Common Colds and Respiratory Viruses: Impact on Allergy and Asthma

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Introduction

The association between common colds and acute wheezing episodes has been recognized for decades [1]. Indeed, wheezing associated with colds is the most common form of wheezing at all ages [2]. Although the nature of the debate has evolved over the years, many of the contradictory issues are still relevant, including whether episodic virus-induced wheezing in young children should be considered asthma. Furthermore, the debate continues over whether severe early viral infections have a causative role in the development of asthma, by immune modulation, airway damage, or both, or whether children who present with virus-induced wheezing have a pre-existing predisposition.

The clinical correlation of the common cold with asthma episodes is not recent. However, it is only in the last 10–15 years, with using sensitive methodologies for the detection of the most prevalent respiratory viruses, such as rhinoviruses and coronaviruses, we have been able to appreciate their importance [3, 4]. Furthermore, some of the most obvious changes in affluent societies, such as family structure, congregation, and hygiene, have implications for the epidemiology of infections, leading to speculation that infection-associated factors may be related to the asthma epidemic [5].

Using the polymerase chain reaction (PCR) and detailed analysis of epidemiological data, our understanding of the relationship between viral infections and asthma exacerbations has improved [6]. Nevertheless, there still are some apparently contradictory effects and many unexplored aspects to be addressed. The mechanisms by which respiratory viruses exacerbate asthma are under scrutiny. In addition, the possibility that some viral or intracellular bacterial infections may initiate asthma is still disputed. The subject has become more complicated since it was recognized that early exposure to microorganisms and/or different infections may actually protect against asthma, the so-called "hygiene hypothesis" [7].

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Understanding the role of viral infections in the development of allergy and asthma may have implications on designing and selecting optimal therapeutic strategies, while indirectly affecting immunization programs, antibiotic use, and marketing of antiviral drugs, as well as the development of new approaches to therapy. This chapter addresses the interplay between viral infections, the immune system, and lung development, focusing on the possible role of respiratory viruses in the origin and exacerbations of asthma and allergy. Also presented are antiviral strategies for the prevention and treatment of virus-induced asthma exacerbations that have been investigated recently.

Viral Infections and the Development of Allergy and Asthma

To understand the potential implications of viral infections in the development of asthma, it has to be kept in mind that asthma has a complex natural history that includes different phenotypes, which may differ in their pathogenesis. It is well established that a majority of asthma cases start early in life. However, a significant proportion of children who wheeze at a young age, mostly after upper respiratory tract infections (URTIs), overcome their problem before school age. These subjects, characterized as 'transient early wheezers', have reduced airway function at birth; thus it is likely that their disease is at least partly mechanical rather than immunological in nature [8]. Other children commence wheezing early and continue to do so at least until adolescence. These persistent wheezers have an altered immune response with a rise in IgE levels during their first reported URTI, and no reduction in eosinophil numbers during the acute phase of the URTI, in contrast to transient wheezers [9].

The virus, most frequently associated with severe bronchiolitis in the first years of life, is the respiratory syncytial virus (RSV). RSV infection occurs in almost all children before their second birthday, and clinical presentations vary from subclinical to severe, life-threatening bronchiolitis [10]. Early studies have pointed out that children suffering from severe bronchiolitis have an increased risk of developing asthma in subsequent years [11]. Whether RSV bronchiolitis represents a marker of susceptibility to wheezing or it can per se divert the immune system or affect the lung and initiate asthma is not yet concluded. It is also possible that these pathways are not mutually exclusive: a pre-existing susceptibility may become clinically relevant once an exceptionally severe infection occurs (Fig. 1).

In 1971, Rooney and Williams [11] found that 56% of children hospitalized for bronchiolitis would continue to wheeze 2–7 years later, and this has been confirmed in several subsequent studies. However, it has been difficult to ascertain whether this is solely an association or whether causation is also involved, resulting from either direct lung damage or an RSV-mediated immunological deviation toward type-2 cytokine production [12]. Although pulmonary function is reduced many years later in children with a history of lower respiratory tract infection, it seems that this is a pre-existing feature of these children [8, 13].

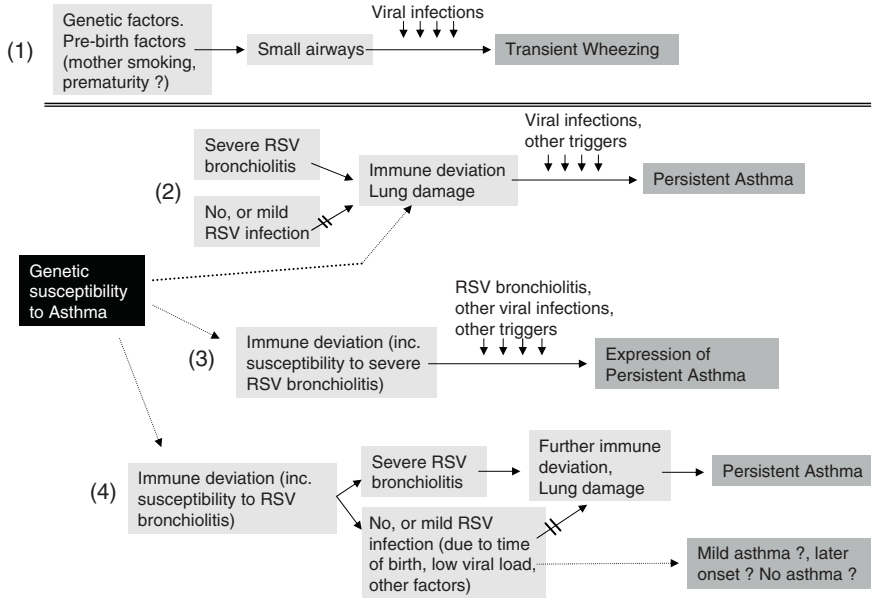

Fig. 1 Transient wheezing may be associated mostly with airway size at birth, resoling before school-age (1) severe RSV bronchiolitis in early life may be either the cause (2) or a marker of susceptibility (3) of asthma. These possibilities are not mutually exclusive, as it is possible that a viral attack may further affect immune programming in a susceptible host (4)

When bronchial responsiveness was assessed, the results were conflicting; either no difference or an increase in bronchial reactivity several years after bronchiolitis has been reported [14]. Equally conflicting are the results relating to the potential effects of RSV in allergic sensitization. One group has shown that RSV bron-chiolitis is an independent risk factor for the development of asthma and allergic sensitization at age 7 years, in fact a stronger risk factor than a family history of asthma [15]. In contrast, other studies have failed to establish such an effect [16, 17]. Differences in disease severity and age of evaluation may partly account for this discrepancy. It is conceivable that severe RSV disease may be required for the establishment of long-lasting effects. In another study, the correlation of RSV bronchiolitis with sensitization that was observed at the age of 6 was not present at age 9–10 years [18]. Importantly, RSV-related effects on wheezing and asthma decline with age, becoming nonsignificant by adolescence [17–19]. These findings should also be interpreted with caution: asthma symptoms may be undervalued in adolescence or may relapse later in life [20]; thus, prospective evaluation of the current cohorts is required to evaluate these possibilities. Most data relating to acute severe infections early in life and the increased risk of asthma implicate RSV, but most of the studies on which these conclusions are based have not adequately looked for other respiratory viruses, in particular rhinovirus (RV). Stein et al. [17] reported a fourfold increased risk of asthma later in life in children with RSV infections that were severe enough to lead to a pediatric consultation

early in life. However, increased risks, —twofold to threefold, were observed with other respiratory viruses, suggesting that any single acute infection severe enough to lead to a pediatric consultation early in life is also a risk factor for asthma later in life. Although important as an observation, this lasting effect, possibly associated with human RV infections that were not virologically confirmed at the time, has not gained enough attention [17]. Nevertheless, it is supported by more recent findings showing that infection with human RV is more frequent during infancy than previously thought [21], can induce severe bronchiolitis [22], is associated with increased airway resistance [23], and is also more strongly associated with persistence of wheezing in the first 3 years of life [24].

A type-2/type-1 cytokine imbalance in favor of type-2 responses or with impaired type-1 responses in acute RSV bronchiolitis has been reported in several instances [25, 26]. These findings could be explained as either an inherent defect or a direct result of the RSV infection itself. A profound imbalance in infants with acute RSV bronchiolitis has been observed, with significantly reduced production of the type-1 cytokines interferon (IFN)-γ, interleukin (IL)-12, and IL-18 and increased production of IL-4 [27]. This imbalance was associated with impaired virus clearance, suggesting that it may be an important determinant of disease severity. In addition, because the imbalance was observed as early as the 1st or 2nd day after initiation of disease, the immune deviation was most probably already present in these infants before RSV infection; deviation of the immune response by the virus itself was unlikely to have occurred so early in the course of the illness, when virus-specific immunity was only beginning to develop [27].

From the above, it is obvious that no safe conclusion about whether RSV bronchiolitis may cause or is only associated with asthma in later life (through common causality) can be currently reached. Nevertheless, these two possibilities are not mutually exclusive. Children with a predisposition to asthma may be prone to develop severe RSV bronchiolitis; however, this infection may also further affect their immune responses and/or lung structure, leading to the development of asthma symptoms (Fig. 1) [28]. With the advent of effective RSV prevention modalities it is now possible to design randomized intervention studies. In these studies, confounding factors should be ruled out by randomization; thus they may offer more conclusive evidence. In one such study, modest differences in pulmonary function were observed between infants treated with ribavirin versus placebo-treated control infants, but the number of subjects was small [29]. Later intervention studies are awaited.

The Protective Effect of Respiratory Viruses on the Development of Allergy and Asthma

As respiratory viral infections are frequently the most apparent event in the presentation of asthma, either as a cause or as a marker, it seems contradictory that similar infections may protect against development of the disease. Nevertheless, the “hygiene hypothesis”, first suggested by Strachan [5], is a dominant theory used

to explain the increasing prevalence of allergies and asthma, based on our understanding of these diseases as dysregulations of the immune system. This subject has aroused much interest because of the findings of an effect of birth order and family size, as surrogate markers of infectious load, on the development of allergy [7, 29]. A protective role of infections also has been seen in developing countries, where an inverse relationship between evidence of respiratory infections and later development of atopy has been observed [30]. One possibility is that these findings could have resulted from a genetic bias in confined communities. However, this is unlikely, as there was a similarly reduced prevalence of atopic disease in East Germany compared with the genetically similar population of West Germany [31]. The East German children were assumed to have been exposed to more infections because of the much greater use of early childhood daycare facilities, which has been associated with less subsequent asthma [31].

There is a paradox, however, as several studies show that parental reports of lower respiratory illnesses are associated positively with later asthma [7]. A possible explanation is that daycare use and large family size are associated with an increased microbial load, which includes gastrointestinal viruses and other potentially protective microbes and is independent of the host response [7]. In contrast, parental reporting of symptoms reflects the host response. Children destined to have asthma have an impaired type-1 response to virus infections and are therefore at a risk of more frequent and severe symptoms, which are more likely to be reported.

In a prospective birth cohort study, children who had more than one respiratory illness confined to the upper respiratory tract during the first 3 years of life were at lower risk of having asthma symptoms at age 7 [31]. In contrast, when the infections were located in the lower airways, the risk for asthma increased significantly in a dose-dependent manner. Although the authors concluded that upper respiratory infections early in life may protect infants against the development of asthma, it is also possible that the development of upper or lower respiratory infectious disease is influenced to a considerable extent by the susceptibility of the host.

Another large birth cohort study found that personal and sibling viral infections (e.g., measles, mumps, rubella, hepatitis, chickenpox, herpes, mononucleosis) during the first year of life resulted in a small protective effect against the development of asthma (but not hay fever or eczema) later in childhood [32]. However, a strong protective birth order effect against all atopic diseases was present in that cohort. When respiratory tract infections as a whole were analyzed, a dose-dependent increased risk for asthma and hay fever was noted. Further, exposure to antibiotics was associated with an increased risk of developing allergic disease.

In another study, the detrimental effect of lower respiratory episodes during the first year of life in the expression of asthma at age 4 was confirmed, while no significant protective effect of upper respiratory infection was found in that setting [33]. An increased number of respiratory infections (e.g., measles, mumps, rubella, varicella) conferred increased risk for atopy in a Danish cohort, irrespective of the age of exposure: the presence of asthma was not assessed in that study [34].

It is probably too early to confirm a possible protective effect of respiratory viruses on the development of allergy and asthma. Prospective studies evaluating

exposure to microorganisms, pathogenic or not, as well as symptomatic infection, will be required to differentiate between the effect of these two factors, also taking into account possible confounders such as birth order and antibiotic use.

We recently proposed an alternative explanation for this apparent contradiction, using the term "incoordination" hypothesis [35]: the physiological rate of development and response of the human immune system may not match the rate of exposure to various stimuli as they currently appear in modern (especially "Westernized") environments. Infectious agents, including viruses, are prominent, but not unique among these stimuli, as they are major determinants of immune maturation and their ecology is affected considerably by environmental changes, including, but not uniquely depending on, hygiene.

Epidemiology of Viruses in Asthma Exacerbations

Although the role of viruses in the induction of, or protection from allergy and asthma is still inconclusive, the evidence for the participation of these pathogens in asthma exacerbations is much stronger. The observation that asthma exacerbations often follow common colds is old and a daily experience of practicing physicians, especially pediatricians. Early reports documented that viral shedding decreased soon after the cold, before the patient referred to their physician or the hospital, indicating that early sampling was necessary for viral detection [36]. Furthermore, virus detection rates in these studies fluctuated considerably; this was attributed to difficulties in RV and corona virus identification. With the use of PCR-based detection for RV and prospective designs, the magnitude of the problem was revealed.

In a prospective study in the community, asthmatic children aged 9–11 years were followed up for 1 year and sampled as soon as they reported cold symptoms [37]. The percentage of asthma exacerbations following virologically confirmed colds was 80–85%. In children hospitalized with severe asthma exacerbations, the viral detection rate was 82% [38]. In adults, the proportion of virus-attributed asthma exacerbations was generally lower. However, it was possible that viral shedding was less or of a shorter duration in adults than in children. In one of the first community-based prospective studies using polymerase chain reaction (PCR) detection for rhinoviruses, virus detection rates were 44%, although cold symptoms preceded 70% of the episodes [39]. In another study (with a combined longitudinal and cross-sectional design) of inner-city asthmatic adults [40], virus detection was once again 44% in followed-up subjects and 50–55% in subjects presenting to the Emergency Department. In another study, virological confirmation was achieved in 60% of asthma exacerbations in adults [40]. Additional studies of similar designs have confirmed the high prevalence of viral infections in association with asthma exacerbations, with RV being the dominant pathogen [41–43].

The conclusion from the above is that respiratory viruses are the most common triggers of asthma attacks, and it is shown that such attacks can be severe, leading

to hospital admissions [44]. Peak in hospital admissions for asthma and virus isolation occurs, in most instances, immediately after school vacations. This pattern of a segregation-dependent disease is a characteristic of rhinovirus colds [45]. Similar seasonal variation has been partly observed in asthma mortality, especially among young children and the elderly, who are most susceptible to viral infections [46].

Another important point, on which all of these studies agree, is that RVs are the most prevalent agents, accounting for 50–60% of all detected viruses. This is thought to reflect the prevalence of these viruses in common colds, rather than any specific asthmagenic properties, because there are minor or no differences in symptoms produced by different viruses [37] or in the proportion of asthma episodes resulting from colds by any specific virus [36]. However, recent evidence from our laboratory suggests that RVs may have increased propensity toward inducing asthma in comparison to influenza viruses (NG Papadopoulos, unpublished data).

Virus-Induced Changes in Airway Reactivity

The above epidemiological data have raised considerable interest regarding the mechanisms of virus-induced asthma exacerbations, the understanding of which may suggest potential therapeutic targets. Airway hyperresponsiveness is one of the most prominent functional abnormalities in asthma that can be objectively assessed in human and animal models. An increase in airway responsiveness to histamine in normal subjects after URTIs, lasting as long as 7 weeks, was observed more than 20 years ago [47]. Although results have varied, probably because of differences in methodology, models, viral strains, and so on; increased airway reactivity has since been documented after RSV, influenza, parainfluenza, and adenovirus infections, mostly in animal models [48].

Because of the lack of appropriate animal models, human experimental infections have been used as a model for RV infection. Using this model, the increased airway responsiveness to histamine after RV infection in atopic asthmatic subjects was correlated with the severity of the experimental cold, which was paralleled by an increase in IL-8 in nasal lavage fluid [49]. In addition, when daily forced expiratory volume in 1 s (FEV_1) was monitored, a variable airway obstruction was observed [50]. When normal and atopic rhinitic subjects were compared, lower airway responsiveness was more affected in the allergic group [51]. However, in another study, experimental RV infection induced small changes in either upper or lower airway symptoms in normal and asthmatic subjects, with no effect on bronchial reactivity, leading the authors to suggest that RV infection by itself may not be sufficient to provoke clinical worsening of asthma [52]. Exposure to allergens during respiratory viral infection is the most obvious cofactor, because it is well known that rhinovirus experimental infection enhances the responses to inhaled allergens [53] and potentiates inflammation after segmental allergen bronchoprovocation [54]. Most surprisingly, Avila et al. [55], using the same human model in allergic rhinitis subjects, showed that pre-exposure of the nose to an allergen significantly delayed the onset of cold symptoms, reduced

the duration of the illness, and delayed the appearance of proinflammatory cytokines locally. An inverse correlation between nasal eosinophils at the time of inoculation and eventual cold symptoms was also observed, suggesting that an allergic response might protect from RV colds. Although this evidence seemed contradictory to most previous findings, it could prove helpful in several ways. First, it suggested that eosinophils could be involved in RV immunity [56]. Furthermore, it indicated that allergen exposure and viral infection did not have a simple additive effect, and timing or dosage may be important.

Mechanisms of Virus-Induced Inflammation

Several characteristics of virus-mediated pathology can also be seen in asthma. Among these, direct virus-mediated damage to lower airway epithelium is a characteristic of several viruses including influenza and RSV. Dead epithelial cells drop into the airway lumen, inducing or increasing airway obstruction. Although to a lesser extent, this is also the case for RV, which can infect the lower airways and induce cytotoxicity [57–59] (Fig. 2). Furthermore, RV infection induces the production of several cytokines and chemokines, including IL-6 and IL-8, regulated upon activation normal T cell expressed and secreted (RANTES), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-16 [58, 59]. These studies strongly suggest that lower airway infection and local inflammation may represent the first step in the pathogenesis of an asthma episode, adding to and clarifying previous attempts to prove this notion [60, 61]. It is possible that the degree of inflammation, which is similar to other respiratory viruses [62], and not the degree of cytotoxicity, is more relevant in the induction of an exacerbation.

Epithelial damage and mediator production after viral infection are only some of the mechanisms that could initiate or sustain an asthma exacerbation. A dysfunction of the inhibitory M2 muscarinic receptor has been documented after viral infection, which could lead to increased reflex bronchoconstriction [63]. The role of tachykinins has also been suggested, partly explained by the reduction of neutral endopeptidase activity, which is the major metabolizing enzyme for substance P and neurokinin A

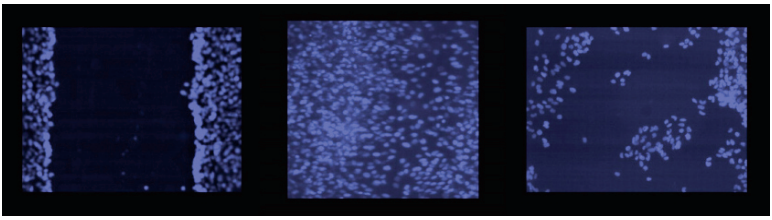

Fig. 2 In a simple model of epithelial wound healing, damaged epithelium ($t = 0$) is suboptimally repopulated after RV-infection in comparison to sham-infected control. DAPI stained cells (Published in *Respiratory Research* [57])

[64]. Most of the above studies have been performed in animal models with cytotoxic viruses such as influenza, parainfluenza, and RSV. A neurally mediated effect does not seem to be a prominent feature of human experimental RV infections [65].

Most puzzling is the potential involvement of the immune response to respiratory viruses in asthma exacerbations. Although the asthmatic phenotype is paradigmatically related to type-2 lymphocyte responses, predominantly IL-4 and IL-5, viral infections induce strong type-1 responses with high levels of IFN-γ that would be expected to downregulate, rather than augment, "allergic" immune responses. Among respiratory viruses, RSV, influenza, and parainfluenza are more extensively studied in animal models. Sensitization of BALB/c mice to the virus attachment protein G of RSV, followed by live virus infection, leads to pulmonary eosinopihlia and type-2 cytokine production. Although IFN-γ is still the dominant T-cell cytokine, a localized relative reduction of IFN-γ mRNA expression with a concomitant increase in IL-4 and IL-5 transcripts has been reported [66]. When *Dermatophagoides farinae*-sensitized mice were repeatedly infected with RSV, an increased production of type-2 cytokines was observed [67]. In the presence of IL-4, virus-specific CD8 T cells can switch to IL-5 production and induce airway eosinophilia [68]. Interestingly, IL-4 can also inhibit antiviral immunity, delaying both influenza [69] and RSV clearance.

RV-infected peripheral blood mononuclear cells from atopic asthmatic subjects produce significantly lower IFN-γ and IL-12 and significantly higher IL-10 and IL-4 than do cells from normal individuals [70]. Although IFN-γ remained the dominant T cell cytokine in this model, a shift toward a type-2 response may be involved in the induction of an asthma exacerbation, by mechanisms similar to the ones described above, for RSV in the mouse.

The recent discovery that airway epithelial cells are deficient in their capacity to generate INF-β when infected with RV raises the possibility that a defect in innate immunity might underlie exacerbations of asthma [71]. The normal response of the airway epithelium to virus infection is the induction of primary IFNs, such as IFN-β, through activation of the Toll-like receptor 3, which recognizes viral double-stranded RNA, leading to apoptosis that is able to effectively eliminate the infected cell and therefore limit viral replication and release. However, in asthmatic epithelial cells a major defect in this pathway leads to enhanced viral replication and virus-induced cell cytotoxicity [72]. Evidence that this pathway might be relevant to the persistence of asthma, as well as exacerbation, comes from the demonstration that in asthmatic patients RV can persist up to 6 weeks after infection [73] and in patients with severe asthma, RV is detectable in airway biopsy specimens between exacerbations [74].

Can Virus-Induced Wheeze Predict Later Asthma?

Most young children who wheeze initially present with episodes related to viral infections. Only a proportion of these children will continue to wheeze in later childhood and adulthood. For the first few years of life, these latter children remain

clinically indistinguishable from those with a transient wheeze. Therefore, it has been a long-standing aim of pediatricians to predict those who will have asthma later in life. In theory, anti-inflammatory remedies given early might modify the outcome [75], although recently it has been shown that early use of inhaled steroids for wheezing in preschool children had no effect on the natural history of asthma or wheeze later in childhood and did not prevent lung function decline or reduce airway reactivity [76]. From population studies, risk factors such as personal atopy or a family history of asthma could predict the relative risk of developing asthma with considerable accuracy, but for use in an individual, this approach is not sufficiently sensitive [77].

Because increased IgE levels and relative eosinophilia can be seen in infants that continue to have persistent wheeze, information obtained from a blood test during the initial episode could have some predictive potential [9]. However, this information has relatively low specificity and may not be ideal for day-to-day practice. It seems, therefore, that currently, a precise prediction of asthma persistence cannot be attempted from the initial virus-induced wheezing episodes.

Antiviral Strategies

Although possible, it is not certain whether a window of opportunity exists from the occurrence of a viral upper respiratory infection to the development of an acute asthma exacerbation, during which an antiviral strategy may be effective. In addition, we cannot predict whether immunization against one or more of the viruses involved will reduce virus-induced asthma exacerbations or it may simply shift the problem to different strains. To answer the above questions, reliable antiviral tools are required.

RVs represent the major causes of virus-induced asthma exacerbations; however, immunization options remain unsatisfactory, mainly due to the large number of

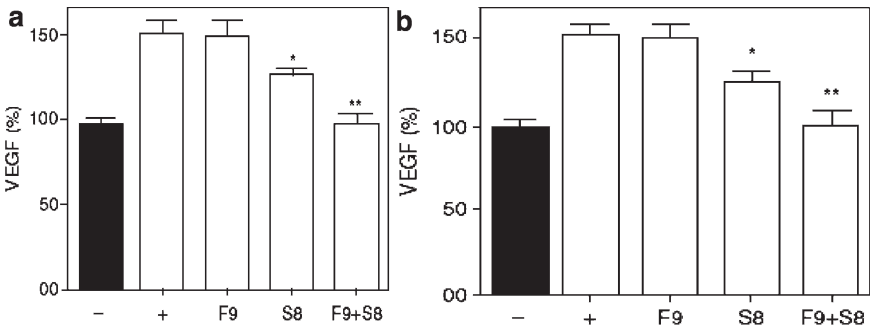

Fig. 3 Addition of salmeterol (S) either at 10^{-8}(A) or 10^{-9}(B) M to fluticasone propionate (F) at 10^{-9} M resulted in a significant reduction of RV-mediated VEGF production by epithelial cells, in a synergistic manner. $n = 4–6$, $^{*}p < 0.05$, $^{**}p < 0.001$ (Adapted from Volonaki et al. [84])

different serotypes [29, 78]. A variety of antiviral agents against RV have been studied. Additional antiviral and anti-inflammatory strategies against common cold have been suggested with varying, but usually little, success [79]. Such strategies include the regularly used ascorbic acid, zinc, and Echinacea, which have little therapeutic value [78]. Macrolide antibiotics, batilomycin A1, erythromycin, and telithromycin have been shown to be effective as potential anti-inflammatory agents in vitro, but clinical proof is still insufficient [80, 81]. During the past few years antirhinoviral compounds such as pleconaril, acting by preventing the uncoating of picornaviruses [82], and the RV protease inhibitor ruprintrivir [83], have shown promising results, but only in early-stage clinical trials.

Based on the above, strategies against virus-induced asthma and related exacerbations are, in principle, anti-inflammatory, following strategies against persistent asthma. Steroids combined with long-acting beta agonists may be effective in this setting: recent studies have shown that such combinations are synergistically effective in reducing RV-induced inflammation in vitro [84] (Fig. 3). Nevertheless, the extent to which these findings translate in clinical practice is still unspecified [85].

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