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Viral infections of the respiratory tract in patients with cystic fibrosis

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The role of viruses and bacteria in the development of respiratory tract infections causing acute deteriorations in lung function in patients with cystic fibrosis (CF) was investigated. Over a period of 30 months, 29 viral respiratory diseases were proven serologically by testing 275 sporadically collected sera from 75 patients with cystic fibrosis. The influenza A virus was the most frequent responsible viral pathogen (11 ×), followed by adenovirus (8 ×), influenza B virus (5 ×), parainfluenza virus type 3 (3 ×), parainfluenza virus type 1 and respiratory syncytial virus (RSV) (each of 1 ×). There was no serological evidence for infections with parainfluenza virus type 2, *Mycoplasma pneumoniae* or *Coxiella burnetii*. Deterioration of the clinical condition was found in 78% of the viral infections leading in 70% to hospital admission. Patients with cystic fibrosis and viral respiratory illnesses showed significantly more admissions to the hospital (3.2 ± 2.7) with a longer stay (90.6 ± 99.6 days). Nearly all viral episodes (93%) were accompanied or followed by a significant change of the microbial flora in the sputum especially by colonisation with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae*. Seventy-two per cent of the viral infections occurred at home and 28% seemed to be hospital acquired. Our study emphasises the importance of improving antibacterial therapy at home to reduce the number of hospital admissions. Efforts for prophylaxis by vaccination or the use of chemotherapeutic agents should be made for the patients with cystic fibrosis.

Keywords: cystic fibrosis, viruses, respiratory tract infections, *Pseudomonas aeruginosa*, sIgA.

Introduction

Respiratory viral infections are frequently responsible for the acute deterioration in the clinical condition of patients with underlying pulmonary disease eg chronic obstructive pulmonary disease or asthma¹. They may also predispose to bacterial infections with *Haemophilus influenzae*, *Streptococcus pneumoniae* or *Staphylococcus aureus*². Viral infections of the upper airways are not more frequent in patients with cystic fibrosis than in healthy controls, but the consequences tend to be more serious³. The major cause of acute deterioration of lung function in patients with cystic fibrosis is considered to be

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bacterial, especially *P. aeruginosa*, and in one-third this is preceded by a viral infection⁴⁻⁶. No previous study has compared quantitative bacteriological analyses of the sputum of patients with cystic fibrosis with a study of the immune responses against different viral agents in these patients. We therefore decided to determine to what extent respiratory tract infections with different viruses, *Mycoplasma pneumoniae* and *Coxiella burnetii* contribute to the development of the acute bronchopulmonary exacerbations of cystic fibrosis.

Material and methods

Seventy-five patients with laboratory and clinical signs of cystic fibrosis (38 female, 37 male; mean age 15.6 ± 5.6 , range 4–28 years) were included in this study. The Shwachman scores⁷ for these patients lay between 35 and 95.

During the study period (from November 1984 until April 1987), 275 sera were collected—between one and 18 serum samples from each patient, with a median value of 2. Fifty-two per cent of the serum samples were taken as outpatients at the Dr. von Hauner'sches Kinderspital München, 22% on admission to hospital and 26% during the hospital stay.

The majority of the CF-patients (62/75) had a chronic infection (>6 months) with *Pseudomonas aeruginosa* at the beginning of the study. Five patients harboured *P. aeruginosa* only intermittently in their airways, whilst six patients were free of the pathogen throughout the study. Two patients with cystic fibrosis developed infection due to *P. aeruginosa* at the start of the study.

Antibodies against influenza A, influenza B, influenza C viruses, parainfluenzae viruses (types 1, 2 and 3), respiratory syncytial virus (RSV), adenovirus, *M. pneumoniae* and *C. burnetii* were determined by complement fixation⁸. According to Enders⁹, titers > 1:64 for adenovirus and mycoplasma, > 1:32 for RSV, influenza A and B viruses and *C. burnetii*, and > 1:16 for influenza C and parainfluenza viruses, or at least four-fold rises in paired serum samples, are considered to indicate a recent infection.

Microbiological analyses of sputum¹⁰ were performed at the same time as the serology. Sputum sIgA-antibodies against the different lipopolysaccharide antigens of *P. aeruginosa* were evaluated as described elsewhere¹¹. For statistical calculations the Wilcoxon-Mann-White test, the χ^2 test and Fisher's exact test were used.

Results

With the criteria of Enders⁹, previously described, we found 29 viral respiratory infectious episodes in the patients with cystic fibrosis taken into the study. Infections with two different viral agents at the same time occurred twice: influenza A and adenovirus; influenza B and adenovirus. Multiple respiratory tract viral infections were found in seven patients with at least 5 months between the two episodes (Table 1).

An at least four-fold titer rise in the complement-fixation test was seen in 25 of the 29 viral infections, and in four cases the first serum samples showed significantly elevated antibody titers (Table 2). Influenza A virus was the most frequent serologically proven viral pathogen (11 ×), followed by adenovirus (8 ×), influenza B virus (5 ×), parainfluenza virus serotype 3 (3 ×), parainfluenza virus serotype 1 and RSV (each of 1 ×). We found no serological evidence for infections with influenza C virus, parainfluenza virus of the serotype 2, *M. pneumoniae* or *C. burnetii*.

Table 1. Multiple viral respiratory tract infections in seven patients with cystic fibrosis

Influenza A virus	—15 months later—	Adenovirus	—5 months later—	Adenovirus + influenza A virus
Influenza A virus	—14 months later—	Parainfluenza virus serotype 3		
Influenza Aa virus	—13 months later—	Influenza B virus + adenovirus		
Influenza A virus	—6 months later—	Influenza A virus		
Influenza A virus	—25 months later—	Influenza A virus		
Respiratory syncytial virus	—13 months later—	Influenza B virus		
Adenovirus	—18 months later—	Influenza B virus		

There was no significant difference between the age distribution of the cystic fibrosis group with and without viral infection of the upper airways (mean age 14.4 ± 5.4 compared with 16.0 ± 5.6). Infections with parainfluenzae viruses seem to appear more frequently in younger children, whereas the prevalence of the other viral agents in the different age groups was similar to the age distribution of all cystic fibrosis patients (Table 3). There was no correlation between carriage of *P. aeruginosa* and viral infection.

Viral infections in cystic fibrosis occur during the entire year (Table 4). Infections with influenza A and B viruses are most frequent in winter and spring in contrast to parainfluenzae viruses and adenovirus which peak during the summer period.

Patients with cystic fibrosis and viral infections of the upper airways required more frequent hospital admission ($P < 0.001$) and stayed there longer ($P < 0.001$) than cystic fibrosis patients without proven serological conversion (Table 5). The greatest part of the serologically proven viral infections (21/29) occurred at home (Table 6) and was accompanied by acute exacerbations of respiratory symptoms. Taking into account an incubation period of 1–7 days for the different viral agents and a period of 10–20 days for serological conversion, 28% of the viral respiratory diseases may be hospital-acquired. Infections with parainfluenzae viruses and with RSV were seen only outside the hospital, while infections with influenza B virus and adenoviruses tended to occur in hospital. Three of the four adenovirus infections were probably nosocomial. Two of the three infections with influenza B virus in the hospital were seen at the same time in winter during an influenza B epidemic in Munich.

Analysing the bacterial flora of the sputum in patients with cystic fibrosis we found *S. pneumoniae* to be as common in CF-patients with respiratory viral infections (1/18) as in those without any evidence of infection (5/57). In contrast to *S. pneumoniae*, *H. influenzae* was more frequently detected in the sputum from CF-patients with viral infections of the upper airways (5/18 versus 3/57) ($P < 0.005$).

Table 2. Time course of significantly elevated antibody titers in the complement-fixation test (m = month; d = day)

Viral agent	CF-patient No.	Antibody titers in the complement-fixation test
Influenza A virus	9	$< 1/8 \frac{4m}{1/32} \frac{8d}{1/64} \frac{1m}{1/16} \frac{3m}{1/16} \frac{6m}{1/8}$
	10	$< 1/8 \frac{1m}{1/512} \frac{14d}{1/256} \frac{1m}{1/256} \frac{6m}{1/128} \frac{5m}{1/32} \frac{9m}{1/32} < 1/8 \frac{4m}{1/16}$
	14	$1/8 \frac{1m}{1/16} \frac{6m}{1/32} \frac{6m}{1/32} < 1/8$
	19	$1/16 \frac{14d}{1/32} \frac{5m}{1/128} \frac{1m}{1/128} \frac{1m}{1/32} \frac{1m}{1/32} \frac{10m}{1/16}$
	20	$1/8 \frac{8d}{1/64} \frac{5m}{1/16} \frac{3m}{1/32} \frac{4m}{1/8} \frac{5m}{1/8} < 1/8 \frac{3m}{1/16}$
	25	$1/64 \frac{15d}{1/64} < 1/8 \frac{6m}{1/16}$
	27	$1/64$
	38	$< 1/8 \frac{9m}{1/16} \frac{1m}{1/32} \frac{11m}{1/32} < 1/8$
Influenza B virus	1	$1/8 \frac{2m}{\text{anticompl.}} \frac{6m}{1/32} \frac{1m}{1/16} \frac{5m}{1/32}$
	4	$< 1/8 \frac{1m}{1/64} \frac{1m}{1/64} \frac{14d}{1/32} \frac{2m}{1/16}$
	13	$< 1/8 \frac{2m}{1/8} \frac{1m}{1/64} \frac{14d}{1/32} \frac{5m}{1/8} \frac{4m}{1/16} \frac{6d}{1/8} \frac{3m}{1/8} < 1/8$
	14	$< 1/8 \frac{1m}{1/16}$
	29	$< 1/8 \frac{2m}{1/16}$
Parainfluenza 1	124	$1/128 \frac{1m}{1/16}$
Parainfluenza 3	6	$< 1/8 \frac{7m}{1/16} \frac{1m}{1/16} < 1/8$
	9	$< 1/8 \frac{2m}{1/16} \frac{1m}{1/16} < 1/8$
	39	$1/32 \frac{1m}{1/8} \frac{1m}{1/8} < 1/8$
RSV	4	$< 1/8 \frac{1m}{1/16} \frac{1m}{1/16} < 1/8$
Adenovirus	8	$< 1/8 \frac{2m}{1/32} \frac{1m}{1/32} < 1/8$
	13	$1/8 \frac{3m}{1/32} \frac{2m}{1/32} \frac{2m}{1/32} \frac{1m}{1/16} \frac{1m}{1/16} \frac{5m}{1/8}$
	14	$< 1/8 \frac{1m}{1/16}$
	20	$< 1/8 \frac{3m}{1/16} \frac{2m}{1/8} \frac{3m}{1/32}$
	37	$< 1/8 \frac{2m}{1/16}$
	38	$< 1/8 \frac{1m}{1/16} \frac{11m}{1/16} < 1/8$
	44	$< 1/8 \frac{1m}{1/16} \frac{16m}{1/16} < 1/8$

Table 3. Viral respiratory tract infections (n = 29) in different age groups of patients with cystic fibrosis

Age (years)	Influenza		Parainfluenza virus		RSV	Adenovirus
	A virus	B virus	Type 3	Type 1		
0-4						
5-9	2		2	1		1
10-14	1					3
15-19	6	5	1		1	2
20-24						
25-29	2					2

Table 4. Seasonal variation of viral respiratory tract infections (n = 29) in cystic fibrosis

	Influenza		Parainfluenza virus			Adenovirus	Total Number
	A virus	B virus	Type 3	Type 1	RSV		
Winter months 12/1/2	4	3			1		7
Spring months 3/4/5	4					2	6
Summer months 6/7/8	1	1*	3			5*	10
Autumn months 9/10/11	2*	1		1		1*	5

* Double infection in one patient.

Table 5. Hospital admissions of patients with cystic fibrosis during a 24-month period

	With viral respiratory tract infections	Without viral respiratory tract infections	<i>P</i>
Number of admissions, $\bar{x} \pm s$	3.2 ± 2.7	1.1 ± 1.3	<0.001
Range	0-10	0-5	
Duration of stay (days), $\bar{x} \pm s$	90.6 ± 99.6	20.6 ± 31.1	<0.001
Range	0-333	0-132	

Deterioration of the clinical condition with pronounced cough, increased expectoration, loss of weight, reduction of lung function was observed in 78% of the cystic fibrosis patients during their viral infection (Table 7).

The microbial flora of the sputum of the cystic fibrosis patients was unchanged, if the ratio of the number of colony forming units (cfus) per millilitre of the different bacterial or fungal species in the sputum after the viral infection to the number of cfus/per millilitre prior to their onset was smaller than 3.0 or greater than 0.33. At least a three-

Table 6. Clinical features of viral respiratory tract infections ($n = 29$) in patients with cystic fibrosis ($n = 18$)

	Influenza		Parainfluenza virus		RSV	Adenovirus	Total number
	A virus	B virus	Serotype 3	Serotype 1			
Leading to hospital admission	6	1*	3	1	1	2*	14
Occurrence during hospital stay	1	3				4	8
Occurrence and treatment at home	4*	1				2*	7

* Double infection in one patient.

Table 7. Change of the microbial flora of sputum and clinical condition of cystic fibrosis patients with viral respiratory tract infections ($n = 27$ episodes)

	Clinical deterioration		No clinical deterioration	
	Number	% of total	Number	% of total
No significant change of the microbial flora of sputum	2	7		
Significant increase of:				
<i>P. aeruginosa</i>	13	48	6	22
<i>H. influenzae</i>	2	7		
<i>S. aureus</i>	1	4		
<i>P. aeruginosa</i> and <i>S. aureus</i>	1	4		
<i>P. aeruginosa</i> and <i>S. aureus</i> and <i>H. influenzae</i>	2	7		
Total	21	78	6	22

fold increase of cfus per millilitre of sputum was found in 25/27 (93%) viral infections, mainly by *P. aeruginosa* (22/27; 82%) even in those cases without clinical deterioration (Table 7).

In two cases, as well as an increase of the number of cfus per millilitre of *P. aeruginosa*, *S. aureus* and *H. influenzae* appeared in the sputum, whereas in two other patients the only significant change was the detection of *H. influenzae* or *S. aureus*. In another case there was colonisation by a penicillin resistant strain of *S. aureus*. Where either *S. aureus* or *H. influenzae* was isolated from the sputum during the viral infections, this was most likely just prior to the acute exacerbation.

The isolation rates of fungi in sputum samples during these acute exacerbations did not parallel the viral infection or the acute exacerbation.

In 21/27 serologically evident viral diseases there was enough sputum to determine anti-*P. aeruginosa* lipopolysaccharide antibodies in paired sputum samples. O-Specific sIgA-antibodies showed an increase in 13 of the 21 (62%) viral respiratory tract infections. An increase of sIgA-antibodies in sputum was seen in both double infections in 2/2 infections due to parainfluenza virus serotype 3, in 4/5 (80%) infections with adenovirus, 2/4 (50%) infections with influenza B virus, and 3/7 (43%) with influenza A virus.

Discussion

Evaluation of the serum antibody titers against different viral and bacterial respiratory tract pathogens revealed 29 viral infections in 18 of the 75 patients with cystic fibrosis. This frequency does not completely reflect the real incidence of respiratory tract infections in these patients as not all the required sera were available. Assays were not performed for antibodies against rhinoviruses, coronavirus, echovirus, *Chlamydia trachomatis* and *Legionella pneumophila*. The purpose of the study was to show whether there is "microbial synergism"¹² between the viruses causing respiratory tract infections and the bacterial microflora of the sputa. We found strong evidence for microbial synergism as significant changes (at least three-fold increases of the cfus per millilitre of sputum) of the bacterial sputum flora occurred in 93% and a clinical deterioration in 78% of patients with viral illnesses. These infections may have decreased mucociliary clearance, damaged the respiratory epithelium and suppressed other host defence mechanisms facilitating bacterial adherence and multiplication. *Pseudomonas aeruginosa*, with its known ability to adhere to damaged epithelial cells¹³, accounted for the greatest part (82%) of the acute bronchopulmonary exacerbations. This was reflected by the increase of sputum sIgA-antibodies directed against the O-antigens of *P. aeruginosa* at the same time. Even in those cases without apparent deterioration of the clinical condition (22%) the number of cfus of *P. aeruginosa* per millilitre of sputum increased significantly. There were also cases where *S. aureus* and/or *H. influenzae* were additional pathogens in the sputum without change in the number of cfus of *P. aeruginosa*. This increase of colonisation with *S. aureus* or *H. influenzae* secondary to viral infection agrees with the work by Eichenwald *et al.*¹⁴. These authors found a correlation between adenoviral infection of the upper airways in newborn infants and bacterial colonisation of the nasopharynx with *S. aureus*, *H. influenzae*, *S. pneumoniae* and β -hemolytic streptococci.

In the treatment of acute respiratory infection in cystic fibrosis, antibiotics effective against *S. aureus* and *H. influenzae* should be considered. Quantitative bacteriological examination of the sputum with the testing of the antibiotic susceptibility of the bacterial isolates is also necessary to optimize antibacterial therapy. In contrast to chronic obstructive pulmonary disease, *S. pneumoniae* was not found. As the detection of *S. pneumoniae* in a sputum of a patient with cystic fibrosis is rather difficult, even with a selective procedure to inhibit the growth of *P. aeruginosa*¹⁰, serological studies are in progress to determine the prevalence of *S. pneumoniae* in these acute exacerbations. We found no evidence of significant changes concerning the number of cfus for *Candida albicans* or *Aspergillus fumigatus* in the sputum after viral respiratory illnesses, although *C. albicans* was the second most frequent microorganism. There were high antibody titers against *C. albicans*¹⁵. The role for respiratory viral or bacterial (mycoplasma, *C. trachomatis*, *L. pneumophila*) infections in the establishment of colonization with

P. aeruginosa in cystic fibrosis is still unclear. In one six-year-old girl *P. aeruginosa* could be detected for the first time from a nasopharyngeal swab after an infection with parainfluenza virus type 1. Another patient intermittently colonized with *P. aeruginosa* harboured *P. aeruginosa* in her bronchial system after an infection with influenza A virus. After disappearing from the sputum, *P. aeruginosa* reappeared after a second influenza A virus infection.

In contrast to Petersen *et al.*⁵ who emphasised the importance of infections due to RSV we found the highest incidence to be influenza A virus followed by adenovirus. This predominant role of influenza A virus may be associated with a higher level of respiratory-tract proteases in cystic fibrosis, including proteolytic cleavage of influenza virus hemagglutinin which is required for the penetration of the virus into the epithelial cell¹⁶. The low rate of significantly increasing O-specific sIgA-antibodies after infections with influenza A virus in contrast to the other viral agents might be related to a suppression of the humoral immune system by this virus¹².

Taking the incubation period and the time required for seroconversion into account, about one-third of the serologically evident viral infections seemed to be nosocomial infections. As we have not determined serotype-specific antibodies against adenovirus we could not prove whether the three infections caused by adenovirus were due to cross infection. Donati *et al.*¹⁷ demonstrated that home therapy for cystic fibrosis patients with pulmonary exacerbations is as effective as hospital therapy. The possibility that patients with cystic fibrosis acquire viral respiratory illnesses in hospital emphasises the importance of home therapy.

The results of this study show clearly the importance of viral respiratory tract infections in cystic fibrosis. All cystic fibrosis patients should be vaccinated against influenza. Chemoprophylaxis with amantadine¹⁸ or treatment with ribavirin¹⁹ should be considered. Future developments should include the diagnosis of respiratory viral illnesses as well as the production of vaccines against adenovirus, parainfluenza virus and RSV²⁰. Studies are in progress to elucidate the role of viral or other bacterial infections for the establishment of primary colonisation with *P. aeruginosa* in the bronchial system of patients with cystic fibrosis.

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