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PRO AND CON EDITORIALS

Rhinovirus vaccination: the case against

A. Papi and M. Contoli

Rhinoviruses are the aetiological agents of the most common acute infectious disease in humans, the common cold [1]. In the last few decades, several studies have provided clear evidence for a major role of respiratory viruses in triggering exacerbations of obstructive lung diseases [2], and rhinoviruses have emerged as the most frequently identified viruses during such acute episodes [3, 4]. Consequently, since it is now recognised that rhinovirus infection can lead not only to a mild and self-limiting disease of the upper airways (*i.e.* the common cold) but also to a more severe disease involving the lower airways, with a relevant impact on patient quality of life and healthcare-related costs (*i.e.* asthma and chronic obstructive pulmonary disease (COPD) exacerbations), the medical attention to rhinovirus infections has changed profoundly.

Current pharmacological treatments, including inhaled glucocorticoids, are not very effective in the prevention of these acute events [5, 6]. Furthermore, apart from influenza virus infection and, probably, respiratory syncytial virus [7–9], we do not have effective and safe antiviral treatments against the most common respiratory viruses [2]. Prevention, using vaccination, has long been thought to be potentially the most effective way of controlling virus-induced diseases. However, vaccine development programs for respiratory viruses have so far had little success, with the notable exception of influenza viruses.

Identification of the specific viral serotypes responsible for the development and manifestation of a disease is the *conditio sine qua non* to develop and set up a viral vaccine. This is particularly evident for the influenza vaccine, which is updated every year after the isolation of the major serotypes responsible for epidemics, and for other vaccines, such as papillomavirus [10], where a few specific serotypes are associated with cervical cancer. For this reason, rhinoviruses have been a very difficult target for vaccine design, since >100 rhinovirus serotypes exist [11]. Recent molecular studies found that the most important *in vivo* role of neutralising antibodies against rhinovirus is to bind the virion and work synergistically with other immune system components [12]. While these results may simplify the goal of creating a vaccine by focusing on capsid recognition rather than possible antibody-induced conformational changes, developing vaccines against all 100 serotypes remains a daunting task. Indeed, all these serotypes are potentially simultaneously present in the environment and, thus, potentially responsible for the clinical manifestations associated with rhinovirus infection.

Following the discovery of the antigenic diversity of rhinoviruses in the 1950s and 1960s, the hope for the development of a common cold vaccine was considerably reduced. ''Is a rhinovirus vaccine possible?''; this relevant question was the title of a prescient editorial [13] by J.P. Fox in 1976. The author highlighted the need for defining the full extent and strength of cross-relations, and for identifying the more closely related groups of serotypes as the basis for formulation of a broadly effective rhinovirus vaccine containing a limited number of serotypes. Thus the answer to this question relies upon the identification of antigenic epitopes common to different serotypes, able to induce the production of cross-reactive antibodies.

Early studies on naturally occurring or experimentally induced human rhinovirus infection ascertained that rhinovirus infections in antibody-naïve individuals are followed by the development of serotype-specific neutralising serum antibodies (immunoglobulin (Ig)G) and also that similar, secretory antibodies (IgA) are induced in the airways [14, 15]. Further studies in humans and in animal models have demonstrated that serum cross-reactive neutralising antibodies can develop following infection with different serotypes [16–18]. This raised the hope that a few serotypes were responsible for most of the clinical burden, but the problem could not be simplified by selecting a few strains that were clinically important, or even by selecting a small set of strains that induced immunity against a range of phenotypes [1, 19]. Also, the attempt to achieve enhanced and broadened antigenicity through the use of polyvalent rhinovirus vaccines has been disappointing [19].

Cross-reactivity has, thus, become the key word in the search for feasible and effective rhinovirus vaccines. From time to time, antigenic peptides derived from one or the other of the four serotype-specific capsid proteins (VP1, VP2, VP3, VP4) [20, 21], have been claimed to have cross-immunogenicity between different serotypes and induce cross-reactive antibodies against several different rhinovirus serotypes [22, 23]. Thus, in the past and more recently, they have been proposed as putative candidates for the development of wide-spectrum vaccines protecting against rhinovirus infection and its clinical consequences. However, virtually all data available so far are based on the development of *in vitro* neutralising antibodies. Despite many decades of investigation, none of these preclinical studies has yet produced robust data to support the

Research Centre on Asthma and COPD, Dept of Clinical and Experimental Medicine, Section of Respiratory Disease, University of Ferrara, Italy.

CORRESPONDENCE: A. Papi, Research Centre on Asthma and COPD, Dept of Clinical and Experimental Medicine, Section of Respiratory Disease, University of Ferrara, Via Savonarola 9, 44121 Ferrara, Italy. E-mail: ppa@unife.it

entry of rhinovirus vaccine in its complete clinical development. So far, not a single large-scale, randomised, placebo-controlled clinical trial has been conducted to test the clinical value of these hypothetical broadly cross-reacting candidates.

The study by EDLMAYR *et al.* [24] published in the current issue of the *European Respiratory Journal* makes a step towards this difficult target. They found that recombinant VP1 proteins from the phylogenetically distant human rhinovirus (HRV) strains HRV14 and HRV89 (*i.e.* the entire 14VP1 and 89VP1 virion capsid proteins) induced antibodies 1) reacting with natural VP1 and 2) cross-neutralising different rhinovirus strains. The latter activity is widely discussed and emphasised by the authors, who propose recombinant VP1 as a candidate for the development of a vaccine to prevent rhinovirus induced clinical manifestations, including asthma and COPD exacerbations. In particular, the authors show that antisera against recombinant (entire 14 and 89)VP1 show higher reactivity and stronger neutralising activity compared to sera raised against highly conserved amino acid sequences (peptides, PVP) of the HRV14 capsid proteins VP1 and VP3. Notably, the antibodies against these peptides have been found previously to be capable of neutralising the majority of rhinovirus serotypes and have been proposed, in 1987 [22], as rhinovirus vaccine candidates.

The authors selected VP1 protein for this study, as it is the most exposed of the four capsid proteins, and it is critically involved in rhinovirus binding to its receptor and in infecting respiratory cells [20]. Indeed, it has been shown that VP1 is primarily recognised by the rhinovirus-neutralising antibodies [25, 26], although with some controversy: for example, recent studies found that antibodies against the N-terminus of VP4, but not of VP1, successfully neutralise rhinovirus infectivity *in vitro* [23].

Though of remarkable interest, the study by EDLMAYR *et al.* [24] clearly reveals how slowly the search for a cross-protective pan-rhinovirus vaccine progresses. The results of the study undoubtedly represent a promising start in this field, but they can hardly be considered conclusive.

First of all, the advantages of VP1-specific antibodies over other peptide-specific antisera (PVP1A, PVP1B and PVP3A) in neutralising rhinovirus infectivity, are questionable. Unfortunately, at variance with all the other neutralisation assays presented in the study, the degree of cell protection offered by these peptide-specific antisera was not properly quantified in this experimental setting. The rather small differences observed between the different antisera were evaluated by means of a subjective, semiquantitative analysis (table 1 in [24]), and for this reason, they should be interpreted with caution in the absence of any statistical confirmation. More specifically, as in this case, the apparent differences occur in a range of antiserum dilutions where anti-14VP1 antibodies are almost totally ineffective in neutralising (in carefully quantified manner) HRV14-induced cytopathic effects (as shown in figs 5 and 6b of [24]).

A concern is the discrepancy between 14VP1 immunogenicity and ability to induce specific immune responses in animals (figs 2 and 4 in [24]) on the one hand, and the inability of the resulting specific antisera to show any protective effect in a biological system where HRV14 cytopathic effects are evaluated (figs 5 and 6b in [24]) on the other. Once more, these results underline the distance that needs to be covered between achieving an effective serological response in animals, and developing efficacious and protective antisera/vaccines.

On the same line, the authors emphasise the broad cross-neutralising effects of the polyclonal immune responses raised against the entire VP1 proteins, but overlook the absence of protection exerted by anti-14VP1 antiserum against HRV14 infectivity and, more generally (fig. 6b), the lacking, or at the best, modest protection offered by both the anti-89 VP1 and anti-14 VP1 antisera against several other rhinovirus serotypes (HRV 1A, 3, 14, 18 and 37). Doesn't this call into question the broadness of their neutralising effect across serotypes?

There are also some worrisome inconsistencies in the results of the study. The authors comment on the results of anti-VP1 antisera cross-protecting against distantly related rhinovirus strains, by pointing out that in one set of experiments (fig. 6b in [24]) anti-14VP1 antibodies are able to inhibit HRV89 infection more strongly than the anti-89VP1 antibodies themselves. However, the opposite is reported in another experimental setting (fig. 5 in [24]), again raising uncertainty over the conclusions.

If such variability in the results occurs in a very controlled experimental system, what is going to happen in a much more complex system (*i.e. in vivo* vaccination) where an endless number of variables and pathways need to be taken into account? Animal models testing the efficacy of rhinovirus vaccination *in vivo* would be of help. However, for a long time the development of small-animal models of rhinovirus infection in many species, from mice to monkeys, has been unsuccessful [2]. Indeed, human rhinoviruses belonging to the major rhinovirus group (>90% of rhinovirus serotypes) recognise human intercellular adhesion molecule (ICAM)-1 as their cellular receptor and do not bind nonhuman ICAM-1 [27–29]. Such a limitation has recently been overcome by the development of the first mouse model of rhinovirus-induced disease, in which transgenic mice expressed a mouse–human ICAM-1 chimera [30]. This model represents an invaluable tool for the development of future interventions for the prevention and treatment of rhinovirus infections.

To complicate matters further, molecular detection methods have revealed the existence of a novel rhinovirus group, designated human rhinovirus species C, that is now known to be highly prevalent and widely distributed worldwide. Species C variants have not been cultured and, therefore, cannot as yet be formally assigned into separate serotypes. However, sequence data has revealed a large number of distinct genetic lineages identifiable by sequence comparisons in the VP1 and VP4/VP2 gene regions [31]. If these sequence variations correspond to major antigenic differences and different serotypes of rhinovirus C, the range of rhinovirus serotypes may be even wider than was previously thought.

Therefore, although progress has been made, we are only slowly climbing the slippery slope that has characterised the search for the right antigen to be targeted for effective protection against rhinovirus infections. There is still a long

way to go and no clinically effective rhinovirus vaccine is on the horizon yet.

STATEMENT OF INTEREST

None declared.

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