11

Viral Respiratory Tract Infections in Cystic Fibrosis

Dennis Wat
Adult Cystic Fibrosis Unit, Papworth Hospital, Cambridge, United Kingdom

1. Introduction

Cystic Fibrosis (CF) is the most commonly inherited potentially lethal disease amongst Caucasian children and young adults. In Europe, approximately 35,000 children and adults are affected by CF. The prevalence in the US and in Canada is approximately 30,000 and 3,000, respectively. CF is an autosomal recessive disorder and is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator gene (CFTR) (Riordan, Rommens et al. 1989). The main function of CFTR in many tissues is to regulate and participate in the transport of chloride ions across epithelial cell membranes. To date, more than 1,800 mutations have been described in this gene, but the most common mutation worldwide is caused by deletion of phenylalanine at position 508 (Delta F508) of the CFTR on chromosome 7. The dramatic improvement in survival from CF has taken great strides over the past 40 years with the introduction of specialist centre care, optimising nutritional status and preventing pulmonary inflammation. The median survival of children born in the 1990s is estimated to exceed 40 years of age with more than 85% of them achieving adulthood. CF is a multisystem disorder and is characterised by chronic suppurative lung disease and by exocrine pancreatic insufficiency which affects gastrointestinal function and causes restricted growth and maturation. CF also causes obstructive azoospermia and thus male infertility. However, in most individuals with CF the major burden is on the lungs. The absence of CFTR in airway epithelium leads to malfunction of chloride conductance and subsequent airway surface liquid (ASL) volume reduction, mucins are concentrated, the periciliary liquid depleted, and mucous clearance by ciliary and cough dependent mechanisms diminished, which leads to airflow obstruction and eventually bacterial colonisation. Bacteria implicated in the morbidity and mortality of CF include *Pseudomonas aeruginosa, Burkholderia cepacia complex, Achromobacter xylosoxidans, Staphylococcus aureus, Haemophilus influenzae, Stenotrophomonas maltophilia* and *non-tuberculous mycobacteria*. However despite the appropriate use of antibiotic therapy, chronic obstructive airway disease continues to develop in patients with CF and is the major cause of morbidity and mortality.

However, the use of appropriate antibiotic therapy has had a limited effect in slowing the progression of pulmonary disease. As a result, recent studies have hypothesised that respiratory viral infection may be a contributing factor to pulmonary exacerbations. Respiratory viruses implicated in the respiratory exacerbations of CF include *influenza A and*

B, respiratory syncytial virus (RSV), parainfluenza virus (PIV) types 1 to 4, rhinovirus, metapneumovirus, coronavirus and *adenovirus*.

The role of respiratory viruses in the aetiology of respiratory exacerbations in CF is not fully understood and may have been underestimated as many previous studies have used insensitive techniques to isolate respiratory viruses, therefore undermining their prevalence. New viral detection techniques have further enhanced the awareness of respiratory viral aetiology in CF exacerbations. A recent in vitro study illustrating the interaction of respiratory virus and *P. aeruginosa* may contribute to the pathogenesis of CF exacerbations. No doubt more work will be required in this area to further understand their relationship so as to allow the development of potential novel treatment. If respiratory viruses do lead to secondary bacterial infection in CF, this may rationalize the treatment of CF in future. Although there are commercially available vaccines and anti-virals for the prevention and treatment of respiratory viral infections, they are mainly limited to influenza viral infection. A number of studies are currently underway looking at the development of new vaccines and anti-virals, hopefully it will not be long before treatment becomes available for different types of respiratory viruses.

This chapter will provide an overview on the epidemiology of respiratory viruses in CF, the available detection techniques for viruses and their differences in sensitivities, the clinical implications of viral infection in CF, the interaction between viruses and bacteria, and the management of viral infections.

CF is the most commonly inherited, potentially lethal disease amongst Caucasian children and young adults (Mearns 1993). Pathological changes occur in all exocrine glands (Vawter and Shwachman 1979), however, in most individuals with CF the major impact is on the lungs (Oppenheimer and Esterly 1975). Chronic lung infections may start very early in the lives of patients with CF. It has been hypothesised that impaired mucociliary clearance and low airway surface liquid (ASL) volume is pivotal for the pathogenesis of lung infections. These in turn lead to impaired bacterial clearance from respiratory epithelial cells (Saiman and Siegel 2004). Pulmonary infections remain to be the greatest cause of morbidity and mortality leading to premature death in CF (Rajan and Saiman 2002).

The incidence of CF in the United Kingdom is around 1 in 2500 live births and 1 in 25 of the population carry a mutation in their CF genes (Dodge, Morison et al. 1993). CF is a multisystem disorder and is characterised by chronic suppurative lung disease and by exocrine pancreatic insufficiency which affects gastrointestinal function and causes restricted growth and maturation. CF also causes obstructive azoospermia and thus male infertility.

CF is an autosomal recessive disorder and is caused by mutations in the CFTR (Riordan, Rommens et al. 1989). The main function of CFTR in many tissues is to regulate and participate in the transport of chloride ions across epithelial cell membranes (Barasch and al-Awqati 1993). So far more than 1,800 mutations have been described in this gene (http://genet.sickkids.on.ca/cgi-bin/WebObjects/MUTATION), but the most common mutation worldwide is caused by deletion of phenylalanine at position 508 (Delta F508) of the CFTR on chromosome 7.

Survival from CF is increasing rapidly as exemplified by the median life expectancy of CF children born in 1990 to be around 40 years which is double that of 20 years ago (Elborn, Shale et al.). The prolonged life expectancy might be attributed to multi-disciplinary care, improved nutritional status, use of antibiotics and better understanding of disease pathology.

CF pulmonary exacerbations represent decreased host defences within the lungs leading to alterations in airway microbiology, airway obstruction related to increased sputum production and ventilatory failure (Goss and Burns 2007). Pulmonary exacerbations are associated with acquisition of new organisms and increased concentration of airway flora (Aaron, Ramotar et al. 2004). The presences of some organisms including S. aureus, P. aeruginosa and B. cepacia in the airways have been shown to lead to clinical deterioration (Thomassen, Demko et al. 1985; Nixon, Armstrong et al. 2001; Sawicki, Rasouliyan et al. 2008). The new acquisitions of *P. aeruginosa* in CF have been demonstrated to occur in the winter months coinciding with the peak of respiratory viral infections (Johansen and Hoiby 1992). *Influenza* is a substantial health threat, it is associated with approximately >36,000 deaths and 220,000 hospitalisations in the United States yearly (Thompson, Shay et al. 2004). The emergence of novel *influenza virus (H1N1)* further heightened the awareness of influenza-like illness. CF Pulmonary exacerbation rates have been shown to be significantly increased during the winter and are highly associated with the influenza season (Ortiz, Neuzil et al. 2010).

2. Viral respiratory infections in CF

Early studies looking at respiratory viruses in CF relied on repeated serological testing, either alone (Petersen, Hoiby et al. 1981) or in combination with viral cultures for viral detection (Wang, Prober et al. 1984; Ramsey, Gore et al. 1989; Pribble, Black et al. 1990; Armstrong, Grimwood et al. 1998; Hiatt, Grace et al. 1999). These methods are relatively insensitive and more recent studies have utilised PCR based methodologies (Smyth, Smyth et al. 1995; Collinson, Nicholson et al. 1996; Punch, Syrmis et al. 2004; Olesen, Nielsen et al. 2006; Wat, Gelder et al. 2008). All these studies produced different results in terms of prevalence of respiratory viruses in CF. The differences can be due to different methodologies. There are also likely to be differences in the populations studied as the prognosis for CF has improved with each successive birth cohort.

The viruses implicated in causing respiratory symptoms in CF include *RSV, adenovirus, PIV (Types 1 to 4) , influenza A&B, rhinovirus* (Ramsey, Gore et al. 1989; Smyth, Smyth et al. 1995; Collinson, Nicholson et al. 1996; Wat, Gelder et al. 2008) and more recently *metapneumovirus* (Garcia, Hiatt et al. 2007). RSV represents 9-58% of all reported viral infection in CF, with the highest incidence in young children (Armstrong, Grimwood et al. 1998). It is possible that RSV precipitates in the initial infection by *P. aeruginosa* of the CF airway(Petersen, Hoiby et al. 1981), the proposed mechanism of which will be discussed later. A new subtype of human *rhinovirus* was recently identified, *rhinovirus C*, and was shown by de Almeida et al (de Almeida, Zerbinati et al. 2010) that it is significantly associated with respiratory exacerbations in children with CF (Odd ratio- 1.213). *Influenza A and B* take 12-27%, but in one small study, it comprised of 77% of positive samples (Hordvik, Konig et al. 1989). *PIV* are found in lower frequencies with only one study showing a detection rate of 43% from positive samples (Petersen, Hoiby et al. 1981). *Metapneumovirus* has recently been detected in

nasopharyngeal aspirates taken from hospitalised children and infants with respiratory tract infections who had signs and symptoms similar to those of RSV infection (van den Hoogen, de Jong et al. 2001). This virus is also associated with lower respiratory tract infections in patients with CF (Garcia, Hiatt et al. 2007).

It is now nearly 30 years since Wang et al (Wang, Prober et al. 1984) described the relationship between respiratory viral infections and deterioration in clinical status in CF. In this 2 year prospective study (Wang, Prober et al. 1984), viruses were identified through repeated serology and nasal lavage for viral isolation in 49 patients with CF (mean age 13.7 years). Although the CF patients had more respiratory illnesses than sibling controls (3.7 versus 1.7/year), there were no differences in virus identification rates (1.7/year). The rate of proven virus infection was significantly correlated with the decline in lung functions, radiology score, and frequency and duration of hospitalisation.

More recent studies suggest no difference in the frequency of either upper respiratory tract illness (URTI) episodes (Hiatt, Grace et al. 1999) or proven respiratory viral infections (Ramsey, Gore et al. 1989) between children with CF and healthy controls, but children with CF have significantly more episodes of lower airway symptoms than controls (Ramsey, Gore et al. 1989; Hiatt, Grace et al. 1999). Ramsey et al (Ramsey, Gore et al. 1989) prospectively compared the incidence and effect of viral infections on pulmonary function and clinical scores in 15 school-age patients with CF aged between 5 to 21 years and their healthy siblings. Over a two-year period, samples were taken at regular two monthly intervals and during acute respiratory illnesses (ARI) for pharyngeal culture and serology for respiratory viruses. There was a total of 68 ARI episodes occurred in the patients with CF and in 19 episodes there was an associated virus identified. A total of 49 infective agents were identified either during ARIs or at routine testing in the patients with CF; 14 were identified on viral isolation (*rhinovirus* on 11 occasions), whilst 35 were isolated on seroconversion (*PIV* on 12, *RSV* on 9 and *M. pneumoniae* on 6 occasions). There was no significant difference in the rate of viral infections between the patients with CF and their sibling controls, as measured either by culture or serology. The rate of viral infections was higher in younger children (both CF and controls), and the rate of decline in pulmonary function was greater in the younger children with CF with more viral infections. At the time of an ARI, the virus isolation and seroconversion (fourfold increase in titres) rates were 8.8% and 19.1%, respectively in children with CF compared to 15.0% and 15.0% respectively for the non-affected siblings. In contrast the rates of virus isolation and seroconversion at routine 2 monthly visits were 5.6% and 16.2 % respectively for children with CF and 7.7% and 20.2% respectively for the healthy siblings.

Similarly Hiatt (Hiatt, Grace et al. 1999) assessed respiratory viral infections over three winters in 22 infants less than two years of age with CF (30 patient seasons), and 27 age matched controls (28 patient seasons). The average number of acute respiratory illness per winter was the same in the control and CF groups (5.0 versus 5.0). However, only 4 of the 28 control infants had lower respiratory tract symptoms in association with the respiratory tract illness, compared with 13 out of the 30 infants with CF (Odd ratio- 4.6; 95% confidence interval 1.3 and 16.5; p-value <0.05). 7 of the infants with CF cultured *RSV*, of whom 3 required hospitalisation. In contrast, none of the controls required hospitalisation. Pulmonary function measured by rapid chest compression technique was significantly reduced in the infants with CF after the winter months and was associated with two interactions; *RSV* infection with lower respiratory tract infection and male sex with lower respiratory tract infection.

From previous reports, two viral agents appear to have the greatest effect on respiratory status in CF, namely *RSV and influenza*, possibly because the uses of viral culture and serology have underestimated the effects of rhinovirus. In younger children, *RSV* is a major pathogen resulting in an increased rate of hospitalisation. Abman et al (Abman, Ogle et al. 1991) prospectively followed up 48 children with CF diagnosed through newborn screening and documented the effect of *RSV* infection. Eighteen of the infants were admitted into hospital a total of 30 times over a mean follow-up of 28 months (range 5-59). In seven of these infants *RSV* was isolated, and their clinical course was severe with 3 requiring mechanical ventilation and 5 necessitating chronic oxygen therapy. Over the next 2 years these infants had significantly more frequent respiratory symptoms and lower Brasfield chest radiograph (Brasfield, Hicks et al. 1979) scores than *non-RSV* infected counterparts. Brasfield scores air trapping on the lateral chest film, and linear markings, nodular cystic lesions, large lesions, and general severity on the posteroanterior chest film. Twenty five points represent a normal chest radiograph with lower scores indicating increasing disease severity.

In older children and adults with CF, *influenza* seems to have the greatest effect. Pribble et al (Pribble, Black et al. 1990) assessed acute pulmonary exacerbation isolates from 54 patients with CF. Over the year of the study, 80 exacerbations were identified, of which 21 episodes were associated with an identified viral agent (*influenza A*- 5 episodes; *influenza B*- 4 episodes; *RSV*- 3 episodes) with most agents identified on serology. Compared to other respiratory viruses, infection with *influenza* was associated with a more significant drop in pulmonary function (FEV_1 declined by 26% compared with 6%). There were also a higher proportion of patients with a greater than 20% drop in FEV_1 within the *influenza* infected cohort. A retrospective study in older patients with chronic *P. aeruginosa* infection reported an acute deterioration in clinical status in association with *influenza A* virus infection (Conway, Simmonds et al. 1992).

Over a 1-year period, Smyth et al (Smyth, Smyth et al. 1995) prospectively investigated 108 patients with CF (mean age of 7.9 years) using a combination of viral immunofluorescence, culture and seroconversion (fourfold increase in titres) to identify respiratory viruses. With the exception of *rhinovirus*, a seminested reverse transcriptase PCR technique was used. During the study, 76 subjects had 157 respiratory exacerbations (1.5 episodes/patient/year) and a viral agent was identified in 44 episodes, 25 of which were *rhinovirus* and an equal distribution of other viruses identified almost always on seroconversion. Identification of a respiratory virus during the course of the year was associated with a significantly greater decline in Shwachman score (Shwachman and Kulczycki 1958) and days of intravenous antibiotics use. The Shwachman scoring system is an objective measurement of the clinical status of cystic fibrosis patients. This score is based on clinical and radiological evaluation and represented a milestone in the history of CF. Patients with scores of 90 to 100 are classified as 'excellent', 80 to 89 as 'good', 70 to 79 as 'fair', and 50 to 69 reflects a 'poor' clinical status. In addition, those children in whom a non-rhinovirus was identified had a significantly greater decrease in FEV_1 over the year of the study.

Collinson et al (Collinson, Nicholson et al. 1996) followed 48 children with CF over a 15 month period using viral cultures for viral detection, with the exception of *picornaviruses* where PCR was used. 38 children completed the study and there were 147 symptomatic upper respiratory tract infections (2.7 episodes/child/year), with samples available for 119

episodes. *Picornaviruses* were identified in 51 (43%) of these episodes, of which 21 (18%) were *rhinoviruses*. In those children old enough to perform spirometry, there were significant reduction in both FVC and FEV_1 in association with URTIs, with little difference in severity of reduction whether a picornavirus was identified or not. Maximal mean drop in FEV_1 was 16.5%, at 1-4 days after onset of symptoms, but a deficit of 10.3% persisted at 21-24 days. Those with more URTIs appeared to have greater change in total Shwachman score (Shwachman and Kulczycki 1958) and Chrispin-Norman score (Chrispin and Norman 1974) over the study. Chrispin-Norman score (Chrispin and Norman 1974)is a standardized scoring system to assess the severity of CF lung disease on chest radiograph and to allow longitudinal follow-up. Six children isolated a *P.aeruginosa* for the first time during the study, 5 at the time of a URTI and only 1 was asymptomatic at the time of first isolation. However, the data from this study has to be handled with care as the term 'upper respiratory tract illness-URTI' did not necessarily imply a positive viral isolation.

Punch et al (Punch, Syrmis et al. 2004) used a multiplex reverse transcriptase PCR (RT-PCR) assay combined with an enzyme-linked amplicon hybridization assay (ELAHA) for the identification of seven common respiratory viruses in the sputum of 38 CF patients. 53 sputum samples were collected over 2 seasons and 12 (23%) samples from 12 patients were positive for a respiratory virus (4 for *influenza B*, 3 for *parainfluenza type 1*, 3 for *influenza A* and 2 for *RSV*). There were no statistical associations between virus status and demographics, clinical variables or isolation rates for *P. aeruginosa, S. aureus or A. fumigatus*.

Olesen and colleagues (Olesen, Nielsen et al. 2006) obtained sputum/laryngeal aspirates from children with CF over a 12 month period in outpatient clinics. They achieved a viral detection rate of 16%, with *rhinovirus* being the most prevalent virus. FEV_1 was significantly reduced during viral infection (-12.5%, p=0.048), with the exception of *rhinovirus* infection. The authors were not able to demonstrate a positive correlation between respiratory viruses and bacterial infections in their studied population as the type or frequency of bacterial infection during or after viral infections were not altered. They also concluded that clinical viral symptoms had a very poor predictive value (0.39) for a positive viral test.

Wat et al (Wat, Gelder et al. 2008) utilised 'real-time' Nucleic Acid Sequenced Based Amplification to examine the role of respiratory viruses in CF. They achieved the highest detection rate of 46% amongst all existing literature concerning respiratory viruses in the CF population during reported episodes of respiratory illness. The results compare favourably with previous studies and this may be that earlier studies relied heavily on repeated serological testing, either alone(Petersen, Hoiby et al. 1981) or in combination with viral isolation (Wang, Prober et al. 1984; Ramsey, Gore et al. 1989; Pribble, Black et al. 1990; Armstrong, Grimwood et al. 1998; Hiatt, Grace et al. 1999). They also achieved a viral detection rate of 18.3% from routine nasal samples and this is comparable to the seroconversion rate of 12.3% as reported by Wang et al(Wang, Prober et al. 1984). This value is similar to the seroconversion rate of 16.2% from asymptomatic samples achieved by Ramsey and colleagues(Ramsey, Gore et al. 1989). Amongst stable asthmatic children, Johnston et al (Johnston, Pattemore et al. 1995) found a viral detection rate of 12% by PCR. Therefore, a laboratory method with a higher sensitivity for viral detection used in this study has not increased the detection rate in asymptomatic samples, implying that the high detection rate of respiratory viruses during exacerbations reinforces their pathogenicity. The authors also demonstrated that *influenza A and B* viruses are major viruses in causing

respiratory exacerbations in CF and both viruses are more commonly detected during pulmonary exacerbations. 22 of 88 (23%) viruses found in this study are *influenza viruses (A & B)*. The result is consistent with majority of the previous studies which showed that *influenza* virus represented between 12 to 27% of all viruses detected. In relation to *influenza* vaccination, the uptake rate was up to 70% during the 2003/4 season (Wat, Gelder et al. 2008) and the significance is that the *influenza* detection rate in this study could easily have been higher had the vaccination uptake rate in the study not been this high.

In 2009, a novel swine pandemic *influenza A virus (H1N1)* was identified. To date very little data exists regarding its impact on patients with CF. Nash et al (Nash, Whitmill et al. 2011) showed the symptoms of CF patients infected with H1N1 tend to be mild. There was no significant reduction in FEV_1 % predicted, FVC % predicted and body mass index regardless of whether the patients were positive or negative for *H1N1*. Colombo et al (Colombo, Battezzati et al. 2011) performed a multi-centre survey showed that diagnostic testing did not identify clinical characteristics specifically associated with *H1N1* infections. Similarly, they did not show a significant decline in lung function associated with this infection.

Experimental data on the effects of viral infections in CF are limited. Toll-like receptors (TLRs) have recently been identified as key mediators of the innate response and they recognise pathogens through detection of conserved microbial structures that are absent from the host. Kurt-Jones et al (Kurt-Jones, Popova et al. 2000) found that *RSV* persisted longer in the lungs of infected TLR4-deficient mice compared to normal mice. Haynes et al (Haynes, Moore et al. 2001) also demonstrated that TLR4-deficient mice when challenged with *RSV* exhibited impaired natural killer cell trafficking and impaired virus clearance compared to normal ones. Limited human studies have demonstrated the important role of TLRs in host response against many major groups of mammalian pathogens(Qureshi and Medzhitov 2003). The relationship between TLR and respiratory virus including *RSV* in humans will require further studies before it can be established.

Some studies have suggested a higher viral replication when there is an impairment of the innate host defence in CF. *Influenza* titres were significantly increased in a mouse model which were chronically infected with *P. aeruginosa* compared to control model (Seki, Higashiyama et al. 2004). Increased virus replication was also found after *PIV* infection of CF human airway epithelial cells, compared to controls (Zheng, De et al. 2003). One of the possible causes of increased virus replication and of virus persistence might be a reduced production of respiratory nitric oxide (NO), which is a vital part of innate antiviral defence mechanism (Zheng, Xu et al. 2004). Increased production of NO protects against viral infections. In CF patients, expression of the NO producing enzyme NO synthase type 2 (NOS2) is considerably reduced.

3. Detection of respiratory viruses

The principal laboratory methods of respiratory virus diagnosis rely on their detection in respiratory secretions and another important factor in respiratory viral diagnosis is to submit an appropriate sample for testing. Inappropriate specimen collection and transport account for the largest source of error in the accuracy of viral detection results (Nutting, Main et al. 1996). Nasal swabs, nasopharyngeal aspirates, nasal wash and sputum specimens are generally considered as the specimens of choice for the detection of

respiratory viruses (Hall and Douglas 1975; Schmid, Kudesia et al. 1998; Covalciuc, Webb et al. 1999; Punch, Syrmis et al. 2004). Performing a nasopharyngeal aspirate or suction can be unpleasant and requires the use of a suction device by a trained individual, which makes it unattractive in widespread clinical applications. In contrast, the collection of a nasal swab is simple, painless and quick and it does not require special equipment and skilled personnel. A prospective study by Heikkinen et al showed that the sensitivity of nasal swabs was comparable to nasopharyngeal aspirates for the detection of all major respiratory viruses by tissue culture with the exception of RSV (Heikkinen, Marttila et al. 2002). In non-sputum producing patients with CF, it has been shown that throat swab is not inferior to nasopharyngeal suction in detecting pathogens (Taylor, Corey et al. 2006).

Molecular techniques have superseded many 'conventional' methods utilised for respiratory viral detection such as viral culture and serology analysis due to their rapid turn-around of results. Traditional virus culture and serology analysis may require 1 to 2 weeks before results are available and direct antigen detection can have variable sensitivity and specificity (Swierkosz, Erdman et al. 1995). Molecular assays also have particular advantages where the starting material available is acellular (swab) or where surveillance samples have a low copy number of the viral target. The rapid turn-over of results allowing diagnostic virology to have an impact on patient management, avoiding the inappropriate prescription of antibiotics and allowing the proper use of anti-virals. It may also play an important role in infection control in the hospital setting.

More recently, Virochip has been shown to be a pan-virus microarray platform that is capable of detection of known as well as novel viruses in a single assay simultaneously (Chiu, Rouskin et al. 2006). The Virochip is very much a research tool at present, and several issues must be addressed before it can be used on a routine basis for virus detection in the clinical setting. These issues include cost, accuracy, reproducibility, and sensitivity/specificity for virus detection in comparison with traditional laboratory techniques. In addition, the implication of novel viruses in the human respiratory tract is not yet defined.

4. Interaction between respiratory viruses and bacteria

In a 25 year retrospective review from the Danish CF clinic, the first isolation of *P. aeruginosa* was most likely between October and March (Johansen and Hoiby 1992) coinciding with the peak of the *RSV* season. These findings must be interpreted with caution by the design of the study, as there are a number of other possible agents that would broadly fit the *RSV* season, most notably *influenza, rhinovirus* and *metapneumovirus*.

An increase in immunoglobulin A (IgA) antibodies to the O-antigen of *P. aeruginosa* is noted in 62% of viral infections (Przyklenk, Bauernfeind et al. 1988). This suggests a possible 'microbial synergism' between bacterial infections and infections with respiratory viruses in CF.

The first bacterial isolation of a given organism in CF has also been shown to often follow a viral infection. In the 17 month prospective study reported by Collinson et al (Collinson, Nicholson et al. 1996), five of the six first isolations of *P. aeruginosa* were made during the symptomatic phase of an upper respiratory tract infection or three weeks thereafter. In

contrast only one of the six initial infections with *P. aeruginosa* was identified during the asymptomatic period. Similarly, *H. influenzae* was recovered for the first time from 3 children within 3 weeks of an upper respiratory tract infection and the one new *S. aureus* infection was identified immediately following a viral infection.

Armstrong and colleagues have reported that 50% of CF respiratory exacerbations requiring hospitalisation are associated with isolation of a respiratory virus (Armstrong, Grimwood et al. 1998). In their prospective study of repeated bronchoalveolar lavage (BAL) in infants over a 5 year period, a respiratory virus was identified in 52% of infants hospitalised for a respiratory exacerbation, most commonly *RSV*. 11 of the 31 hospitalised infants (35%) acquired *P. aeruginosa* in the subsequent 12-60 month follow up, compared to 3 of 49 (6%) non-hospitalised infants (Relative risk 5.8).

Respiratory viruses can disrupt the airway epithelium and precipitate bacterial adherence. *Influenza A* infection has been shown to cause epithelial shedding to basement membrane with submucosal oedema and neutrophil infiltrate (Walsh, Dietlein et al. 1961), while both influenza and adenovirus have a cytopathic effect on cultured nasal epithelium leading to destruction of the cell monolayer (Winther, Gwaltney et al. 1990). This epithelial damage results in an increase in the permeability of the mucosal layer (Igarashi, Skoner et al. 1993; Ohrui, Yamaya et al. 1998) and possibly facilitating bacterial adherence. Bacteria can also utilise viral glycoproteins and other virus induced receptors on host cell membrane as bacterial receptors in order to adhere to virus infected cells (Sanford, Shelokov et al. 1978; Raza, Essery et al. 1999).

Kim et al (Kim, Battaile et al. 2008) found that invariant natural killer T cells induce a type of macrophage activation driving the secretion of interleukin-13 leading to the production of globlet cell metaplasia and airway hyperactivity following infection with Sendai virus. The term 'invariant' stems from the fact that all invariant natural killer T cells in humans and mice use a unique T cell receptor that is essential for interaction with CD1d. CD1d molecules present lipid antigens to T lymphocytes rather than peptide antigens as in the case of major histocompatibility-complex (MHC) class I and II molecules. Historically, MHC class II dependent CD4 and T lymphocytes, through their response to stimulation by environmental allergens, are keys to the pathogenesis of human asthma. The findings by the authors lead to the notion of the use of anti-interleukin-13 therapy as a potential therapy in patients.

Viral infections might predispose to secondary bacterial infections by impairing mucociliary function and triggering host inflammatory receptors (Wilson and Cole 1988; Murphy and Sethi 1992). This phenomenon has been demonstrated both in vivo and in vitro (Jiang, Nagata et al. 1999; White, Gompertz et al. 2003). Avadhanula et al (Avadhanula, Rodriguez et al. 2006) showed that different respiratory viruses use different mechanisms to enhance the adherence of bacteria to respiratory epithelial cells. In particular *RSV* and *PIV type 3* upregulate intercellular adhesion molecule-1 (ICAM-1), carcinoembryonic adhesion molecule 1 (CEACAM1) and platelet activating factor receptor (PAFr) but not mucin on the surfaces of A549, BEAS-2B and NHBE but not SAE cell lines. Much of the increased bacterial adhesion following *RSV* infection could be blocked by antibodies directed against these receptors. A549 and BEAS-2B are transformed cell lines derived from type II alveolar and normal bronchial cells, respectively. NHBE and SAE cells and primary epithelial cells obtained from bronchi and distal bronchial tree and are likely to include a heterogeneous population of cells.

Mechanisms independent of the expression of conventional receptors for bacteria, such as binding to viral proteins, could be responsible for enhanced adhesion (Hament, Kimpen et al. 1999). Immunofluorescence microscopy demonstrates that bacteria binding to *RSV* infected A549 cells adhere not only to these cells expressing viral antigens but also to uninfected epithelial cells. These data suggest that the ability to augment bacterial adhesion may result from a factor served by infected cells that exert a paracrine effect on adjacent epithelium. Cytokines or other inflammatory molecules are potential good candidates for such a mediator.

Rhinovirus has been shown to potentiate bacterial infections by inhibiting the secretion of TNF alpha and interleukin-8 by macrophages in vitro following co-infection with gram negative bacterial products, lipopolysaccharide (LPS), and gram positive bacterial products, lipoteichoic acid (LTA) (Oliver, Lim et al. 2008). This rhinovirus dependent impairment of the macrophage immune response was not mediated by autocrine production of the anti-inflammatory cytokines interleukin-10 and PGE2, or by downregulation of the cell surface receptor for LTA and LPS. In addition, the authors also show that rhinovirus inhibit the phagocytosis of bacterial products by macrophages. These findings support the notion that *rhinovirus* exposure resulted in a reduced ability to innate and adaptive immune responses against bacterial products, hence promoting the occurrence of bacterial and viral co-infections.

The lower respiratory tract is protected by local mucociliary mechanisms that involve the integration of the ciliated epithelium, periciliary fluid and mucus. Mucus acts as a physical and chemical barrier onto which particles and organisms adhere. Cilia lining the respiratory tract propel the overlying mucus to the oropharynx where it is either swallowed or expectorated. *Influenza* viral infection has been shown to precipitate the loss of cilial beat, and shedding of the columnar epithelial cells generally within 48 hours of infection (Thompson, Barclay et al. 2006). Pittet et al (Pittet, Hall-Stoodley et al.) showed that a prior *influenza* infection of tracheal cells in vivo does not increase the initial number of *pneumococci* found during the first hour of infection, but it does significantly reduce mucociliary velocity, and thereby reduces *pneumococcal* clearance during the first 2 hours after *pneumococcal* infection at both 3 and 6 days after an *influenza* infection. The defects in *pneumococcal* clearance were greatest at 6 days after *influenza* infection. Changes to the tracheal epithelium induced by *influenz*a virus may increase susceptibility to a secondary *S. pneumoniae* infection by increasing *pneumococcal* adherence to the tracheal epithelium and/or decreasing the clearance of *S. pneumoniae* via the mucociliary escalator of the trachea, and thus increasing the risk of secondary bacterial infection.

De Vrankrijker et al (de Vrankrijker, Wolfs et al. 2009) showed that mice that were co-infected with *RSV* and *P. aeruginosa* had a 2,000 times higher colony-forming units (CFU) counts of Pseudomonas aeruginosa in the lung homogenates compared to mice that were infected with *P. aeruginosa* alone. Co-infected mice also had more severe lung function changes. These results suggest that *RSV* can facilitate the initiation of acute *P. aeruginosa* infection.

Another study also showed that *H. influenzae* and *S. pneumoniae* bind to both free *RSV* virions and epithelial cells transfected with cell membrane-bound G protein, but not to secreted G protein. Pre-incubation with specific anti-G antibody significantly reduce bacterial adhesion to G protein-transfected cells (Avadhanula, Wang et al. 2007).

Stark et al (Stark, Stark et al. 2006) showed that mice that were exposed to *RSV* had significantly decreased *S. pneumonia*, *S. aureus* or *P. aeruginosa* clearance 1 to 7 days after *RSV* exposure. Mice that were exposed to both *RSV* and bacteria had a higher production of neutrophil-induced peroxide but less production of myeloperoxidase compared to mice that were exposed to *S. pneumoniae* alone. This suggests that functional changes in the recruited neutrophils may contribute to the decreased bacterial clearance.

More recently, Chattoraj et al (Chattoraj, Ganesan et al., 2011) demonstrated that acute infection of primary CF airway epithelial cells with rhinovirus liberates planktonic bacteria from biofilm. Superinfection with *rhinovirus* stimulates robust chemokine responses from CF airway epithelial cells that were pretreated with mucoid *P. aeruginosa*. The authors also showed that these chemokine responses lead to a liberation of bacteria from mucoid *P. aeruginosa* biofilm and transmigration of planktonic bacteria from the apical to the basolateral surface of mucociliary-differentiated CF airway epithelial cells. Planktonic bacteria, which are more proinflammatory than their biofilm counterparts, stimulate increased chemokine responses in CF airway epithelial cells which, in turn, may contribute to the pathogenesis of CF exacerbations and subsequent prolonged intravenous antibiotic use and hospitalisation.

Taken together, these findings suggest that respiratory viruses may lead to epithelial disruption, increased or decreased cytokine production, neutrophil influx, inhibition of macrophage phagocytosis, destruction of mucociliary escalator, increased cytokine production, and increased neutrophil induced peroxide release, indirectly facilitating bacterial infection of the airway.

5. Prevention and treatment for respiratory viruses

The existence of diverse viral serotypes in causing infection has made vaccine preparation very difficult. Frequent mutations of viral proteins of RNA viruses (for example genetic drift and shift of *influenza*) have further hampered the prevention of the illness.

Influenza associated death is between 13,000 to 20,000 per year in the winter months in the UK (Fleming 1996), though some of the deaths may be attributed to *RSV*. *Influenza* vaccines are the only commercially available vaccines against common respiratory viruses. They have been used since mid 1940s and they now have an established role in prevention of *influenza A and B* infections. Inactivated *influenza* vaccine is effective even in young children including those younger than 2 years (Heinonen, Silvennoinen et al. 2010). The waning of vaccine-induced immunity over time requires annual re-immunisation even if the vaccine antigens are unchanged.

Recent vaccines contain antigens of two *influenza A* subtypes, strains of the currently circulating *H3N2 and H1N1 (Swine flu)* subtypes, and one *influenza B* virus. The current recommendation for *influenza* vaccination in the UK is to offer it to those over the age of 65, those with chronic heart, respiratory (including CF) or renal diseases and those who are diabetic or immunosuppressed.

Wat et al (Wat, Gelder et al. 2008) recently showed that *influenza* vaccination provides protection against *influenza* acquisition in patients with CF, with 1 of 41 patients vaccinated had a positive nasal swab for influenza compared to 4 of the 22 non-vaccinated patients

(p=0.046). Although *influenza* vaccination does not appear to have any impact on respiratory exacerbation rates, it does have a role in preventing live infections. In this study, respiratory exacerbation rates in the preceding 10 months before the study between the vaccinated and non-vaccinated groups were similar, indicating that these were unlikely to be the reasons influencing the decision on immunisation. The decision may be down to a combination of patient/ parent education, social background, awareness of vaccination and accessibility of vaccination.

Due to the lack of randomised controlled studies looking at the efficacy of *influenza* vaccine in CF, the Cochrane review recommends clinicians to make their own judgements on the benefits and risks of this therapy in this cohort of patients (Dharmaraj and Smyth 2009). In addition to vaccine, neuraminidase inhibitors have been shown to have a role in preventing *influenza A and B* infections (Harper, Bradley et al. 2009).

Rhinovirus has more than 100 serotypes; it is unlikely that a unifying vaccine will be developed. VP4, one of the nonenveloped capsids, is highly conserved among all of the rhinoviruses; anti-VP4 antibodies have recently been generated and been shown to have the potential for future vaccine development(Katpally, Fu et al. 2009).

The development of an *RSV v*accine has been hampered by the experience with formalin-inactivated whole *RSV* vaccine in the 1960s, as it caused 80% of *RSV* vaccinees to become hospitalised compared with 5% of controls, as well as two fatalities (Kim, Canchola et al. 1969). Current major research work has focused on a prophylaxis using a humanised mouse monoclonal antibody, Palizivumab. In patients with CF, monthly Palizivumab injection significantly reduce the hospitalisation rate for acute respiratory illness during the *RSV* season compared to those who were not immunised ($p<0.05$). The former group also had fewer hospital days for acute respiratory illness (Giebels, Marcotte et al. 2008).

There is currently no licensed *PIV* vaccine to date. The formalin-inactivated vaccine generated in the 1960s was not able to prevent *PIV* infection and was soon abandoned. Recently, recombinant bovine *PIV type 3* and human *PIV type 3* attenuated vaccines are being evaluated in animal models as vectors for the delivery of other viral antigens such as *RSV*-G and *RSV*-F proteins. This bivalent vaccine combination provides high level of resistance to challenges with *PIV type 3* and *RSV* in animal models (Schmidt, McAuliffe et al. 2001).

The conventional methods of vaccination are via the intramuscular and subcutaneous routes. Mucosal immunisation has recently been explored as it represents an attractive manner of delivering vaccines. It is fast, simple, non-invasive and can be carried out by unskilled individuals. The use of mucosal vaccination seems logical in that most of respiratory viral infections initially start at the mucosal sites and therefore inducing local immunity.

So far, there has been inconclusive evidence to support the use of vitamin C and extracts of the plant Echinacea in common cold prevention. Daily supplementation with large doses of vitamin C does not seem to prevent common colds, however there seems to be a modest (8 to 9%) reduction in the number of symptom days in individuals with established cold symptoms, with larger doses having greater effect (Douglas, Chalker et al. 2000). In vitro studies have shown that Echinacea can activate macrophages, increase phagocytosis,

enhance cytokine production (Sharma, Arnason et al. 2006), and natural killer cell activity, and improve lymphocyte and monocyte cell counts (Goel, Lovlin et al. 2005). Current data is available in the adult population and has reported positive findings both in the treatment and prevention of upper respiratory tract infection. However, variations in the design of the clinical trial and in Echinacea preparations have to be taken into account (Giles, Palat et al. 2000).

Zinc has been shown to possess anti-viral properties in vitro and different preparations of zinc have been proposed for the treatment of common cold. Postulated mechanisms in the common cold include interfering with rhinovirus protein cleavage or capsid binding to ICAM-1 in nasal epithelium (Novick, Godfrey et al. 1996). Zinc lozenges appeared to have positive effects on adults but negative effects on children in terms of duration and severity of common cold symptoms (Macknin, Piedmonte et al. 1998; Marshall 2000). Higher doses were found to have a greater impact in reduction of symptom duration and reduced symptom severity (Godfrey, Conant Sloane et al. 1992; Mossad, Macknin et al. 1996). Zinc nasal spray appears to reduce the total symptom score but has no effect on the duration of common cold (Belongia, Berg et al. 2001). Irritation by nasal sprays limits their use; they also seem to have lower concentrations in the nasopharynx (Godfrey 1988).

Amantadine has been the conventional anti-viral against *influenza*. However it is strain specific as it is only effective against *influenza A* and has common side-effects such as insomnia, poor concentration and irritability. It is now largely being replaced by neuraminidase inhibitors such as Zanamivir and Oseltamivir which are licensed for the treatment of *influenza A and B*, including *avian flu H5N1* and *swine flu H1N1*. However, Amantadine still has a role in dealing with Oseltamivir resistant H1N1 virus. In children and adults, early initiation of neuraminidase inhibitors within 48 hours of the onset of symptoms can reduce the duration of flu-like symptoms by 0.5 to 2.5 days (Shun-Shin, Thompson et al. 2009). Early use of these medications can also reduce development of complications such as pneumonia (Yu, Liao et al. 2010). The 2009 pandemic *H1N1* virus remains susceptible to neuraminidase inhibitors, and Oseltamivir has been used extensively for treatment related to this viral infection. Resistance to Oseltamivir has been reported with *H1N1* viral infection but this is mainly restricted to immunocompromised individuals (Bautista, Chotpitayasunondh et al. 2010). Zanamivir has a poor oral bioavailability, and intranasal application has been shown to be effective in treating experimental *influenz*a infection with the reduction in symptoms caused, virus shedding and development of otitis media (Hayden, Treanor et al. 1996). Intravenous use of Peramivir or Zanamivir could be lifesaving in critically ill patients with *influenza* infection (Birnkrant and Cox 2009; Harter, Zimmermann et al. 2010).

Ribavarin, a synthetic guanosine nucleoside that has a broad spectrum of antiviral activity, has been used for treatment of infections related to *RSV*, *metapneumovirus, and parainfluenza and influenza viruses* (Yin, Brust et al. 2009). Potential benefits of ribavarin therapy include the inhibition of RSV-specific IgE production in nasal secretions, which has been associated with the development of hypoxaemia and wheezing (Rosner, Welliver et al. 1987) and it has improved pulmonary functions (Hiatt 1990). Controlled studies also show that the use of ribavarin is effective in reducing the clinical severity score, duration of mechanical ventilation, supplemental oxygen use and days of hospitalisation (Smith, Frankel et al. 1991). Aerosolised ribavarin has been used for the treatment of *RSV* related bronchiolitis

and pneumonia. Intravenous formulation could be used for treatment of severe pneumonia, caused by infection *RSV, metapneumovirus, or parainfluenza virus*, on the basis of experience in immunocompromised patients (Hopkins, McNeil et al. 2008). Bonney et al has shown that *metapneumovirus* can be successfully treated with a combination of intravenous ribavarin and immunoglobulin (Bonney, Razali et al. 2009).

Although *rhinovirus* is the major cause of colds, its vast amount of serotypes has made development of anti-virals against it problematic. 90% of *rhinovirus* serotypes gain entry into epithelial cells using ICAM-1 cellular receptors and blockade of these receptors in experimental studies have shown reduced infection severity (Turner, Wecker et al. 1999), but further study is required before this treatment option becomes widely available. Macrolide antibiotics, Bafilomycin A1 and Erythromycin have been shown to inhibit ICAM-1 epithelial expression and hypothesis about their potential as anti-inflammatory agents have yet to be definitive, as clinical proof is either negative or inconclusive (Suzuki, Yamaya et al. 2002).

Recently, an anti-rhinoviral agent known as Plecoranil, which acts by inhibiting the uncoating of Picornaviruses (Ledford, Patel et al. 2004), the RV 3C protease inhibitor, Ruprintrivir(Hayden, Turner et al. 2003) and soluble ICAM-1, Tremacamra(Turner, Wecker et al. 1999) have shown promising results in early-stage clinical trials, but each of these medications was derailed by a combination of cost, pharmacokinetics, toxicity, drug interactions, and limited efficacy (Turner 2005).

6. Conclusion

With the available knowledge regarding the impact of respiratory viruses in the exacerbation of CF, screening for respiratory viruses during pulmonary exacerbations should be implemented as part of routine clinical assessment. This assessment should include obtaining specimens from the respiratory tract and using molecular viral detection methods to reach a rapid diagnosis. The identification of respiratory viruses may allow appropriate anti-virals to be used.

With gene therapy still undergoing further research with regards to its validity and specificity, gaining further understanding in the pathogenesis of virus induced respiratory exacerbations in CF may allow the development of new therapeutic techniques. If viral infection does predispose to bacterial infection, then influencing the interaction between viruses and bacteria could be a next pathway to diminish respiratory morbidity in patients with CF. The development of novel therapies will be exciting and this may further prolong the lifespan of patients with CF and more importantly improve their quality of life.

In light of the above, future research in respiratory viruses in CF is urgently required to address a number of important questions: 1) What is the optimal way for viral sampling? 2) What is the most efficient and rapid method to detect a range of respiratory viruses? 3) How do respiratory viruses influence bacterial behaviour in chronically infected airways? 4) What is the efficacy of *influenza* vaccination in CF? 5) What are the roles of anti-virals in CF?

Further understanding in the pathogenesis of viral infection in CF would be beneficial as this may provide insight to the above unresolved mysteries. At the moment it appears that *influenza* vaccination remains the mainstay of management of viral infections in CF.

7. References

Aaron, S. D., K. Ramotar, et al. (2004). "Adult cystic fibrosis exacerbations and new strains of Pseudomonas aeruginosa." *Am J Respir Crit Care Med* 169(7): 811-5.

Abman, S. H., J. W. Ogle, et al. (1991). "Early bacteriologic, immunologic, and clinical courses of young infants with cystic fibrosis identified by neonatal screening." *J Pediatr* 119(2): 211-7.

Armstrong, D., K. Grimwood, et al. (1998). "Severe viral respiratory infections in infants with cystic fibrosis." *Pediatr Pulmonol* 26(6): 371-9.

Avadhanula, V., C. A. Rodriguez, et al. (2006). "Respiratory viruses augment the adhesion of bacterial pathogens to respiratory epithelium in a viral species- and cell type-dependent manner." *J Virol* 80(4): 1629-36.

Avadhanula, V., Y. Wang, et al. (2007). "Nontypeable Haemophilus influenzae and Streptococcus pneumoniae bind respiratory syncytial virus glycoprotein." *J Med Microbiol* 56(Pt 9): 1133-7.

Barasch, J. and Q. al-Awqati (1993). "Defective acidification of the biosynthetic pathway in cystic fibrosis." *J Cell Sci Suppl* 17: 229-33.

Bautista, E., T. Chotpitayasunondh, et al. (2010). "Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection." *N Engl J Med* 362(18): 1708-19.

Belongia, E. A., R. Berg, et al. (2001). "A randomized trial of zinc nasal spray for the treatment of upper respiratory illness in adults." *Am J Med* 111(2): 103-8.

Birnkrant, D. and E. Cox (2009). "The Emergency Use Authorization of peramivir for treatment of 2009 H1N1 influenza." *N Engl J Med* 361(23): 2204-7.

Bonney, D., H. Razali, et al. (2009). "Successful treatment of human metapneumovirus pneumonia using combination therapy with intravenous ribavirin and immune globulin." *Br J Haematol* 145(5): 667-9.

Brasfield, D., G. Hicks, et al. (1979). "The chest roentgenogram in cystic fibrosis: a new scoring system." *Pediatrics* 63(1): 24-9.

Chattoraj, S. S., S. Ganesan, et al. (2011) "Rhinovirus infection liberates planktonic bacteria from biofilm and increases chemokine responses in cystic fibrosis airway epithelial cells." *Thorax* 66(4): 333-9.

Chiu, C. Y., S. Rouskin, et al. (2006). "Microarray detection of human parainfluenzavirus 4 infection associated with respiratory failure in an immunocompetent adult." *Clin Infect Dis* 43(8): e71-6.

Chrispin, A. R. and A. P. Norman (1974). "The systematic evaluation of the chest radiograph in cystic fibrosis." *Pediatr Radiol* 2(2): 101-5.

Collinson, J., K. G. Nicholson, et al. (1996). "Effects of upper respiratory tract infections in patients with cystic fibrosis." *Thorax* 51(11): 1115-22.

Colombo, C., P. M. Battezzati, et al. (2011). "Influenza A/H1N1 in patients with cystic fibrosis in Italy: a multicentre cohort study." *Thorax* 66(3): 260-1.

Conway, S. P., E. J. Simmonds, et al. (1992). "Acute severe deterioration in cystic fibrosis associated with influenza A virus infection." *Thorax* 47(2): 112-4.

Covalciuc, K. A., K. H. Webb, et al. (1999). "Comparison of four clinical specimen types for detection of influenza A and B viruses by optical immunoassay (FLU OIA test) and cell culture methods." *J Clin Microbiol* 37(12): 3971-4.

de Almeida, M. B., R. M. Zerbinati, et al. (2010). "Rhinovirus C and respiratory exacerbations in children with cystic fibrosis." *Emerg Infect Dis* 16(6): 996-9.

de Vrankrijker, A. M., T. F. Wolfs, et al. (2009). "Respiratory syncytial virus infection facilitates acute colonization of Pseudomonas aeruginosa in mice." *J Med Virol* 81(12): 2096-103.

Dharmaraj, P. and R. L. Smyth (2009). "Vaccines for preventing influenza in people with cystic fibrosis." *Cochrane Database Syst Rev*(4): CD001753.

Dodge, J. A., S. Morison, et al. (1993). "Cystic fibrosis in the United Kingdom, 1968-1988: incidence, population and survival." *Paediatr Perinat Epidemiol* 7(2): 157-66.

Douglas, R. M., E. B. Chalker, et al. (2000). "Vitamin C for preventing and treating the common cold." *Cochrane Database Syst Rev*(2): CD000980.

Elborn, J. S., D. J. Shale, et al. (1991). "Cystic fibrosis: current survival and population estimates to the year 2000." *Thorax* 46(12): 881-5.

Fleming, D. M. (1996). "The impact of three influenza epidemics on primary care in England and Wales." *Pharmacoeconomics* 9 Suppl 3: 38-45; discussion 50-3.

Garcia, D. F., P. W. Hiatt, et al. (2007). "Human metapneumovirus and respiratory syncytial virus infections in older children with cystic fibrosis." *Pediatr Pulmonol* 42(1): 66-74.

Giebels, K., J. E. Marcotte, et al. (2008). "Prophylaxis against respiratory syncytial virus in young children with cystic fibrosis." *Pediatr Pulmonol* 43(2): 169-74.

Giles, J. T., C. T. Palat, 3rd, et al. (2000). "Evaluation of echinacea for treatment of the common cold." *Pharmacotherapy* 20(6): 690-7.

Godfrey, J. C. (1988). "Zinc for the common cold." *Antimicrob Agents Chemother* 32(4): 605-6.

Godfrey, J. C., B. Conant Sloane, et al. (1992). "Zinc gluconate and the common cold: a controlled clinical study." *J Int Med Res* 20(3): 234-46.

Goel, V., R. Lovlin, et al. (2005). "A proprietary extract from the echinacea plant (Echinacea purpurea) enhances systemic immune response during a common cold." *Phytother Res* 19(8): 689-94.

Goss, C. H. and J. L. Burns (2007). "Exacerbations in cystic fibrosis. 1: Epidemiology and pathogenesis." *Thorax* 62(4): 360-7.

Hall, C. B. and R. G. Douglas, Jr. (1975). "Clinically useful method for the isolation of respiratory syncytial virus." *J Infect Dis* 131(1): 1-5.

Hament, J. M., J. L. Kimpen, et al. (1999). "Respiratory viral infection predisposing for bacterial disease: a concise review." *FEMS Immunol Med Microbiol* 26(3-4): 189-95.

Harper, S. A., J. S. Bradley, et al. (2009). "Seasonal influenza in adults and children--diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America." *Clin Infect Dis* 48(8): 1003-32.

Harter, G., O. Zimmermann, et al. (2010). "Intravenous zanamivir for patients with pneumonitis due to pandemic (H1N1) 2009 influenza virus." *Clin Infect Dis* 50(9): 1249-51.

Hayden, F. G., J. J. Treanor, et al. (1996). "Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza." *Jama* 275(4): 295-9.

Hayden, F. G., R. B. Turner, et al. (2003). "Phase II, randomized, double-blind, placebo-controlled studies of ruprintrivir nasal spray 2-percent suspension for prevention and treatment of experimentally induced rhinovirus colds in healthy volunteers." *Antimicrob Agents Chemother* 47(12): 3907-16.

Haynes, L. M., D. D. Moore, et al. (2001). "Involvement of toll-like receptor 4 in innate immunity to respiratory syncytial virus." *J Virol* 75(22): 10730-7.

Heikkinen, T., J. Marttila, et al. (2002). "Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses." *J Clin Microbiol* 40(11): 4337-9.

Heinonen, S., H. Silvennoinen, et al. (2010). "Effectiveness of inactivated influenza vaccine in children aged 9 months to 3 years: an observational cohort study." *Lancet Infect Dis* 11(1): 23-9.

Hiatt, P. T., D Laber, L (1990). "Pulmonary function (PF) following treatment with ribavarin in infants hospitalised with RSV bronchiolitis." *Am Rev Respir Dis* 141: A624.

Hiatt, P. W., S. C. Grace, et al. (1999). "Effects of viral lower respiratory tract infection on lung function in infants with cystic fibrosis." *Pediatrics* 103(3): 619-26.

Hopkins, P., K. McNeil, et al. (2008). "Human metapneumovirus in lung transplant recipients and comparison to respiratory syncytial virus." *Am J Respir Crit Care Med* 178(8): 876-81.

Hordvik, N. L., P. Konig, et al. (1989). "Effects of acute viral respiratory tract infections in patients with cystic fibrosis." *Pediatr Pulmonol* 7(4): 217-22.

http://genet.sickkids.on.ca/cgi-bin/WebObjects/MUTATION.

Igarashi, Y., D. P. Skoner, et al. (1993). "Analysis of nasal secretions during experimental rhinovirus upper respiratory infections." *J Allergy Clin Immunol* 92(5): 722-31.

Jiang, Z., N. Nagata, et al. (1999). "Fimbria-mediated enhanced attachment of nontypeable Haemophilus influenzae to respiratory syncytial virus-infected respiratory epithelial cells." *Infect Immun* 67(1): 187-92.

Johansen, H. K. and N. Hoiby (1992). "Seasonal onset of initial colonisation and chronic infection with Pseudomonas aeruginosa in patients with cystic fibrosis in Denmark." *Thorax* 47(2): 109-11.

Johnston, S. L., P. K. Pattemore, et al. (1995). "Community study of role of viral infections in exacerbations of asthma in 9-11 year old children." *Bmj* 310(6989): 1225-9.

Katpally, U., T. M. Fu, et al. (2009). "Antibodies to the buried N terminus of rhinovirus VP4 exhibit cross-serotypic neutralization." *J Virol* 83(14): 7040-8.

Kim, E. Y., J. T. Battaile, et al. (2008). "Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease." *Nat Med* 14(6): 633-40.

Kim, H. W., J. G. Canchola, et al. (1969). "Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine." *Am J Epidemiol* 89(4): 422-34.

Kurt-Jones, E. A., L. Popova, et al. (2000). "Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus." *Nat Immunol* 1(5): 398-401.

Ledford, R. M., N. R. Patel, et al. (2004). "VP1 sequencing of all human rhinovirus serotypes: insights into genus phylogeny and susceptibility to antiviral capsid-binding compounds." *J Virol* 78(7): 3663-74.

Macknin, M. L., M. Piedmonte, et al. (1998). "Zinc gluconate lozenges for treating the common cold in children: a randomized controlled trial." *Jama* 279(24): 1962-7.

Marshall, I. (2000). "Zinc for the common cold." *Cochrane Database Syst Rev*(2): CD001364.

Mearns, M. (1993). Cystic fibrosis: the first 50 years: a review of the clinical problems anf theit management. *Cystic Fibrosis Current Topics*. J. A. Dodge, D. J. H. Brock and J. H. Widdicombe, John Wiley and Sons Ltd. 1.

Mossad, S. B., M. L. Macknin, et al. (1996). "Zinc gluconate lozenges for treating the common cold. A randomized, double-blind, placebo-controlled study." *Ann Intern Med* 125(2): 81-8.

Murphy, T. F. and S. Sethi (1992). "Bacterial infection in chronic obstructive pulmonary disease." *Am Rev Respir Dis* 146(4): 1067-83.

Nash, E. F., R. Whitmill, et al. (2011). "Clinical outcomes of pandemic (H1N1) 2009 influenza (swine flu) in adults with cystic fibrosis." *Thorax* 66(3): 259.

Nixon, G. M., D. S. Armstrong, et al. (2001). "Clinical outcome after early Pseudomonas aeruginosa infection in cystic fibrosis." *J Pediatr* 138(5): 699-704.

Novick, S. G., J. C. Godfrey, et al. (1996). "How does zinc modify the common cold? Clinical observations and implications regarding mechanisms of action." *Med Hypotheses* 46(3): 295-302.

Nutting, P. A., D. S. Main, et al. (1996). "Toward optimal laboratory use. Problems in laboratory testing in primary care." *Jama* 275(8): 635-9.

Ohrui, T., M. Yamaya, et al. (1998). "Effects of rhinovirus infection on hydrogen peroxide-induced alterations of barrier function in the cultured human tracheal epithelium." *Am J Respir Crit Care Med* 158(1): 241-8.

Olesen, H. V., L. P. Nielsen, et al. (2006). "Viral and atypical bacterial infections in the outpatient pediatric cystic fibrosis clinic." *Pediatr Pulmonol* 41(12): 1197-204.

Oliver, B. G., S. Lim, et al. (2008). "Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages." *Thorax* 63(6): 519-25.

Oppenheimer, E. H. and J. R. Esterly (1975). "Pathology of cystic fibrosis review of the literature and comparison with 146 autopsied cases." *Perspect Pediatr Pathol* 2: 241-78.

Ortiz, J. R., K. M. Neuzil, et al. (2010). "Influenza-associated cystic fibrosis pulmonary exacerbations." *Chest* 137(4): 852-60.

Petersen, N. T., N. Hoiby, et al. (1981). "Respiratory infections in cystic fibrosis patients caused by virus, chlamydia and mycoplasma--possible synergism with Pseudomonas aeruginosa." *Acta Paediatr Scand* 70(5): 623-8.

Pittet, L. A., L. Hall-Stoodley, et al. "Influenza virus infection decreases tracheal mucociliary velocity and clearance of Streptococcus pneumoniae." *Am J Respir Cell Mol Biol* 42(4): 450-60.

Pribble, C. G., P. G. Black, et al. (1990). "Clinical manifestations of exacerbations of cystic fibrosis associated with nonbacterial infections." *J Pediatr* 117(2 Pt 1): 200-4.

Przyklenk, B., A. Bauernfeind, et al. (1988). "Viral infections in the respiratory tract in patients with cystic fibrosis." *Serodign Immunother Infect Dis*(2): 217-25.

Punch, G., M. W. Syrmis, et al. (2004). "Method for detection of respiratory viruses in the sputa of patients with cystic fibrosis." *Eur J Clin Microbiol Infect Dis*.

Qureshi, S. T. and R. Medzhitov (2003). "Toll-like receptors and their role in experimental models of microbial infection." *Genes Immun* 4(2): 87-94.

Rajan, S. and L. Saiman (2002). "Pulmonary infections in patients with cystic fibrosis." *Semin Respir Infect* 17(1): 47-56.

Ramsey, B. W., E. J. Gore, et al. (1989). "The effect of respiratory viral infections on patients with cystic fibrosis." *Am J Dis Child* 143(6): 662-8.

Raza, M. W., S. D. Essery, et al. (1999). "Infection with respiratory syncytial virus and water-soluble components of cigarette smoke alter production of tumour necrosis factor

alpha and nitric oxide by human blood monocytes." *FEMS Immunol Med Microbiol* 24(4): 387-94.

Riordan, J. R., J. M. Rommens, et al. (1989). "Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA." *Science* 245(4922): 1066-73.

Rosner, I. K., R. C. Welliver, et al. (1987). "Effect of ribavirin therapy on respiratory syncytial virus-specific IgE and IgA responses after infection." *J Infect Dis* 155(5): 1043-7.

Saiman, L. and J. Siegel (2004). "Infection control in cystic fibrosis." *Clin Microbiol Rev* 17(1): 57-71.

Sanford, B. A., A. Shelokov, et al. (1978). "Bacterial adherence to virus-infected cells: a cell culture model of bacterial superinfection." *J Infect Dis* 137(2): 176-81.

Sawicki, G. S., L. Rasouliyan, et al. (2008). "The impact of incident methicillin resistant Staphylococcus aureus detection on pulmonary function in cystic fibrosis." *Pediatr Pulmonol* 43(11): 1117-23.

Schmid, M. L., G. Kudesia, et al. (1998). "Prospective comparative study of culture specimens and methods in diagnosing influenza in adults." *Bmj* 316(7127): 275.

Schmidt, A. C., J. M. McAuliffe, et al. (2001). "Recombinant bovine/human parainfluenza virus type 3 (B/HPIV3) expressing the respiratory syncytial virus (RSV) G and F proteins can be used to achieve simultaneous mucosal immunization against RSV and HPIV3." *J Virol* 75(10): 4594-603.

Seki, M., Y. Higashiyama, et al. (2004). "Acute infection with influenza virus enhances suspectibility to fatal pneumonia following Streptococcus pneumoniae infection in mice with chronic pulmonary colonisation with Psudomonas aeruginosa." *Clin Exp Immunol* 137(1): 35-40.

Sharma, M., J. T. Arnason, et al. (2006). "Echinacea extracts modulate the pattern of chemokine and cytokine secretion in rhinovirus-infected and uninfected epithelial cells." *Phytother Res* 20(2): 147-52.

Shun-Shin, M., M. Thompson, et al. (2009). "Neuraminidase inhibitors for treatment and prophylaxis of influenza in children: systematic review and meta-analysis of randomised controlled trials." *BMJ* 339: b3172.

Shwachman, H. and L. L. Kulczycki (1958). "Long-term study of one hundred five patients with cystic fibrosis; studies made over a five- to fourteen-year period." *AMA J Dis Child* 96(1): 6-15.

Smith, D. W., L. R. Frankel, et al. (1991). "A controlled trial of aerosolized ribavirin in infants receiving mechanical ventilation for severe respiratory syncytial virus infection." *N Engl J Med* 325(1): 24-9.

Smyth, A. R., R. L. Smyth, et al. (1995). "Effect of respiratory virus infections including rhinovirus on clinical status in cystic fibrosis." *Arch Dis Child* 73(2): 117-20.

Stark, J. M., M. A. Stark, et al. (2006). "Decreased bacterial clearance from the lungs of mice following primary respiratory syncytial virus infection." *J Med Virol* 78(6): 829-38.

Suzuki, T., M. Yamaya, et al. (2002). "Erythromycin inhibits rhinovirus infection in cultured human tracheal epithelial cells." *Am J Respir Crit Care Med* 165(8): 1113-8.

Swierkosz, E. M., D. D. Erdman, et al. (1995). "Isolation and characterization of a naturally occurring parainfluenza 3 virus variant." *J Clin Microbiol* 33(7): 1839-41.

Taylor, L., M. Corey, et al. (2006). "Comparison of throat swabs and nasopharyngeal suction specimens in non-sputum-producing patients with cystic fibrosis." *Pediatr Pulmonol* 41(9): 839-43.

Thomassen, M. J., C. A. Demko, et al. (1985). "Pseudomonas cepacia colonization among patients with cystic fibrosis. A new opportunist." *Am Rev Respir Dis* 131(5): 791-6.

Thompson, C. I., W. S. Barclay, et al. (2006). "Infection of human airway epithelium by human and avian strains of influenza a virus." *J Virol* 80(16): 8060-8.

Thompson, W. W., D. K. Shay, et al. (2004). "Influenza-associated hospitalizations in the United States." *JAMA* 292(11): 1333-40.

Turner, R. B. (2005). "New considerations in the treatment and prevention of rhinovirus infections." *Pediatr Ann* 34(1): 53-7.

Turner, R. B., M. T. Wecker, et al. (1999). "Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: a randomized clinical trial." *Jama* 281(19): 1797-804.

van den Hoogen, B. G., J. C. de Jong, et al. (2001). "A newly discovered human pneumovirus isolated from young children with respiratory tract disease." *Nat Med* 7(6): 719-24.

Vawter, G. F. and H. Shwachman (1979). "Cystic fibrosis in adults: an autopsy study." *Pathol Annu* 14 Pt 2: 357-82.

Walsh, J. J., L. F. Dietlein, et al. (1961). "Bronchotracheal response in human influenza. Type A, Asian strain, as studied by light and electron microscopic examination of bronchoscopic biopsies." *Arch Intern Med* 108: 376-88.

Wang, E. E., C. G. Prober, et al. (1984). "Association of respiratory viral infections with pulmonary deterioration in patients with cystic fibrosis." *N Engl J Med* 311(26): 1653-8.

Wat, D., C. Gelder, et al. (2008). "Is there a role for influenza vaccination in cystic fibrosis?" *J Cyst Fibros* 7(1): 85-8.

Wat, D., C. Gelder, et al. (2008). "The role of respiratory viruses in cystic fibrosis." *J Cyst Fibros* 7(4): 320-8.

White, A. J., S. Gompertz, et al. (2003). "Chronic obstructive pulmonary disease . 6: The aetiology of exacerbations of chronic obstructive pulmonary disease." *Thorax* 58(1): 73-80.

Wilson, R. and P. J. Cole (1988). "The effect of bacterial products on ciliary function." *Am Rev Respir Dis* 138(6 Pt 2): S49-53.

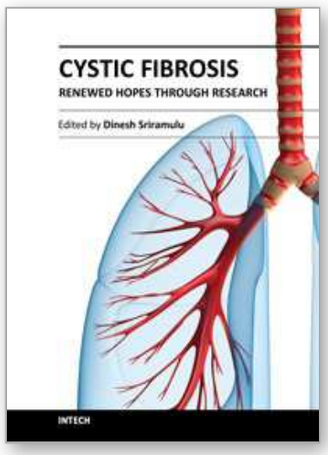
Winther, B., J. Gwaltney, et al. (1990). "Respiratory virus infection of monolayer cultures of human nasal epithelial cells." *Am Rev Respir Dis.* 141(4 Pt 1): 839-45.

Yin, M., J. Brust, et al. (2009). Antiherpes, anti-hepatitis virus, and anti-respiratory virus agents. *Clinical virology, 3rd edition.* D. Richman, R. Whiyley and F. Hayden. Washington, ASM Press: 217-64.

Yu, H., Q. Liao, et al. (2010). "Effectiveness of oseltamivir on disease progression and viral RNA shedding in patients with mild pandemic 2009 influenza A H1N1: opportunistic retrospective study of medical charts in China." *BMJ* 341: c4779.

Zheng, S., B. De, et al. (2003). "Impaired innate host defense causes suspectibility to respiratory virus infections in cystic fibrosis." *Immunity* 18(5): 619-30.

Zheng, S., W. Xu, et al. (2004). "Impaired nitirc oxide synthase-2 signaling pathway in cystic fibrosis airway epithelium." *Am J Physiol Lung Cell Mol Physiol* 287(2): L374-81.



Cystic Fibrosis - Renewed Hopes Through Research

Edited by Dr. Dinesh Sriramulu

ISBN 978-953-51-0287-8

Hard cover, 550 pages

Publisher InTech

Published online 28, March, 2012

Published in print edition March, 2012

Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen