

Allergens, Viruses, and Asthma Exacerbations

Clare S. Murray, Angela Simpson, and Adnan Custovic

North West Lung Centre, Wythenshawe Hospital, Manchester, United Kingdom

In adults and children with asthma, viral infections (rhinovirus [RV] infection being the most prevalent) will often trigger an increase in symptomatology. The mechanisms responsible for viral-induced exacerbations remain uncertain. Proposed mechanisms include direct infection of the lower respiratory tract, the inflammatory response to viruses, increases in bronchial responsiveness and up-regulation of intercellular adhesion molecule-1 expression in bronchial epithelium. In addition, exposure to allergens, especially seasonal allergens, in sensitized asthmatic individuals have been implicated in asthma attacks. Increased levels of exposure in sensitized asthmatics have been related to increases in hospital admissions and emergency room visits, increased bronchial hyperresponsiveness, increased levels of exhaled nitric oxide, lower levels of lung function, increased treatment requirements, and even increased mortality. In recent years studies have suggested that viruses and allergens may have a synergistic effect on individuals with asthma, thus having a greater influence on exacerbation rate together than either factor alone. Models of experimentally induced RV infection in both allergic and nonallergic individuals using bronchoalveolar lavage and segmental allergen challenge have helped researchers to investigate the possibility of an interaction between allergen sensitization, exposure, and virus infection and their role in the induction of an asthma exacerbation. This review aims to summarize the evidence supporting the role of viruses (in particular RV) as well as the role of, and interaction with, allergen sensitization and exposure on exacerbations of asthma.

Keywords: rhinovirus; allergy; respiratory hypersensitivity

For many patients with asthma (both adults and children), the disease is characterized by a series of exacerbations interrupting otherwise good control of symptoms. The cause of such exacerbations is hotly debated by doctors and patients alike. Some investigators have focused on the role of allergens, and with the advent of reverse transcription-polymerase chain reaction (RT-PCR) assays, studying the role of viruses has become feasible. More recently a model of experimentally induced viral infection has been developed and studied in allergic and nonallergic subjects to investigate the possibility of interaction between allergen sensitization, exposure, and viral infection in the induction of an asthma exacerbation. This review summarizes the evidence supporting the role of viruses and of allergen sensitization and exposure in exacerbations of asthma, and possible interactions between the two.

What constitutes an exacerbation of asthma? Although practicing clinicians can recognize one, there is no universally accepted definition. The studies reviewed have used a range of different end points from reported symptoms, through a drop in peak flow, to hospital admission, and therefore particular outcomes used are highlighted throughout the text.

VIRUSES AFFECTING THE RESPIRATORY TRACT

The most common viral infection of the respiratory tract is the common cold, which is most often caused by a rhinovirus (RV), although other viruses (e.g., coronavirus, respiratory syncytial virus [RSV], parainfluenza virus Types I–IV, coxsackie virus A21 and B3, echovirus Types 11 and 20, adenovirus, and other picornaviruses) can also cause symptoms of a common cold. Infections of the lower respiratory tract can be caused by viruses such as influenza A, B, and C, parainfluenza virus (Types I–IV), RSV, adenovirus, measles, and varicella-zoster.

Rhinoviruses (of which there are about 100 serotypes) are part of the family Picornaviridae (which also includes enteroviruses) and are small (about 25 nm in diameter), nonenveloped viruses with an icosahedral (20-sided) shape. The genome consists of a single-stranded sense RNA. They become unstable and noninfectious in an acidic (pH less than 5) environment, but are hardy and can survive for up to 3 hours on surfaces (1).

Rhinoviruses account for 30–50% of all acute respiratory illnesses. Incidence gradually declines with age, with children being infected several times per year and the elderly every few years. Infections are prevalent throughout the year, but the incidence is highest in the early autumn and in mid to late spring. Transmission occurs by respiratory droplets during sneezing, coughing, or close contact, and can also be spread by the hands or by fomites contaminated with secretions from a case.

VIRAL INFECTIONS AND EXACERBATION OF ASTHMA

Almost 30 years ago the association between viral infections and exacerbation of asthma in children was demonstrated, using culture and serological methods of detecting viruses (2). Subsequently, other studies using similar methods reported similar findings in both adults and children (3–5). However, respiratory viruses, especially rhinovirus, are difficult to detect by standard methods, and therefore earlier studies may have underestimated the impact of viral infection on wheezing.

In more recent years RT-PCR assays, which are extremely sensitive in detecting respiratory viruses, have been developed (6, 7). These techniques have been applied in studies investigating the relationship between viral infections and asthma severity. In a longitudinal study of 138 adults with asthma, nose and throat swabs and blood samples were collected to coincide with either symptoms of acute upper respiratory tract infection or worsening of asthma (8). One-quarter of confirmed viral infections were associated with mean decreases in peak expiratory flow rate (PEFR) of 50 ml/minute or more, and one-half were associated with mean decreases in PEFR of 25 ml/minute or more. Respiratory pathogens were implicated in almost half of all severe asthma exacerbations (decrease in PEFR of 50 ml/minute or more), and the most commonly identified virus was RV.

Johnston and coworkers performed a similar study in 9- to 11-year-old children (9). Viruses were detected in 80% of reported episodes of a decrease in PEFR of 50 ml/minute or more, 80% of reported episodes of wheeze, and 85% of all reported episodes of upper respiratory symptoms, cough, wheeze, and fall in PEFR. Again, the most commonly identified virus was rhinovirus. The same group reported that the seasonal patterns of upper respiratory tract infection correlated with hospital admissions with

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Correspondence and requests for reprints should be addressed to Adnan Custovic, M.D., Ph.D., North West Lung Centre, Wythenshawe Hospital, Manchester M23 9LT, UK. E-mail: a.custovic@man.ac.uk

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asthma, but that the relationship was stronger for pediatric than for adult admissions (7). By recruiting couples (one with and one without asthma) and measuring rhinovirus in nasal secretions at 2-week intervals over a 3-month period, Corne and coworkers demonstrated that rhinoviral infections are no more common in those with than in those without asthma (10% with asthma versus 9% without asthma for all samples collected). They did, however, find that subjects with asthma were significantly more likely to have lower respiratory tract symptoms (43 versus 17%), and also that these symptoms were likely to last longer in subjects with asthma (10). Zambrano and coworkers inoculated subjects with mild asthma (bronchodilators only) and healthy control subjects with human RV-16. Those with asthma had significantly more upper and lower respiratory tract symptoms than control subjects over the 3-week period of study (11).

Other studies have investigated the role of respiratory viruses in the exacerbation of asthma leading to hospital admissions or attendance at the emergency room. Atmar and coworkers examined 122 adults presenting at the emergency room with an acute asthma attack for viral respiratory tract infection (12). Of 148 asthma exacerbations, 55% were associated with a viral respiratory tract infection, and of 42 patients who required hospitalization 50% had a viral respiratory tract infection identified. Sixty percent of all infections were caused by RV. Rakes and coworkers performed a cross-sectional study of 70 children presenting to the emergency room with wheeze and 59 control subjects (13). Respiratory viruses were detected in more than 80% of the cases, with the predominant pathogen in children less than 2 years of age being RSV (68%), followed by RV (41%). Rhinovirus was the most frequently detected pathogen in older children (71%), and was also detected in 35% of control subjects. However, this study might have overestimated the importance of viral infections, as it was performed between January of one year and April of the next, but excluded the months of June to August, when one would expect fewer respiratory infections. Freymuth and coworkers examined nasal aspirates from 75 children admitted to hospital in France for an acute attack of asthma (14). A virus, *Chlamydia pneumoniae*, or *Mycoplasma pneumoniae* was detected in 71.9% of cases. Rhinovirus was the most frequently detected agent (46.9%) followed by RSV (21.2%).

Despite their widely differing design, these studies suggest that viral infections are involved in about 50% of asthma exacerbations among adults and in probably substantially more childhood asthma exacerbations. The virus most commonly implicated in asthma exacerbations appears to be RV.

MECHANISMS OF VIRUS-INDUCED ASTHMA EXACERBATIONS

Several mechanisms by which rhinovirus induces asthma exacerbations have been postulated, including direct infection of the lower respiratory tract, induction of inflammatory responses to RV, reduction in lung function, exacerbation of bronchial reactivity, and upregulation of surface intercellular adhesion molecule 1 (ICAM-1) expression in bronchial epithelium.

Do Viruses That Trigger Asthma Infect the Lower Airway?

An important question is whether viruses exacerbate asthma by local mechanisms consequent on lower airway infection, or whether they infect only the upper respiratory tract and cause their effects indirectly. Clearly some viruses infect the lower airways (e.g., influenza, RSV, and parainfluenza), causing tissue inflammation and airway obstruction, but until more recently it was presumed that rhinoviruses were unable to infect the lower airways. In the nose, RV replicates in the epithelium and the

lymphoepithelium of the adenoid. The optimal temperature for replication is 33°C. Rhinovirus in the nose can be readily detected by tissue culture, RT-PCR, or *in situ* hybridization. Evidence of the presence of RV in lungs has been more difficult to obtain. Gern and coworkers used PCR to assess lower airway RV load in experimental infections, in a study designed to control for upper airway contamination (15). Bronchoalveolar lavage (BAL) cells were positive for RV during infection in 80% of samples, whereas nasal lavage fluid in the same patients was positive in 100%, and BAL fluid in only 37%. This suggests that RV is able to infect the lower airways and that RV RNA is largely cell associated.

Cultured respiratory epithelial cells can be infected with RV (16), and although a more recent study was able to infect only 10% of cultured bronchial epithelial cells with RV, a similar proportion of cultured adenoidal cells were also infected, the adenoids being the site *in vivo* from where most RV is recovered (17).

This work has not, however, been mirrored by clinical findings in natural infections. Although there are case reports of fatal RV infection of the lower airways in children (18, 19), a study of postmortem tissue specimens from 12 subjects who had died of asthma (age, 12–69 years), found no evidence of RV by RT-PCR (20). Also, in a review of BAL samples collected from a tertiary referral center over a 10-year period, RV was detected only in samples collected from immunosuppressed subjects (21). Although the *in vitro* and experimental infection work is suggestive, conclusive evidence of RV infection of bronchial epithelial cells *in vivo* in natural infections is lacking.

RV and Inflammation

Cultured bronchial epithelial cells produce inflammatory cytokines and chemokines (interleukin [IL]-6, IL-8, granulocyte-macrophage colony-stimulating factor, IL-16, RANTES [regulated on activation, normal T cell expressed and secreted], and epithelial-neutrophil activating peptide-78) when infected with RV (16, 22, 23). Increased IL-8 in nasal lavage samples has been found in infants with natural RV infections (24) and in adults with asthma or rhinitis, with experimental RV infection (25).

Fraenkel and coworkers collected endobronchial biopsy samples from normal volunteers (n = 11) and subjects with mild atopic asthma (n = 6) after inoculation with RV. They found increases in submucosal lymphocytes, and a decrease in circulating lymphocytes that correlated with changes in bronchial hyperresponsiveness (BHR). The authors concluded that RV infection initiates T cell-mediated immune responses that are linked to lower airway dysfunction (26). After the development of *in situ* hybridization techniques, they were able to identify RV in 50% of bronchial biopsy samples, suggesting that it is infection with RV that causes the proinflammatory response (22).

Peripheral blood mononuclear cell responses to RV have been shown to be different between healthy volunteers and subjects with atopic asthma; IL-4 was induced only in those with asthma, and the IFN- γ :IL-4 ratio was lower in those with asthma than in those without. Higher IL-10 and lower IL-12 levels were also seen in those with asthma as well as different expression of costimulatory molecules (27, 28).

Effects on Lung Function

Several experimental studies have failed to demonstrate a significant increase in airway obstruction in patients with asthma and RV infection, as measured by spirometry under laboratory conditions (11, 29, 30). However, more recently, Grunberg and coworkers examined the effects of experimental RV-16 infection on daily home recordings of FEV₁ in nonsmoking subjects with atopic, mild asthma. The subjects recorded FEV₁ three times daily from 4 days before until 10 days after infection. The FEV₁

(expressed as a percentage of personal best) decreased significantly after infection, reaching a minimum 2 days after inoculation (31). In addition, the lowest FEV₁ correlated significantly with cold symptom score, asthma symptom score, and decrease in airway responsiveness.

Effects on Bronchial Hyperresponsiveness

Increases in BHR have been found in normal subjects (32) and subjects with asthma (33) after experimental infection with influenza A. Increases in BHR have also been found in normal individuals after naturally acquired upper respiratory tract infections (34). Viral infections have been shown to enhance both the reactivity of the lower airway and the magnitude of bronchoconstriction in response to inhaled contractile substances in asthma, and the latter effect can persist for several weeks after infection (30). Host factors appear to influence the effects of viral infections. Studies of subjects with naturally acquired common cold symptoms have indicated that allergic individuals experience greater changes in airway responsiveness than do nonallergic individuals (35). Gern and coworkers reported that allergic individuals, compared with normal subjects, had a significantly greater change in BHR during experimental RV infection and that this effect was more marked in those with a lower baseline FEV₁ (36). However, a study of experimental RV infection in individuals with mild asthma (bronchodilators only) and normal individuals reported no significant increases in BHR (11).

ICAM-1 Interaction

ICAM-1, in its dual role as an intercellular adhesion molecule (on antigen-presenting cells, lymphocytes, eosinophils, mast cells, submucosal glands, airway smooth muscle cells, and epithelial cells) and as the major RV receptor, has been the focus of much attention in rhinovirus-induced inflammation. There is evidence to suggest that rhinoviruses have the ability to upregulate surface ICAM-1 expression on the basal cells of bronchial epithelium in both normal and asthmatic individuals (37). This is associated with enhanced adherence of polymorphic mononuclear cells and migration of T cells. Such upregulated expression is partly mediated by cytokines such as IL-1, which is an inducer of ICAM-1 expression. Thus it is possible that increased ICAM-1 promotes both rhinovirus spreading and influx of inflammatory cells, thereby potentially contributing to the development of an exacerbation of asthma. In addition, patients with asthma have been reported to have an enhanced expression of ICAM-1 in the bronchial epithelium. *In vitro* studies suggest that glucocorticoids may downregulate both basal and cytokine-induced epithelial ICAM-1 expression (38). However, Grunberg and coworkers found that although RV-16 infection in subjects with atopic asthma increased ICAM-1 expression in the bronchial epithelium, pretreatment with inhaled glucocorticoids had no effect on rhinovirus-increased expression (39).

ALLERGENS AND ASTHMA

Sensitization to allergens is a risk factor for asthma. Different allergens predominate in different areas, such as cat allergen in Sweden, mite allergen in Australia, and cockroach allergen in U.S. inner cities. Patients with asthma are often exposed to high levels of allergens to which they are sensitized throughout the year—a situation quite different from contact with viruses. Data are presented that support the role of exposure to indoor inhalant allergens in sensitized individuals causing more severe asthma. Some seasonal allergens are also implicated in exacerbations of asthma.

Allergen Sensitization and Asthma Severity

Of 450,000 emergency department adult asthma admissions per year in the United States, 200,000 have been attributed to the risk associated with sensitization to mite, cat, or cockroach allergen (40). However, it is often difficult to differentiate clearly between the effect of sensitization and that of exposure. Sensitization to mites, cats, and cockroaches was found to be a significant risk factor for acute asthma in patients requiring treatment in hospital emergency room in several American studies (40, 41). However, none of these studies unequivocally established a quantitative relationship between current allergen exposure and the exacerbation of asthma requiring admission to hospital.

Allergen Exposure and Hospital Presentation

In the American study of inner city children with asthma, those who were sensitized and highly exposed to cockroach allergen were more likely to be hospitalized with their asthma. They also had more unscheduled medical visits, and had more time off school, compared with either the nonexposed or the nonsensitized (42). Rosas and coworkers found a correlation between asthma-related emergency room visits and the presence of *Leptospira* ascospores in Mexico City (43). In addition, grass pollen exposure was related to asthma admissions in both children and adults, independent of air pollutants.

Among children admitted to hospital with an acute attack of asthma in the United Kingdom, those who were sensitized and exposed to dust mite were at increased risk of readmission during the following month (44). In the Childhood Asthma Management Program study, children who were sensitized to dog and exposed to high levels of *Canis familiaris* I (*Can f* I) were more likely to have had a hospital admission for asthma in the univariate analysis, but this effect was lost in the multivariate analysis (45).

Allergen Exposure and Lung Function

Several studies have examined the effect of allergen exposure on various parameters of lung function in subjects with asthma. Zock and coworkers examined 228 school children with wheezing in the last year or doctor-diagnosed asthma and found a positive relationship between *Dermatophagoides pteronyssinus* I (*Der p* I) levels and both PEFR variability and symptom scores (46). Chan-Yeung and coworkers found similar results in 120 adults with asthma (47). Increased PEFR variability and BHR with decreased FEV₁ was observed in adults with asthma who were both sensitized and exposed to mite allergens (48). In addition, a significant negative correlation was observed between allergen levels in beds and PD₂₀. Maestrelli and coworkers reported that exposure to *Der p* I levels of 2 µg/g or less in the bedrooms of asthmatic subjects allergic to mites and adequately treated with antiasthma drugs for 1 year was associated with a significant fall in bronchial responsiveness to methacholine. In contrast, bronchial responsiveness was unchanged after 1 year in comparable subjects with asthma exposed to higher levels of *Der p* I allergen (49). Higher levels of exhaled nitric oxide were measured in adults with mild asthma (not using inhaled steroids) who were both sensitized and exposed to the relevant allergen compared with sensitized, but not exposed, subjects (50). A larger, similar study has confirmed this finding and also found lower FEV₁ and increased BHR in those both sensitized and exposed to indoor allergens (51).

In an experimental situation, after a controlled challenge with either a high or low dose of allergen, sensitized subjects experience an increase in nonspecific airway reactivity (52). Low-dose allergen challenge of patients with allergic asthma (which mimics “real life” situations) can produce the characteristic eo-

sinophilic infiltration of the airways and an increase in proinflammatory cytokines (e.g., IL-5) (53).

Allergen Exposure and Medication Requirements

Vervloet and coworkers investigated, in mite-sensitized patients with asthma, the relationship between treatment requirements to control symptoms and mite allergen levels in mattresses (54). With increasing mite allergen levels, patients required increasing amounts of treatment (mean mite allergen of 1.3 $\mu\text{g/g}$ dust in patients with no treatment, 5.4 $\mu\text{g/g}$ dust in patients who used β -agonist only when required, and 17.8 $\mu\text{g/g}$ dust in patients who were receiving daily inhaled corticosteroid treatment). Similar relationships were demonstrated between mean allergen levels and number of asthma attacks. Sensitization and exposure to common indoor allergens (mite, cat, and dog) appear to be associated with severe asthma (55). Patients with severe asthma were taking at least 1,500 μg of beclomethasone per day and had more than 40% diurnal variation in peak flow for more than 50% of days, patients with mild asthma were taking 500 μg of beclomethasone or less per day and had less than 25% diurnal variation in peak flow for more than 50% of days. The proportion of patients both sensitized and exposed to sensitizing allergen at each site was significantly greater among those with severe asthma than those with mild asthma. *Can f1* levels were about 30-fold higher in dog-sensitized patients with severe asthma compared with dog-sensitized patients with mild asthma.

Allergen Exposure and Asthma Mortality

Some data suggest that exposure to seasonal allergens may be implicated in sudden asthma deaths. Two studies, one from the United States and one from the United Kingdom, have demonstrated a seasonal pattern among asthma deaths in young adults with asthma, the peak death rate occurring when the mold spore rates are highest (56, 57). Targonski and coworkers found that the odds of an asthma-related death occurring among 5–34-year-olds in Chicago were 1.2 times higher for every increase of 1,000 spores per cubic meter in the daily mold spore level (58). In addition, O'Hollaren and coworkers reported sudden onset of respiratory arrest occurring in adolescents and young adults in the peak *Alternaria* spore season, and found sensitivity to *Alternaria* to be associated with a 200-fold increased risk of respiratory arrest (59).

INTERACTION BETWEEN VIRAL INFECTION AND ALLERGEN EXPOSURE IN INDIVIDUALS WITH ASTHMA

Green and coworkers have reported that the risk of admission to hospital with acute asthma in adults was markedly increased with the combination of sensitization and current exposure to high levels of sensitizing allergens and the presence of viral infection (60). These results indicate that there is a synergism between allergen sensitization, exposure to a high level of sensitizing allergen, and viral infection in inducing deterioration in asthma requiring hospital admission. Viral infection per se was not an independent risk factor for asthma admission, but significantly increased the risk in those patients who were also both sensitized and exposed to allergens. Interestingly, in this study in adults only 16 of the 61 acute asthma cases had a positive PCR for a respiratory virus. This is in contrast to a number of previous studies in children, which have shown a strong relationship between viral infection and exacerbations of asthma (7). However, 22 of the admitted patients with asthma reported symptoms that they attributed to a cold before admission, but had negative virus PCR. These symptoms may have been due to an allergic response that was mistaken for infection, or they

could also have been true viral infections that were not detected (e.g., sampling late in the course of the illness, as the gap between infection and admission could be longer in adults than in children, nasal lavage producing less mucus than an aspirate, or taking a nasal sample instead of sputum).

Possible Mechanisms of Interaction

The use of experimental infection of volunteers with and without allergic rhinitis/asthma has enabled direct comparisons of common cold symptoms in these two groups. Furthermore, techniques such as BAL and segmental antigen challenge have been used to directly sample lower airway fluids and tissues during acute viral infection and thus allow us to understand the mechanisms underlying the combined effects of viral infection and allergen exposure on airway physiology and inflammation.

Lemanske and coworkers studied 10 adult subjects with allergic rhinitis (61). Patients were examined for both early- and late-phase response airway reactivity to histamine and ragweed antigen, and 1 month later were intranasally inoculated with live RV-16. During the acute illness, there was a significant increase in airway reactivity to both histamine and ragweed (before rhinovirus inoculation only 1 patient had a late-phase response to ragweed, compared with 8 of 10 after inoculation). Calhoun and coworkers performed a similar study in which plasma levels of histamine and tryptase were measured after inhaled antigen challenge (62). Those patients, whose pattern of response after antigen challenge changed from an immediate response only before infection to a dual response (immediate and late phase) during infection, had significantly greater plasma histamine concentrations after challenge than those whose pattern of response did not change. This indicates that a mechanism by which rhinovirus increases the likelihood of a late-phase response could include enhanced mediator release from pulmonary mast cells or from circulating or recruited basophils.

The same group performed a further study using segmental antigen bronchoprovocation and BAL to investigate the effect of allergen challenge on sensitized and nonsensitized individuals before, during, and after inoculation with rhinovirus (63). Seven patients with allergic rhinitis and five healthy volunteers were infected with RV-16. Segmental challenge with saline and antigen was performed 1 month before infection, during the acute infection, and 1 month after infection. BAL fluid obtained from the patients with allergic rhinitis during the acute viral infection and after 1 month showed a significantly enhanced release of histamine immediately after local antigen challenge and histamine leak 48 hours afterward, and a significantly greater recruitment of eosinophils to the airway 48 hours postchallenge. These changes were not seen in the nonallergic volunteers, nor were they observed in allergic individuals before infection. It appears that RV-16 infection results in persistently enhanced airway inflammation after local antigen challenge in allergic subjects, which persists for up to 1 month after infection. De Kluijver and coworkers have investigated the effect of inhaling low-dose house dust mite allergen for 10 days on response to experimental RV infection in adults with mild asthma in a double-blind placebo-controlled study. Although the allergen inhalation alone resulted in reduced FEV₁, increased BHR, and increased expired NO and the RV-16 infection alone caused a fall in FEV₁ and increased sputum IL-8, there was no additive effect seen in those subjects who received both active treatments (64).

CONCLUSIONS

A number of experimental studies (lung segmental allergen challenge, experimental rhinoviral infection) suggest potential synergistic effects between allergens and respiratory viral infection. Few studies have investigated the possible interaction between

sensitization, allergen exposure, and viral infections in real-life exacerbations. It has been reported that admission to hospital with acute asthma was strongly associated with the combination of sensitization and current high exposure to sensitizing allergens and the presence of viral infection. The results confirm experimental data that allergens and viruses may act together to exacerbate asthma, indicating that domestic exposure to allergens acts synergistically with viruses in sensitized patients, increasing the risk of exacerbation. Thus, strategies to reduce the impact of asthma exacerbations should include interventions directed at both viruses and reducing allergen exposure. However, factors other than allergens and viruses (such as air pollution, cigarette smoking, compliance with and availability of treatment, and psychological factors) are undoubtedly relevant in some exacerbations of asthma and make the elucidation of possible interactions more complex.

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