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The potential for a protective vaccine for rhinovirus infections

Gareth R. Williams[1], Ilona Kubajewska[1], Nicholas S. Glanville[1], Sebastian L. Johnston[1] and Gary R Mclean[12]*

1) University College London, London, United Kingdom of Great Britain and Northern Ireland
2) London Metropolitan University, London, United Kingdom of Great Britain and Northern Ireland

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Immunity to Human Rhinoviruses

Rhinovirus (RV) infections impose a major disease burden as they cause around three out of four common colds and are responsible for the majority of acute exacerbations of chronic obstructive pulmonary disease (COPD) and asthma [1, 2]. RVs therefore are associated with an enormous economic cost in missed work or school and medical attention. Prophylactic vaccination against infection is arguably the most effective medical intervention ever developed, and has proven enormously effective in protecting against a large number of diseases. However, at the present time no effective vaccine exists for RVs. This is largely due to the existence of 100 serotyped antigenically distinct RV strains - such variability means that a vaccine designed to elicit immune responses against a particular RV is unlikely to be able to provide protection against the full range of virus subtypes successfully [3]. In fact, this phenomenon was observed as early as 1965 when immunising with formalin inactivated whole RV and is confirmed by the knowledge that the immunity induced following RV infection does not significantly protect from future infection by different RV serotypes [4]. More sophisticated attempts at immunisation with multiple inactivated RV serotypes also failed to induce significant cross-serotype protection [5]. Thus, an effective cross-serotype responsive RV vaccine has remained elusive. The relatively recent description of a new clade of RV types (RV-C) has increased the number of identified strains/serotypes to ~160 [6]. Perhaps the quest for a RV vaccine has been dismissed as too difficult or even impossible, but new developments suggest that it may be feasible to generate a significant breadth of immune protection.

Subunit Vaccines and Adjuvants

The most effective existing viral vaccines are “live” formulations in which the patient is exposed to an attenuated form of the virus. These replicate in the body mimicking the natural infection without the induction of serious disease and thus lead to prolonged exposure to antigens, inducing protective immunity. However, live vaccines are unlikely to deliver broadly reactive immunity to RVs, for many of the same reasons that inactivated vaccines failed.

Thus, the application of subunit vaccines, which have been developed for other significant human viral pathogens, such as hepatitis B virus and human papilloma virus [7], has now

been explored for RVs. In subunit vaccines, the immunogen comprises a small subunit or a virus-like particle (VLP) form of the pathogen. By their very nature these do not cause infection. However, most such non-infectious peptide-based vaccines are poorly immunogenic and rely on the presence of an adjuvant to inculcate robust immunity by mimicking the danger signals that naturally trigger immune responses. The addition of adjuvants to vaccines enhances the immunogenicity of antigens, and reduces the number of immunizations required.

At present, inorganic compounds known as 'alums' (usually either AlOOH or amorphous aluminium hydroxyphosphate) are used as adjuvants in the vast majority of cases [8]. Alum can promote strong antibody responses that are effective in inducing immunity to bacteria and parasites (a Th2 response). However, it does not provoke strong Th1 immunity, which is intrinsically linked to the cellular immune responses that are most potent against intracellular pathogens. With respect to RVs, a Th1-biased immune response is likely to be desirable because human memory T cell responses to RV have been demonstrated to be primarily Th1 orientated [9] and memory Th1 responses were found to be protective against virus shedding after experimental infection [10]. RV infections are also known to enhance the Th2 responses already found in asthma [11, 12]. A reconfiguration of this undesirable immune response to Th1 could alleviate RV triggered exacerbations of allergic airway diseases.

Only alum, the oil-in-water emulsion adjuvants AS03 and MF59, and a new adjuvant containing alum and monophosphoryl lipid A (AS04) are currently licensed for use in human vaccines. These often do not provoke the correct pattern of immune response needed to most effectively target a viral disease, although mixed Th1/Th2 responses can be promoted by AS03 and MF59 [13]. In addition, despite the fact that it has been used for around 80 years in the clinic, the mechanisms of action of alum are not well understood. Not for nothing is it known as the immunologist's "dirty little secret" [14].

Thus in addition to the immunogen, the adjuvant represents a key challenge to developing a subunit RV vaccine.

Novel Adjuvants and Immune Response Tailoring

AlOOH is an inorganic material, but until very recently few attempts had been made to employ materials chemistry approaches to develop new adjuvants. However, some important steps forward have been taken to this end in the last 5 years. The effect of varying the particle size and shape of alum has been explored, and nanoparticles found to be more potent as adjuvants than particles on the micron scale [15]. In other work, rod shaped particles have been found to promote stronger immune responses than plates or polyhedra [8]. A range of other inorganic particles such as zinc and cobalt oxides, silver and gold nanoparticles have also been discovered to possess adjuvanticity [8]. In the latter case, rod shaped particles were again found to be more potent than other shapes.

Perhaps the most exciting finding is the recent discovery that layered double hydroxides (LDHs), inorganic materials with compositions similar to alum, can act as potent adjuvants [16]. LDHs contain metal hydroxide sheets which bear a positive charge, and charge-balancing anions between these layers. They have the general formula $[M^{z+}_{1-q}M^{3+}_{q}(OH)_2]^{q+}(X^{n-})_{q/n}\cdot yH_2O$, and typically contain mixtures of mono-/di- and trivalent cations with the interlayer anions comprising halides, nitrate, *etc*. A wide variety of metal ions and anions can be incorporated into the LDH structure (*e.g*. $[LiAl_2(OH)_6](CO_3)_{0.5}\cdot yH_2O$; $[Mg_2Al(OH)_6]Cl\cdot yH_2O$; $[Ca_2Al(OH)_6]NO_3\cdot yH_2O$).

Different LDHs have been found to promote different responses across a range of cytokines (e.g. IL-1β, IL-6, IL-12p70), chemokines (e.g. IL-8), costimulatory molecules (CD40, CD86) and antibodies (e.g. IgG1, IgG2c) [16]. The latter is particularly important: some LDHs

stimulate IgG1 (a Th2-associated antibody) at levels similar to or greater than alum while others stimulate both IgG1 and IgG2c (a Th1-associated antibody) [16]. Thus, LDHs can drive both Th2 and Th1 responses. Moreover, it proved possible to use mathematical modelling to develop an equation linking the immune response provoked *in vitro* and *in vivo* to certain physicochemical properties of the LDHs, permitting the response induced by an as-yet untested adjuvant to be predicted *a priori* [16].

Other work has further shown that the $[Mg_2Al(OH)_6]Cl{\cdot}yH_2O$ LDH conjugated with CpG can also drive a strong Th1 response [17]. The discovery of such advanced adjuvants offers great potential to incrementally tune the immune response, and might ultimately yield materials able to produce a particular spectrum of immune response (cytokines, chemokines, costimulatory molecules) at a greater level of specificity than a simple Th1/Th2 polarisation.

Recent Developments in Subunit Rhinovirus Vaccines

In recent work, areas of the RV capsid proteins VP4 and VP2 (VP0) and the 3' region of the viral polymerase were identified as being broadly conserved across RV serotypes [18]. These regions have been assessed as potential subunit immunogens to induce broadly reactive immunity to RV [18]. Using small animal models, immunization with RV-A16 VP0 protein in combination with the Th1-directing incomplete Freund's adjuvant and CpG promoted potent Th1 immune responses, resulting in VP0-specific T cell IFN-γ production and induction of antibodies which bound multiple virus serotypes [18]. Robust cross-serotype (both to the closely related serotype RV-A1 and importantly also distantly related serotypes RV-B14 and RV-A29) RV-specific Th1 responses were seen systemically after VP0 immunisation, and also in the lungs with VP0 immunisation followed by live intranasal heterologous RV challenge. Furthermore, immunisation and RV challenge enhanced RV-neutralising antibody titres and was associated with reduced viral load *in vivo* [18]. In other studies, immunisation of rabbits with the RV VP1 capsid proteins of RV-B14 or RV-A89 generated antisera containing cross-serotype RV-neutralising antibodies that weakly neutralised several distantly related RV serotypes, suggesting that known regions of higher sequence similarity can induce cross-neutralising antibodies even though neutralising antibodies tend to recognise native virion structural epitopes [19]. Additionally, repeated immunisation of mice with inactivated RV-A1 preparations followed by live RV-A1 infection induced antibodies that predominantly bound VP1 of multiple serotypes, and induced weak (up to a 1:32 dilution) neutralising activity against RV-A16, further implicating VP1 as a potential vaccine candidate [20]. Thus, conserved protein antigens exist within RVs and these can induce cross-reactive cellular and humoral immune responses with protective abilities. These regions of RVs (VP0 or VP1) are applicable as candidate subunit vaccines for RVs and warrant further investigation.

Future Outlook

The twin discoveries of tuneable adjuvants and potential immunogens which can provide cross-serotype immunity to RV offer potential for future developments of a vaccine formulation against this widespread disease. The combination of an adjuvant able to drive a highly Th1 polarised response and an immunogen which could lead to broad spectrum protection could lead to robust protective immunity and minimal side effects in terms of undesirable airway inflammation in individuals suffering from RV-induced exacerbations of asthma - the most critical unmet medical need relevant to RVs.

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