

RHINOVIRUS, ALLERGY AND ASTHMA – WHAT ARE SOME OF THE KEY QUESTIONS?

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ABSTRACT

Rhinovirus (RV) is a cardinal virus pathogen, particularly in the context of pre-existing lung disease. It is likely to cause disease by subversion of key elements of the host tissue immune response, particularly innate immune responses. It is now clear that RV may cause asthma exacerbations through excessive virus replication in patients following initial infection. This augmented virus replication is probably the result of both host and pathogen factors and new therapeutic advances will have to target both the pathogen and the host. It seems likely given the development of molecular techniques, their refinement and advances in reversed genetics that preventative treatments for RV will be available in future. A rapid, simple 'cure' for RV seems doubtful, but it may be possible in future to attenuate the effects of infection and to 'protect' the airways of patients with asthma and chronic obstructive pulmonary disease (COPD).

INTRODUCTION

Rhinovirus (RV) is now acknowledged to be of critical medical importance, particularly in allergy, asthma and chronic obstructive pulmonary disease (COPD). Not only is RV the most common virus infection in the general population, but it has been shown to play a cardinal role in exacerbations of asthma and COPD. RV is therefore an infection of particular importance in patients with pre-existing respiratory disease states. Paradoxically, it is one of the least studied viruses and until fairly recently relatively little was known about its basic biology and relevant disease associations.

RV is the most frequent cause of 'common colds'. Epidemiological studies done in the early 1960s found that RV caused in excess of 60% of all upper respiratory infections associated with a cold syndrome. Other viruses cause only a relatively small percentage of colds in a given calendar year, except when epidemic strains of other viruses occur – as in the recent influenza H1N1 outbreak. Until recently, the role of RV as a cause of colds was well established, but the important role of this ubiquitous RNA virus in the genesis of acute episodes of obstructive airway disease was not known or appreciated. Recent research has changed that perception.

Studies in the late 1990s showed for the first time that RV is associated with asthma exacerbations. Johnston

and co-workers¹ found that RV was associated with most exacerbations in children aged 6 to 12 years. In 292 asthma episodes associated with increases in asthma symptoms, cold symptoms or reductions in peak flow, a virus was identified in 226 patients with the majority (>70%) being RV. This brought about a paradigm shift in the understanding of the role viruses play in asthma exacerbations, made possible by the development of a polymerase chain reaction (PCR) to identify RV and other virus infections. The technique has subsequently become the benchmark to identify viruses associated with disease states and has been shown to be 5-50 times more sensitive than virus culture. Subsequent studies have shown that the association of RV with exacerbations is also present in all age groups, adult patients with asthma and in COPD.²⁻⁴

In summary, the key role of RV has been underestimated but recently research has burgeoned and detailed information has become available. As a case in point: 25 years ago publications focused on 'virus research' totalled 13 900, of which RV made up 0.04%. The landscape has changed significantly and the percentage now approaches 5%.

HOW DOES RHINOVIRUS CAUSE INFECTION AND INITIATE DISEASE?

We have gained a good understanding of how RV causes infection in humans (Fig. 1). After the initial contact, the virus attaches itself to cellular receptors. There are two receptors identified in humans, intercellular adhesion molecule-1 (ICAM-1) for the major group of RV and low-density lipoprotein (LDL) receptor for the minor group RV. After the receptor is occupied, RV is internalised and then uncoats with release of virus genome into the cytoplasm – RV is a positive single-strand RNA virus. This is followed by translation of the virus RNA forming a polyprotein which is sequentially processed by two viral proteases – 2A and 3C – that are included within the polyprotein, resulting in generation of func-

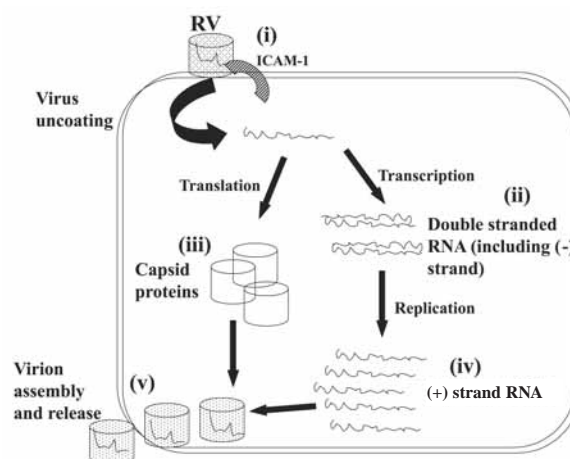


Fig. 1. Rhinovirus replication is demonstrated. After attachment to the cellular receptors (ICAM-1 or LDL), virus is internalised, naked RNA is released followed by production of negative-strand RNA and virus structural proteins. After positive-strand RNA is made, the new viruses are constituted and are released following lysis of the host cell.

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tional structural proteins as well as the viral polymerase. The viral polymerase acts to replicate the viral genome resulting in intermediate negative-strand RNA and double-stranded RNA moieties. The resultant progeny genomes are encapsidated by the structural proteins to form fully assembled virions that are released on lysis of the cell.

Although the RV genome is elegant and efficient, many questions remain, particularly with regard to the activities and function of some of the functional virus proteins such as proteins 2A and 3C. After RV enters the cell, a polyprotein is translated from virus RNA and this protein is then processed by virus proteases into a group of structural proteins as well as a number of other proteins with uncertain function, including the non-structural 2A and 3C/3CD proteins. These virus proteins may have various activities including interfering with cellular functioning and reducing efficacy of host immune responses. We have recently demonstrated that 3C damages the nuclear membrane leading to altered traffic into the nucleus, which in turn may affect cellular transcription and other vital cellular survival functions.⁵ Much remains to be understood about how the virus manages to hijack the cellular 'machinery' for its own ends.

Recently it has become established that RV may cause cellular infection, not only in the surface epithelial layers but also beyond the airway surface. Infection of surface epithelial cells was established in our initial studies,⁶ but it was initially unclear whether other airway cells are infected. Macrophage infection has been shown to occur,⁷ and our own studies have recently demonstrated that airway fibroblasts can be efficiently infected with high levels of viral replication.⁸ The likely outcome of epithelial and mesenchymal cell infection, particularly infection of fibroblasts, is likely to be an explosion of pro-inflammatory activities resulting from the infection itself – and as a result of host defensive responses to infection.

Many studies have now demonstrated production of multiple pro-inflammatory mediators in response to RV infection. Cytokines produced in response to infection include interleukin (IL)-8, RANTES, interferon (IFN)- α and - β , tumour necrosis factor (TNF)- α and many other mediators.^{4,9} Our own studies have demonstrated an important role for the chemokine epithelial-neutrophil activating factor (ENA)-78 in response to RV infection.¹⁰ It leads to recruitment of neutrophils shortly after RV infection, probably triggering an influx of other inflammatory cells into tissues.

Infection of fibroblast cells by RV may be of singular importance. Replication was first conclusively demonstrated in fibroblasts using dual antibody staining for RV16 and double-stranded RNA (Fig. 2). Infection with production of virus progeny occurred in fibroblast cells because they were positive by immune-fluorescence for vimentin protein, the marker that characterises these cells. The studies also demonstrated prolific viral replication leading to a lytic death of cells. To date

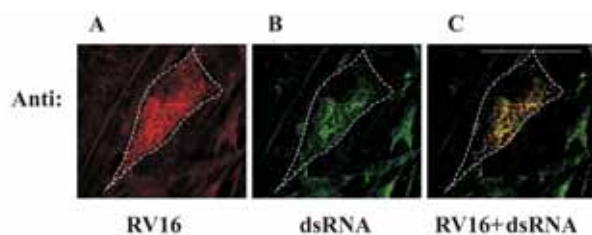


Fig. 2. Confocal microscopy showing RV infection of fibroblast cells. Antibody to virus colocalises with double-stranded RNA signifying RV replication.

infection of other tissue types and cells in the body have not been studied and the distribution of RV infection through the body is poorly understood. A study in infants has suggested that systemic spread of RV infection may occur but it is not clear if this is a frequent event.^{3,11}

A conundrum at this stage is whether RV may persist in asthma. Traditionally it has been assumed that RV (as for all RNA viruses) is eliminated fairly rapidly by the host, and persistence is thought not to occur, at least not in non-asthmatic individuals. This view has been challenged and there is now some evidence that RV may have prolonged nasal residence, particularly in asthmatics. The first study to suggest such a scenario was reported by Kling and co-workers.¹² It was shown that in a cohort of children presenting to an emergency department with acute asthma and associated RV (>80% of children had RV present), almost 40% of children still had RV present after 6 weeks. This was an unexpected finding and assessment of the amounts of virus RNA detected by PCR suggested that these were chronic rather than new infections. A study in an adult asthma cohort reported finding RV in the submucosa in asthmatics with severe asthma, again suggesting the possibility of chronic infections.¹³ Further studies are indicated and will help us understand better how RV plays not only a central role in exacerbations of asthma but possibly also in persistent severe disease.

WHAT ARE THE KEY HOST DEFENCES AGAINST RHINOVIRUS INFECTION?

The chief defence against RV infection is the production by human host cells of type I and III interferons (IFNs). These are mainly type I IFN: IFN α , - β and - λ . These proteins form part of early innate immune responses and lead to cellular apoptosis with non-inflammatory elimination of RV. Innate mechanisms provide a first line of defence, followed by adaptive immune responses that eliminate infected cells by way of IFN- γ production and cytotoxic lymphocyte (CTL) proliferation and activities.

Recent studies in asthmatics have demonstrated that IFN responses may be subtly deficient. Wark and others¹⁴ as well as Contoli and co-workers¹⁵ demonstrated that there was enhanced virus replication in cells obtained from asthmatic airways. This was accompanied by up to 30% reduction in the production of type I IFN. The findings brought about a paradigm shift in our understanding of asthmatic responses to RV (and other infections) and may explain why disease burden is often so much higher in this group of patients with pre-existing disease of the airways.

The reasons for a reduction in IFN responses in asthma have not been clarified and appear unlikely to be associated with genetic polymorphisms or related factors. An intriguing possibility is that an endogenous immunosuppressant may be present in asthma tissue. A strong contender for this role is transforming growth factor- β (TGF- β), a molecule that is part of a large cytokine family that includes activins and bone morphometric proteins. TGF- β is produced by most tissue cells and primarily directs cellular differentiation but also has potent immune-suppressive activities. It is thought to play an important role in asthma because it induces transdifferentiation of fibroblasts to myofibroblasts, the typical hybrid cell found in the remodelled asthmatic airway. TGF- β has been shown to be increased in asthma, both in a study done in bronchoalveolar fluid,¹⁶ and in tissues.¹⁷ The amount of TGF- β in asthma tissue biopsies also correlated with severity of asthma.

CAN TGF- β ACT AS ENDOGENOUS IMMUNE-SUPPRESSANT IN THE CONTEXT OF RV INFECTION?

Our own recent studies have concentrated on the role of TGF- β in RV infection. Overall, these investigations have found that RV replication is enhanced in the presence of TGF- β and shown that this is mediated via suppression of IFN production.¹⁸

Incubation of fibroblast cells with TGF- β for 24 hours prior to infection with RV leads to a marked increase of viral replication, both as measured by PCR and viral titration (Fig. 3). Longer term incubation with TGF- β induced transdifferentiation of airway fibroblasts with formation of myofibroblasts. These cells could also be infected and sustained higher levels of RV replication. This was found for both RV16, a major group of RV and RV2, a minor group member. A deficient type I IFN mRNA response was noticed at 24 hours and the IFN-regulated gene proteins RANTES and IP10 were both suppressed. An interesting aspect of these studies was demonstration of dysregulated type I IFN responses (Fig. 4). In cells not exposed to TGF- β , IFN responses were correlated with the number of virus particles. However, in myofibroblasts (i.e. putative asthmatic cells) this association was lost and IFN responses became dissociated from RV replication.

Identification of an intrinsic deficiency of IFN responses in response to RV infection of asthmatic patients suggests possibilities for this immune defect in asthma to be rectified by treatment. In our studies, exogenous recombinant IFN was added in experiments. This strategy resulted in almost complete normalisation of immune responses with reduction of RV replication to baseline levels (Fig. 5). These tantalising findings suggest that further studies using IFN may be indicated,

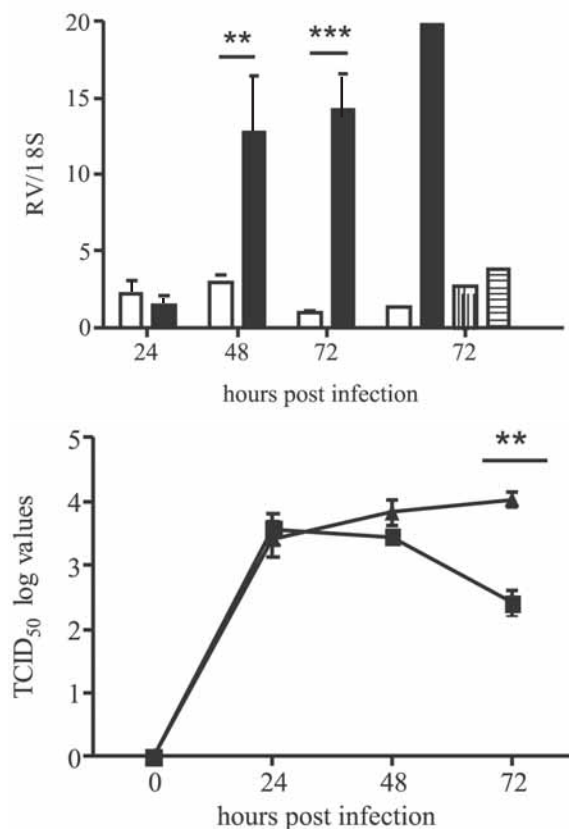


Fig. 3. Virus replication is enhanced by TGF- β as shown by RT-PCR (top panel) and virus titration. Black bars (top panel) and triangles (bottom panel) indicate cells exposed to TGF- β cells prior to infection.

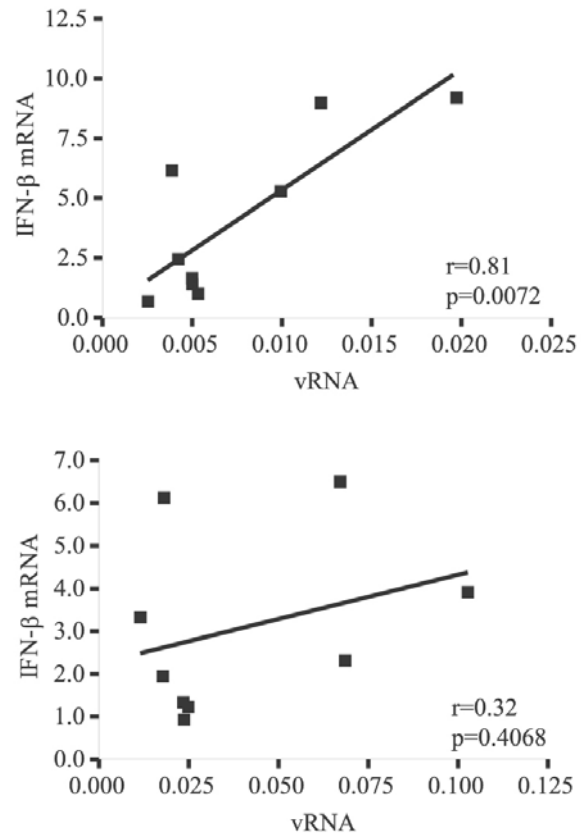


Fig. 4. Dysregulated IFN- β responses in cells exposed to TGF- β (bottom panel) as opposed to cells not influenced by the cytokine (top panel), indicating 'disconnect' between virus RNA production and host innate immune responses.

particularly if the compound can be given by inhalation in order to have direct effects on the lung.

FUTURE THERAPEUTIC POSSIBILITIES

As noted above, treatment in future with IFN may help to correct the subtle immune deficiency that is present in asthma. Further studies will help us to understand both the immune deficiency and how this may be rectified by exogenous IFN treatments. An alternative would be to use TGF- β antagonists or blockers. A number of compounds have been studied in mouse models to date and indicate that it may be possible to antago-

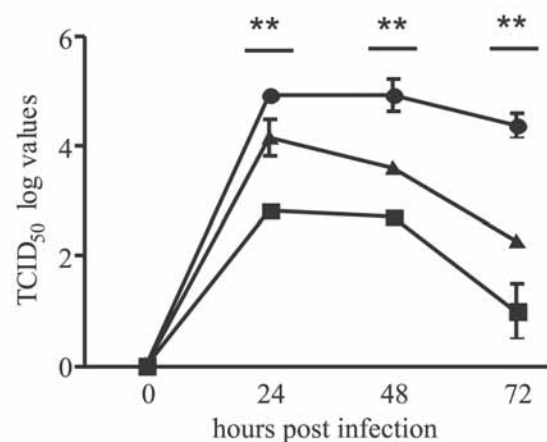


Fig. 5. Addition of recombinant IFN prior to or during RV infection rectifies virus replication levels. Shown are titration curves with RV infection only (circles), IFN during infection (triangles) and 24 hours prior to RV infection (squares).

nise the effects of TGF- β either at receptor or protein level. However, no studies have yet been reported in humans and this aspect awaits further development.

Studies directly targeting the virus have been ongoing, particularly assessing chemical or other antagonists of the virus and RV-binding receptors on human cells. Again, this strategy has proved to be difficult and will take further experimentation and validation in human models to prove efficacy.

Finally, vaccination against RV remains a key approach that merits further investigation. Reverse genetics may make it possible to engineer RV mutants and produce immunogenic virus proteins effective against the more than 100 RV serotypes. These studies are notoriously difficult, particularly because of a lack of good animal and human models, but have significant potential for the future.

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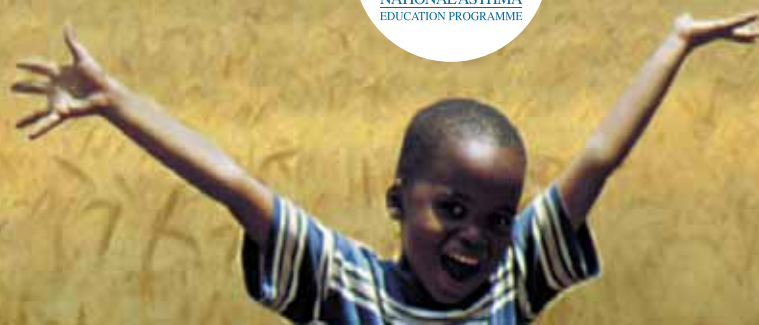
Declaration of conflict of interest

The authors declare no conflict of interest.

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