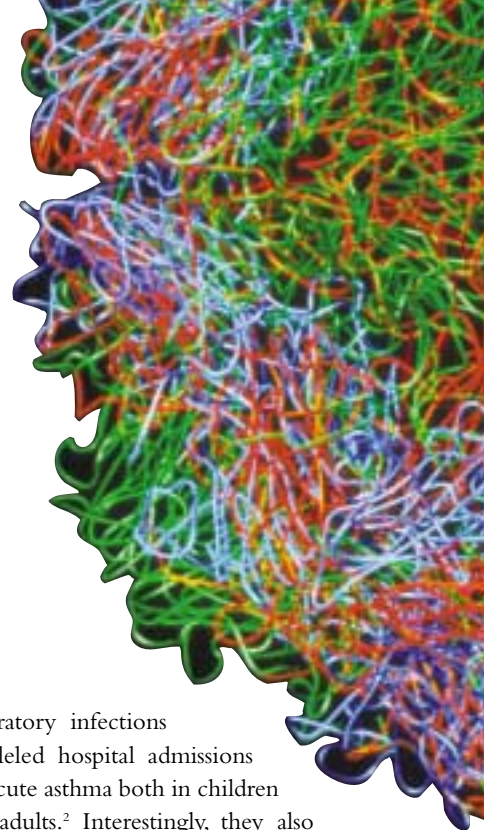


The infection challenge



The technique of experimentally infecting cells with rhinovirus is helping scientists probe the link between the common cold and the acute exacerbations that plague many asthma sufferers. Stephen Holgate looks at the current state of play in this potentially lucrative therapeutic area

It is widely accepted that asthma is a disease involving the recruitment of inflammatory cells and the enhanced release of inflammatory mediators. In addition, a variety of environmental insults can trigger exacerbations of the illness, impacting significantly on the quality of life of asthma sufferers. Poor underlying anti-inflammatory control and interaction with inhaled allergens are well recognised causes of exacerbations which respond to corticosteroids. However, in both children and adults, current understanding is that respiratory virus infections are the most common trigger of acute asthma exacerbations – accounting for up to 80% of episodes in children and 60–80% of cases in adults – particularly in the autumn, winter and spring months. Viral infection is also associated with more severe acute asthma and more serious airflow obstruction, calling for longer stays in hospital.

While it is understood that some exacerbations may respond to an increase in corticosteroid therapy, clinical trials conducted on both adults and children have failed to show the beneficial effect on virus-induced exacerbations that would be expected with an increase in steroid dose. This raises a number of important questions concerning not only the pathogenesis of exacerbations but also how they should be treated. To date, no antiviral therapies have become widely adopted treatments for asthma.

At this point, it is worth considering a number of investigations that have significantly contributed to our understanding of the role viruses play in asthma. In a longitudinal UK study of over 100 schoolchildren in the community, and a total of 280 exacerbations, micro-organisms were detected in 83% of cases.¹ Separately, the same investigators demonstrated that upper

respiratory infections paralleled hospital admissions for acute asthma both in children and adults.² Interestingly, they also observed that virus-induced respiratory exacerbations tended to occur shortly after children returned to school following vacations. Presumably, viruses had been picked up over the holiday period and then

exchanged between children on their return to the school environment. Researchers in New Zealand have made a similar finding.³

Statistics have also been collected for asthma exacerbations in adults in the community. In a 15-month study of 130 atopic asthmatic adults,⁴ asthma exacerbations were associated with a respiratory virus in 60% of cases. In both children and adults, infection with rhinovirus has been shown to be the predominant cause of asthma exacerbations.^{1,4}

And a 2002 Australian study compared exacerbations in asthmatics, for whom there was positive virus identification compared to those where no virus had been identified.⁵ Asthmatics in which a virus had been positively identified were ill for longer, demonstrated less symptomatic allergy, and had a lower baseline lung function on presentation, suggesting that the susceptibility to virus infection is non-allergic in nature.

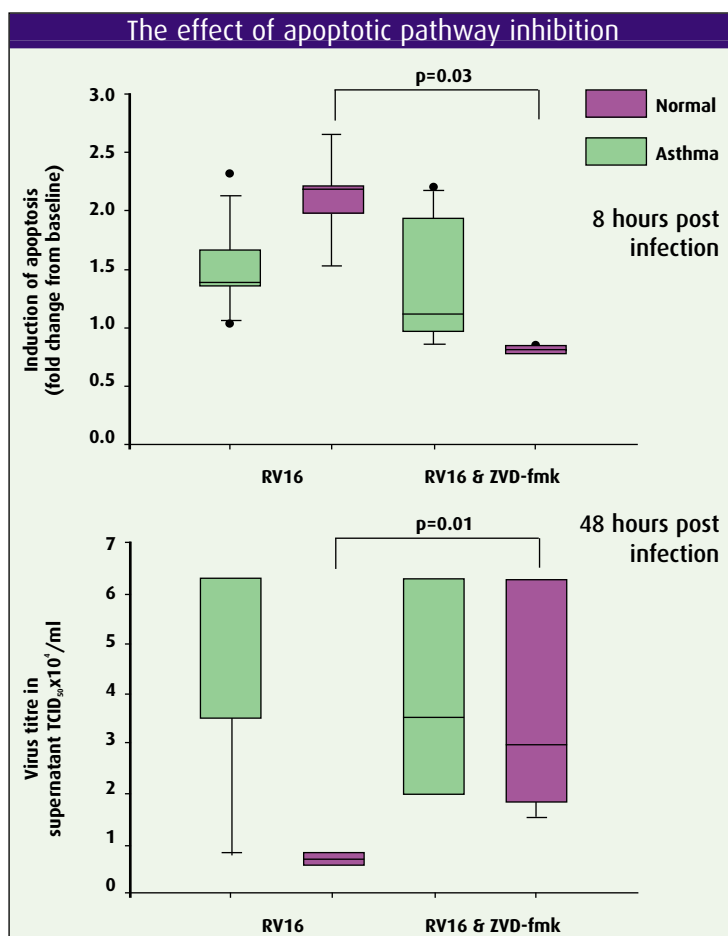


Figure 1: Bronchial epithelial cells were treated with RV16 alone or in the presence of the caspases-3 inhibitor, ZVD-fmk, to block apoptosis. In asthmatic cells, pre-treatment with ZVD-fmk had little effect on apoptosis. But in normal cells, RV-16 infection resulted in a significant induction of apoptosis eight hours after infection; this was abolished by ZVD-fmk pre-treatment. 48 hours later, this block in apoptosis led normal cells to behave like asthmatic cells, with a marked increase in the release of rhinovirus from cells.

Molecular artwork of the surface structure of a single rhinovirus, cause of the common cold as well as other respiratory diseases. Rhinoviruses belong to the Picornaviridae family and are spread readily in the air by droplets produced by talking, coughing and sneezing. The virus may infect mucous membranes of the nose and lungs

Alfred Pasiaka/Science Photo Library

Viral infection as a research tool

To investigate the efficacy of corticosteroids in preventing virus-induced asthma exacerbations, one Dutch group studied 25 atopic mild asthmatics who had been infected with experimental rhinovirus 16 (RV16). The patients were also administered with budesonide 800µg bd, with treatment starting two weeks before the infection.⁶ The inhaled corticosteroid had no significant effect on the respiratory virus-associated changes in lung function or inflammatory cells in airway biopsies. According to these results, inhaled corticosteroids appear to offer limited protection against acute virus-induced asthma exacerbations, underlining an important unmet clinical need for alternative therapeutic approaches. This clinical trial also demonstrated the utility of experimental RV16 challenge as a research tool, which may be used to test the efficacy or otherwise of potential anti-rhinovirus therapies. In addition, important strides are now being made in our understanding of the mechanisms that underlie exacerbations. For example, it has been known for some time that respiratory virus-induced exacerbations are more often associated with a neutrophilic rather than an eosinophilic (allergic) airway inflammatory

response.⁷⁻⁹ Recent studies using bronchial biopsies have shown that experimental rhinovirus infection results in an increase in replicative viral RNA in the airway epithelium of asthmatics,^{7,10} reinforcing the view that the virus preferentially gains access to the asthmatic epithelium and it is through this tissue layer that the exacerbation is initiated. Once in the epithelium, the virus not only replicates itself, it also activates oxidative pathways that result in the release of mediators and cytokines and, ultimately, epithelial cell death and virus shedding.

The author and co-workers have used human asthmatic epithelial cells in tissue culture to explore the connection between asthma exacerbations and common cold viruses.¹¹ The team hypothesised that asthmatic bronchial epithelial cells were more susceptible to the effects of infection with common cold viruses when compared to

healthy controls. This was subsequently confirmed by the finding that RV16 infection of human airway epithelial cells from non-asthmatics promoted controlled programmed cell death (apoptosis). However, in epithelial cells from asthmatics, which were infected with RV16, there appeared to be a defect in the early activation of the apoptotic pathways leading to enhanced survival of the cells. After 48 hours in tissue culture, it was apparent that more of the asthmatic epithelial cells were killed by the virus. This cytotoxic death in the asthmatic epithelial cells provoked by RV16 infection was accompanied by an order of magnitude greater release of rhinovirus into the culture supernatant. Thus it appears that in asthma, there is a fundamental defect in the ability of airway epithelial cells to activate the pro-apoptotic pathway and, in this way, the virus is able to replicate within the epithelium, leading eventually to cell death. The team has also demonstrated that inhibition of the apoptotic pathways in normal epithelial cells results in increased virus replication and cell cytotoxicity similar to that observed in the epithelial cells of asthmatics (see Figure 1).

It is pertinent to ask why asthmatic epithelial cells *in vitro* are so vulnerable to virus-induced cell cytotoxicity when compared to normal epithelial cells, and whether there might be a primary defect in innate immunity peculiar to asthma. Viral infection of both normal and asthmatic epithelial cells produces marked increases in a wide variety of cytokines and chemokines, but there was no difference in the quantity/type produced between the normal and asthmatic cultures. However, infection of normal epithelial cells with RV16 did produce a dramatic increase in the immunoregulatory cytokine, interferon beta (IFN- β), both at RNA and protein level, whereas this was not observed in asthmatic epithelial cells.

Since IFN- β is involved in activating programmed cell death, it seemed to be a key candidate mediator accounting for the differences observed between normal and asthmatic epithelial responses to rhinovirus infection. Further experiments have demonstrated that the addition of exogenous IFN- β to asthmatic epithelial cells *in vitro*

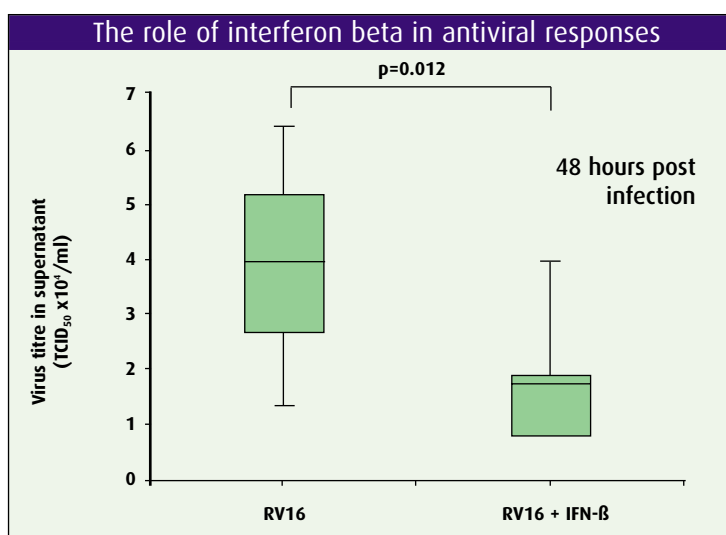


Figure 2: In asthmatic bronchial epithelial cells treated with IFN- β both before and after RV16 infection, there was a significant reduction in virus release compared with cells infected with RV16 alone.

prior to and after infection with rhinovirus, restored the cells' ability to eliminate the virus and enter into programmed cell death (see Figure 2). Thus a primary defect in IFN- β production would seem to be a reasonable explanation for the increased susceptibility of asthmatic lower airways to common cold viruses. Since viral-induced exacerbations of asthma are non-responsive to corticosteroids this represents a potentially significant market, especially in those patients with severe forms of the disease. Administration of an inhaled IFN- β formulation might provide a new way of preventing virus-induced exacerbation of asthma. Clinical efficacy can be demonstrated in a proof-of-concept study utilising the rhinovirus challenge model.



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