection.	[10]

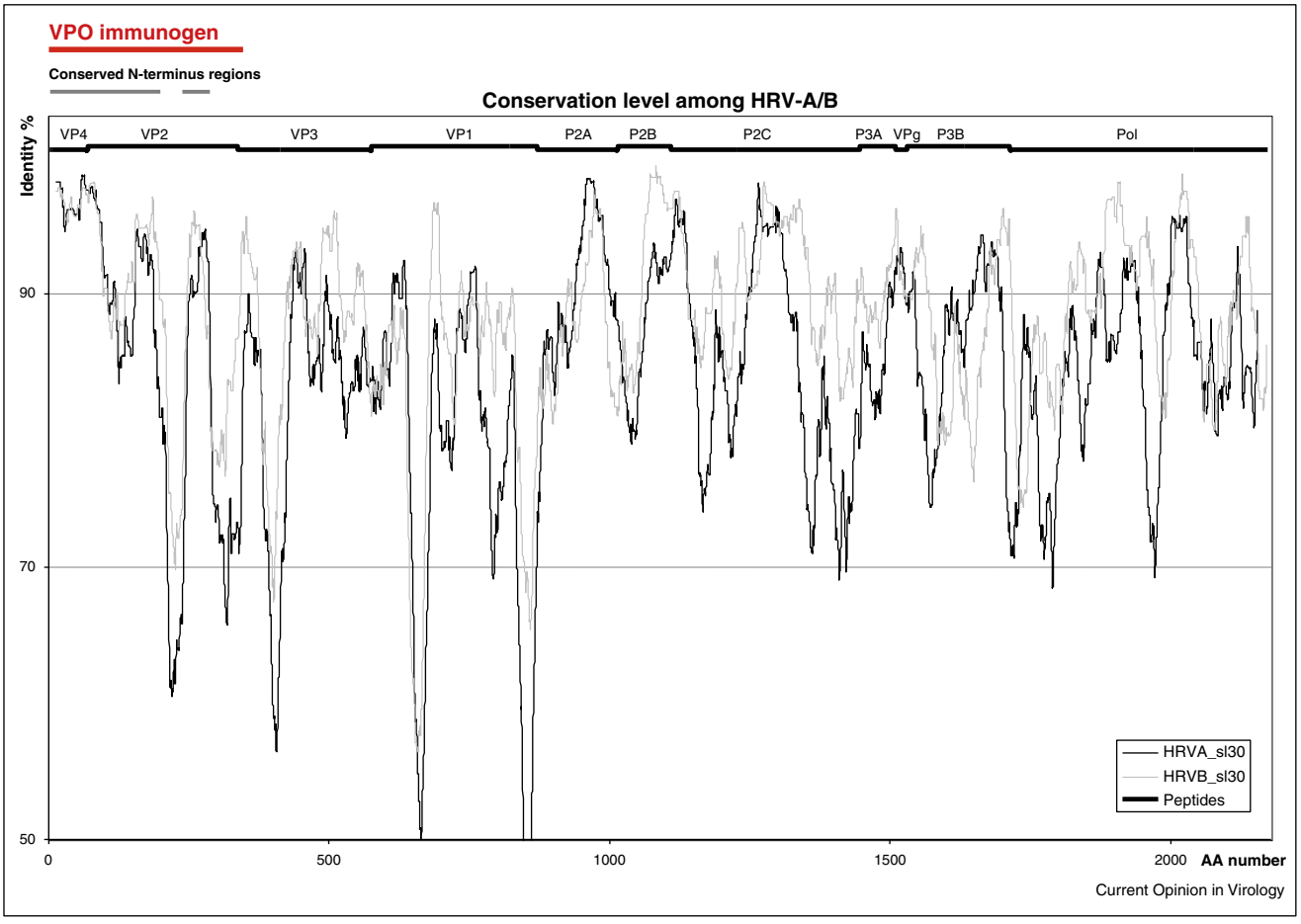
with formalin inactivated HRV led to a reduction in rate of symptomatic colds from 47% in unvaccinated subjects to just 3.5% following homologous virus challenge two weeks later. Although only 3.5% of vaccinated subjects had a symptomatic cold, nearly half had recoverable virus in the nose or rises in neutralising antibody titre post-infection, suggesting that immunisation reduced disease but did not prevent subclinical infection [15]. Administration of formalin inactivated HRV via the nasal route demonstrated similar protection in another study, this time reducing symptomatic cold rates from 85% to 33% upon subsequent RV13 challenge [16]. This protection afforded by intranasal inactivated HRV against common cold symptoms lasted until at least 5 months after vaccination [17]. Table 1 provides a summary of those vaccine studies carried out to date.

Antibody cross-reactivity amongst HRVs

Infection-induced antibody and inactivated, unadjuvanted, whole virus immunisation can therefore provide protection against infection, at least in terms of symptoms. However, the principle reason why these initial vaccine studies were not built upon is the considerable antigenic diversity amongst HRVs. HRV A and B species in fact number 100 immunologically distinct serotypes as determined by serum cross-neutralisation tests [18,19]. The more recently discovered C species comprises a further 50–60 viruses, albeit as defined by genetic analyses only. Studies of HRV evolution further show that intra-species and inter-species recombination is common [20,21]. Additionally, sequencing of clinical isolates in paediatric populations show that a large number of distinct genotypes circulate within a community at any given time [22–24]. Developing a vaccine which induces sufficiently broad immunity therefore represents a very significant challenge.

This is not to say there is not some antibody cross-reactivity between HRVs because this has been demonstrated both for neutralising antibody in immunised animals [25,26] and more recently in serum from human subjects, where both within-species and between species cross-reactivity was demonstrated for naturally occurring VP1-binding IgG1 [12•]. However, in those studies which have assessed a significant number of serotypes, cross-reactivity was shown to be somewhat limited because for example only 13 of 37 tested antisera from whole virus immunised rabbits neutralised a single other virus serotype [26]. The cross-reactive virus binding and virus neutralising antibody responses in humans are also somewhat variable between individuals [12•,27,28]. In terms of protection against virus infection, secondary infection with a heterologous virus serotype could reduce the frequency and severity of symptoms similarly to a homologous virus reinfection in one study [29], but intramuscular inactivated vaccine provided no protection against cold symptoms or virus shedding following heterologous virus challenge in another [15]. Intramuscular live RV immunisation in a cotton rat model caused reduced virus titres upon subsequent homologous, but not heterologous HRV infection [10]. Limited neutralising antibody cross-reactivity also required multiple adjuvanted immunisations plus infection in mice [30]. That high antigenic diversity should be a barrier to vaccine design is perhaps

Figure 1



Mean linear amino acid sequence conservation amongst A (black line) and B (grey line) species HRVs. Mean conservation level calculated at each position as a sliding window of 30 amino acids. Adapted from [33••]. Conserved N-terminus regions (grey line) and VP0 immunogen (red line).

not surprising when one considers that those neutralising epitopes described [31,32] map to VP1, VP2 and VP3 capsid protein regions that recent full length HRV genome analyses have found to be the most highly variable (as indicated by the major troughs in Figure 1) [21,33••]. Intriguingly, a recent study has added further complexity to this issue, suggesting that a large proportion of HRV binding antibodies in human serum may also be misdirected towards a non-protective epitope on VP1 [34•].

A polyvalent vaccine comprising multiple HRV types is one possible way of overcoming the issue of antigenic diversity. A previous attempt at this strategy with formalin inactivated decavalent whole virus preparations however demonstrated rises in neutralising antibody titres to 40% of serotypes at best [27]. A huge number of virus types would also likely need to be contained in such a vaccine because, as noted above, it has been found that large numbers of strains circulate simultaneously, with for example over 100 genetically distinct strains having been found in a small paediatric cohort in just a 2 year period [22]. It seems therefore that an effective HRV vaccine based solely on induction of neutralising antibody would be required to induce a broadly cross-reactive response based on the identification of new and highly conserved neutralising epitopes.

Induction of cross-reactive antibodies using peptide immunogens

Two recent studies have successfully generated cross-reactive neutralising antibodies using peptide immunogens. Edlmayr [35] immunised rabbits with recombinant VP1 based on the sequence of two different viruses and showed that both antisera were capable of inducing cross-reactive neutralising antibodies, neutralising up to half of ten other HRV serotypes tested. This study follows a similar study utilising shorter VP1 and VP3 peptides in which purified antibody from immunised rabbits neutralised 60% of 48 tested serotypes [36]. The relatively low amino acid conservation within VP1 perhaps makes these results somewhat surprising. The VP4 capsid protein in contrast is highly conserved, but because it is located on

the inside of the intact capsid, it should perhaps not represent a good vaccine target. Interestingly then, Katpally *et al.* [37] have defined a region of the n-terminus of VP4 that is transiently exposed during a process termed 'capsid breathing' and showed that anti-serum raised against a short VP4 peptide representing this region of HRV14 neutralised heterologous serotypes. Misgivings have been expressed about the potential efficacy of this strategy of immunisation with short peptides, such as whether immunogens need to recreate the complex discontinuous epitopes likely found on an intact capsid to be efficacious and regarding the modest titres of neutralising antibody short linear peptide immunogens induce [38]. However, given that the minimum protective neutralising antibody titre in man is essentially unknown, these studies provide some promising findings which need to be further tested for their ability to protect against infection and disease.

T cell vaccine strategies

An alternative strategy with perhaps greater potential to induce heterologous responses is to specifically induce HRV-specific T cells because of their potential to respond to highly conserved proteins not exposed on the capsid surface. Amino acid sequences are highly conserved in some non-structural proteins of HRVs as in some other viruses [25,26] and there is some evidence that T cells can be cross-reactive for HRV serotypes, both in man [39] and in mice [40]. For influenza, higher frequencies of cross-reactive T cells recognising internal protein epitopes before infection has been associated with reduced virus shedding and symptom severity upon subsequent infection, independently of antibody [41,42•].

Based on these findings we have employed a mouse HRV infection model [43] to determine if immunisation with conserved HRV proteins can enhance T cell responses to heterologous virus infection. We identified regions of the VP0 (VP4 + VP2 precursor) capsid protein and the virus polymerase as being highly conserved amongst A and B species HRVs (Figure 1). In recombinant HRV16 VP0 immunised mice, antibodies and T cells reactive to multiple other serotypes were detectable systemically. After subsequent infection, activated CD4+ and CD8+ T cell number in the airways was increased. Importantly, enhanced T cell responses were measured when mice were challenged with heterologous A species serotypes and lung cell responses to shorter VP0 peptides showed that immunisation induced T cells responsive to a B species serotype different from both the immunogen and the infecting virus. Whilst the immunogen did not itself induce neutralising antibodies, immunisation enhanced neutralising antibody responses to heterologous infecting virus, indicating that boosting of neutralising antibody was dependent on B cell help elicited by cross-reactive CD4+ T cells and thus that humoral responses to any subsequent infection might be enhanced [33••].

As with the other animal studies discussed, the mouse model in which these studies were undertaken differs to human infection in terms of the comparatively limited and short-lived replication, the measurement of immune responses in the lower rather than upper airways and perhaps importantly, that animals had not experienced the previous infections experienced by a human subject. This work however provides a promising initial proof of concept for a T cell inducing HRV vaccine which can now be tested in human models of HRV challenge in healthy subjects, of asthma exacerbation [44–47] and of COPD exacerbation [48].

Conclusions

A handful of studies some 40–50 years ago remain the only human vaccine trials to have been conducted for HRVs despite their huge disease burden. This lack of progress within the field can be largely attributed to the issue of antigenic heterogeneity. There are however studies now showing that antibodies with limited cross-serotype reactivity can be generated by capsid peptide immunisation and insights from influenza and now HRV itself suggest that induction of cross-reactive memory T cells could have efficacy in generating a broadly cross-reactive vaccine. The availability of complete HRV genome sequences will help in identification of further conserved proteins which might be utilised as immunogens. There is a need however for greater understanding of adaptive immune responses to HRV infection, especially to the poorly characterised C species viruses, to identify the determinants of protection in man. Understanding the role of T cells in disease must be a priority given the few studies that exist. The availability now of murine models of infection can only help in speeding up this process. Finally, it is worth noting that the problem of antigenic heterogeneity is not exclusive to HRV vaccines and has been partially overcome for influenza viruses by a large scale surveillance programme which monitors circulating strains, allowing selection of strains for the coming season's vaccine. Whilst it is already known that a large number of HRV types circulate in a given population at any one time, large scale and systematic monitoring would be enormously beneficial in identifying how many different HRV serotypes might need to be targeted, because despite recent advances it seems unlikely that effective protection against all human HRVs can be achieved with a single immunogen.

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