CHAPTER 1

Viral, Fungal and Atypical Bacterial Infections in Cystic Fibrosis

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Summary. The role of respiratory viral and fungal agents in cystic fibrosis (CF) was investigated. Among the serologically proven viral infections, adenovirus was the most frequent (53%), followed by parainfluenza (24%) and RSV (20%); 54% of the episodes lead to hospital admission. A significant change in microbial flora of respiratory secretions was seen in 88% of the episodes, mainly with *P. aeruginosa*, and 65% of cases with initial colonization with *P. aeruginosa* are associated with a viral respiratory tract infection. It is hypothesised that viral respiratory infections to a predamaged lung in CF predispose to colonization with *P. aeruginosa*. Many of the CF-patients show elevated serum IgG to *C. albicans*, and therefore, *C. albicans* should not be considered a harmless colonizing mold. The prevalence of *A. fumigatus* increases with age. Of the CF-patients with *A. fumigatus*, 73% showed elevated specific serum IgG to this fungus. No correlation was found between culture, specified IgG to *A. fumigatus* and either specific Aspergillus IgE or total serum IgE. Maintaining good pulmonary condition and reduction of exposure to *A. fumigatus* may prevent colonization and infection with this mold.

Introduction

The clinical course of patients with cystic fibrosis (CF) is characterized by progressive pulmonary dysfunction, resulting in respiratory failure and death. However, the time of onset and the course of the lung disease in the individual patient are highly variable. Typically, acute exacerbations of the pulmonary disease which contribute to further lung damage are seen in these patients. Each of these exacerbations may be associated with the isolation of high concentrations of *S. aureus* and/or *H. influenzae* in the respiratory tree in the youngest CF-patients, and of *P. aeruginosa* in older CF-populations. One third of these exacerbations are thought to be induced by viral respiratory tract pathogens [1]. As well as the main bacterial pathogens *P. aeruginosa* and *S. aureus*, various bacterial and fungal species may occasionally be recovered from respiratory secretions of CF-patients. In this study the role of non-bacterial respiratory tract infections in CF-patients is evaluated, especially

those induced by viral and fungal pathogens. Further, the role of occasionally isolated microorganisms will be estimated.

Methods

Viral Respiratory Tract Infections

1289 serum samples from 276 patients with laboratory and clinical signs of cystic fibrosis (139 male, 137 female; age 0.3–34 years, mean age 15.6 ± 7.2 years) were collected during the study period (from November 1984 until November 1991). 902 serum samples were taken from 258 CF-patients, outpatients at the Dr. von Haunersches Kinderspital München.

Of the 276 CF-patients, 161 (58.3%) had been chronically (>6 months continuously) and 19 (6.9%) intermittently colonized with *P. aeruginosa* at their entry into this study. Of the 276 CF-patients, 96 (34.8%) had not been colonized with *P. aeruginosa* before. Of 387 serum samples collected during a hospital stay, 332 were from 110 CF-patients who had been admitted to the hospital because of pulmonary exacerbations (overall, 243 admissions). Of the 110 patients, 70 were chronically colonized, 6 intermittently and 34 not colonized with *P. aeruginosa* at the start of the study. Pulmonary exacerbations were defined as an acute change in health associated with increasing cough, sputum production, dyspnea, hemoptysis or fever. Antibodies to influenza A, influenza B, influenza C, parainfluenza types 1 to 3, and respiratory syncytial virus (RSV) were detected by complement fixation. Antibodies to adenovirus were measured by an IgG-ELISA using purified hexon antigen [2]. Some serum samples were tested in purified IgM-fractions for antibodies to adenovirus, parainfluenza viruses and RSV by indirect immunofluorescence technique. 20% of the serum samples had been tested for antibodies to mycoplasma and *C. burnetti*, as described previously [3]. A fourfold or greater increase of antibody titer between paired serum samples in the complement fixation, or a threefold or greater increase of U/1 in the adeno IgG-ELISA were considered to indicate a recent infection, as well as titers $\geq 1:32$ for influenza A, B and RSV, $\geq 1:16$ for parainfluenza and influenza C [4], a positive IgM-immunofluorescence test and ≥ 80 U/1 in the adeno-IgG-ELISA in single serum samples.

Standardized quantitative microbiological analyses of sputum or throat swabs [5] were performed at the same time as serology.

Fungal and Atypical Bacterial Infections in Patients with Cystic Fibrosis

For consideration of the role of fungal and atypical bacterial infections in patients with cystic fibrosis, the data of 3500 microbiological samples

(sputum samples and throat swabs) of 386 CF-patients (199 female, 187 male) were analyzed over a period of 8 years (November 1984 to November 1992). The mean age of the patients was 10.6 ± 7.8 years (range 0.2–33.5 years). The microbiological analyses were performed by standardized procedures [5]. Cultures for mycoplasma, chlamydiae, typical and atypical mycobacteria were not performed.

Serum IgG-antibodies to *Candida albicans* and *Aspergillus fumigatus* were determined as described previously (6).

Results

Viral Respiratory Tract Infections

In 116 of 276 CF-patients 168 viral respiratory infectious episodes (mean 1.4 infections per patient, range 1–5) were detected, by serological methods. 29 of the 168 (17%) episodes were simultaneous infections with more than one viral agent (Table 1). Infections caused by adenoviruses were the most frequent (89/168; 53%), followed by RSV (34/168; 20%), influenza A (25/168; 15%), parainfluenza type 2 (20/168; 12%), parainfluenza type 3 (14/168; 8%), influenza B (11/168; 7%) and parainfluenza type 1 (7/168; 4%). Deterioration of the pulmonary condition occurred in 144/168 (86%) episodes leading in 78/144 (54%) cases to hospital admission. Infections with RSV, adenovirus and parainfluenza type 2 tended to be more severe than episodes caused by other agents, leading more frequently to hospital admission (Table 2). Ten episodes (adenovirus 4, influenza B 3, RSV once, influenza A once, adenovirus + influenza B + RSV once) were probably nosocomial.

The serum samples tested for antibodies against respiratory viral pathogens were collected regularly during the four seasons of the year

Table 1. Simultaneous infections with more than one viral agent (n = 29) in patients with cystic fibrosis

Serological diagnosis	No. of patients
Adenovirus + RSV	6
Adenovirus + influenza A	5
Adenovirus + parainfluenza type 2	3
RSV + parainfluenza type 3	3
Adenovirus + parainfluenza type 1	2
RES + influenza A	2
RSV + parainfluenza type 2	2
RSV + parainfluenza type 1	1
Adenovirus + influenza B	1
Influenza A + influenza B	1
Adenovirus + RSV + influenza B	1
Adenovirus + RSV + parainfluenza type 1	1
Adenovirus + influenza A + parainfluenza type 1	1

Table 2. Clinical features of viral respiratory tract infections in patients with cystic fibrosis

Virus	Leading to hospital admission	Nosocomial infection	Occurrence and treatment at home	No. of infections
Adenovirus	41	5	43	89
RSV	19	2 ¹	14	34
Influenza A	11	1 ¹	14	25
Parainfluenza type 2	11		9	20
Parainfluenza type 3	7	7		14
Influenza B	2	4 ²	6	11
Parainfluenza type 1	2		5	7

¹One patient with RSV infection and one patient with influenza A, treated at home after discharge from the ward.

²One patient with influenza B, readmitted to the hospital.

(Table 3). Infections with adenovirus, RSV, and parainfluenza occurred throughout the year whereas influenza A and B were more frequent in winter and spring time (Table 3). Taking into account the combined viral infections, the incidence of respiratory infectious episodes was highest in winter (44 episodes/304 serum samples being tested; 14.4%), followed by spring (50/372; 13.4%), fall (37/304; 12.2%) and summer (37/309; 11.9%).

Infections in preschool CF-patients were most frequently caused by adenovirus (39%), followed by RSV (33%) and parainfluenza types 1 to 3 (27%). The prevalence of influenza A and B is higher in the second and third decade of life, whereas infections by RSV and parainfluenza become less frequent (Table 4).

63% (15/24) of the infectious episodes without clinical deterioration and 78% (113/144) of the infections with acute pulmonary exacerbation

Table 3. Seasonal variation of viral respiratory tract infections in patients with cystic fibrosis

	Winter months 12/1/2	Spring months 3/4/5	Summer months 6/7/8	Fall months 9/10/11
Serum samples tested	304	372	309	304
Serological diagnosis				
Adenovirus	20	28	20	21
RSV	9	9	7	9
Influenza A	9	11	3	2
Parainfluenza type 2	5	3	6	6
Parainfluenza type 3	4	1	4	5
Influenza B	5	4	1	1
Parainfluenza type 1	4		1	2
Total number of infections	56	56	42	46
% of serum samples tested	18.4	15.1	13.6	15.1

Table 4. Viral etiology of respiratory tract infections in different age groups of patients with cystic fibrosis

	Age (years)					
	<1	1–2	3–5	6–9	10–19	≥20
Serum samples tested	14	41	88	186	588	372
Serological diagnosis						
Adenovirus	1	2	10	23	32	21
RSV	1	2	8	3	16	4
Influenza A				3	15	7
Parainfluenza type 2	1	1	3	4	6	5
Parainfluenza type 3			2	3	8	1
Influenza B				1	10	
Parainfluenza type 1			2	2	2	1
Total number of infections	3	5	25	39	89	39
% of serum samples tested	21.4	12.2	28.4	21.10	15.0	10.5

were evaluable, as microbiological cultures had been performed at the same time as the serology and the infection did not occur at the beginning of the study. An increase of a bacterial or fungal pathogens in the sputum of CF-patients was considered to be significant if the ratio of the number of colony-forming units (CFUs) per millilitre in the sputum before and after the viral infection was greater than 1:3. A significant change in the microbial flora was found in 88% (99/113) of the infections with pulmonary exacerbation compared with 73% (11/15) of the infections without pulmonary deterioration (Table 5). These changes were mainly caused by *P. aeruginosa* (74/128; 58%), followed by *C. albicans* (38/128; 30%), *S. aureus* (21/128; 16%), *A. fumigatus*

Table 5. Sputum or nasopharyngeal flora and clinical condition of CF-patients with viral respiratory tract infections (n = 168 episodes; 128 microbiologically evaluable)

	Pulmonary exacerbation no. of episodes	No pulmonary exacerbation no. of episodes
No significant change of the microbial flora	14	4
Significant increase of		
<i>P. aeruginosa</i>	67	7
<i>S. aureus</i>	19	2
<i>X. maltophilia</i>	12	
Enterobacteriaceae	6	3
<i>H. influenzae</i> and <i>parainfluenzae</i>	5	1
coagulase-negative staphylococci	5	
Others (<i>S. pneumoniae</i> twice; <i>E. faecalis</i> , <i>A. denitrificans</i> , <i>P. fluorescens</i> , <i>P. vesiculare</i> each once)	5	1
<i>C. albicans</i>	37	1
<i>A. fumigatus</i>	14	

(14/128; 11%) and *X. maltophilia* (12/128; 9%). The proportion of proven viral respiratory tract infections with significant changes of the microbial flora did not differ for the viral agents investigated.

17 of 74 episodes with a significant increase of *P. aeruginosa* were due to the first isolation of *P. aeruginosa* associated with a viral respiratory tract infection (adenovirus 6, parainfluenza 4, RSV 2, RSV + parainfluenza type-3 2, adenovirus + RSV once, adenovirus + parainfluenza type-1 once, adenovirus + RSV + parainfluenza type-1 once). These patients were 12.1 ± 7.9 years (range 0.6–25.1 years) old at the onset of initial colonization with *P. aeruginosa*, which was also the onset of chronic colonization in 6/17 (35%) patients.

Nine CF-patients, who had not been colonized with *P. aeruginosa* at the beginning of the study but became initially colonized during the observation period, did not show elevated antibody titers against the viruses investigated. Thus, 65.4% (17/26) of cases with initial colonization with *P. aeruginosa* in cystic fibrosis seem to be associated with viral respiratory tract infections.

56/60 (93%) CF-patients, who became initially colonized with *P. aeruginosa* between 1984 and 1992 (independently of whether they were tested for antiviral antibodies or not) had the onset of their initial colonization in winter, spring or fall and only 4/60 (7%) during the summer. The onset of chronic colonization shows the same seasonal variation (Figure 1).

Figure 1. Month of onset of initial/chronic colonization with *P. aeruginosa* in patients with cystic fibrosis from 1984 to 1992.

Fungal and Atypical Bacterial Infections in Patients with Cystic Fibrosis

Overall, 72 different bacterial and fungal species were identified in the 3500 samples of 386 CF-patients investigated. The dominating species with a prevalence higher than 5% of patients or an overall mean isolation rate of more than 1% were *P. aeruginosa* (72.8% of the patients; 69.9% of the samples), *C. albicans* (77.9%; 46.8%), *S. aureus* (49.5%; 18.3%), *Aspergillus* spp. (33.4%; 14.1%), *X. maltophilia* (24.4%; 11.8%), *E. coli* (16.8%; 4.4%), *H. influenzae* (14.8%; 2.3%), *E. cloacae* (9.1%; 1.7%), *K. oxytoca* (6.7%; 1.2%), *S. pneumoniae* (6.5%; 0.8%), *S. marcescens* (4.9%; 1.1%), and *B. cepacia* (3.4; 1.8%).

The role of various species in the respiratory secretions of CF-patients may be determined by considering the persistence of these species or the immune response of the patients to these microbial pathogens. The persistence of a species was defined as the ratio of the number of isolates which were recovered in consecutive samples and of the number of all isolates of that species minus the number of isolates recovered only from the last sample examined of the patients. The persistence was most extended for *P. aeruginosa* (95.1%), followed by *C. albicans* (93.8%), *X. maltophilia* (87.5%), *B. cepacia* (86.9%), *A. fumigatus* (77.1%), *S. aureus* (75.6%), *Acinetobacter* spp. (65.6%), *M. catarrhalis* (60%) and enteric bacilli (54.9%). Other species including *Haemophilus* spp., *S. pneumoniae* and other glucose non-fermenting bacilli were intermittently isolated. The serologic response to *C. albicans* in CF-patients has been reported previously [6]. 28/36 (78%) of CF-patients (mean age 16.9 ± 4.3 years) from whom *C. albicans* was recovered in respiratory secretions and each of six CF-patients (mean age 14.8 ± 6.4 years) with repeatedly negative cultures for *C. albicans* showed significantly increased serum IgG to *C. albicans*. In 17 of 22 (77%) patients with more than one serum sample tested significant rises of the specific IgG could be observed concurrently to the isolation of *C. albicans*.

The prevalence of *A. fumigatus* in sputum of CF-patients increases with age from 13% in the first decade to 22% in the second, 25% in the third, and 45% in the fourth decade of life. CF-patients colonized with *P. aeruginosa* in the respiratory secretions are not more frequently colonized by *A. fumigatus* than patients without *P. aeruginosa*. 73% (40/55) of the CF-patients with cultures positive for *A. fumigatus* (mean age 16.3 ± 6.7 years, range 5–33 years) who were tested for specific serum IgG to *A. fumigatus* showed elevated IgG to *A. fumigatus*, whereas only 40% (14/35) of CF-patients without *A. fumigatus* (mean age 16.1 ± 5.9 years, range 4–28 years) and 30% (3/10) of healthy young adults were seropositive. There was no correlation between specific *Aspergillus* IgG and either specific *Aspergillus* IgE or total serum IgE level. This was true in all CF-patients investigated and of those CF-patients from whom *A. fumigatus* could be isolated. In addi-

tion, no correlation was found between the presence or absence of *A. fumigatus* and Aspergillus-specific IgE.

Discussion

Determination of antibodies to different viral respiratory tract pathogens revealed 168 infectious episodes mainly caused by adenoviruses (53%), parainfluenza viruses types 1 to 3 (24%) and RSV (20%) in the CF-patients investigated. Viral cultures and antigen detection procedures from respiratory secretions were omitted, together with assays for antibodies to rhinoviruses, coronavirus, echovirus and *Legionella* species. Furthermore, serum samples were not collected during each acute episode of upper respiratory tract disease. The real incidence of viral respiratory tract infections therefore, in CF-patients, is not reflected by these data. However, the effect of a proven viral respiratory tract infection for the further clinical and microbiological course of the patients can be evaluated. The consequences of viral infections are more severe in CF-patients than in healthy individuals: deterioration of pulmonary condition occurred in 86% of the episodes. A respiratory virus was serologically identified in association with 32% (78/243) of the admissions to hospital brought about by major respiratory exacerbations. These data are comparable with those previously published [7, 8]. Some of the admissions associated with viral disease of the upper respiratory tract were the first required by CF-patients. The detection of ten probable nosocomial infections reveals the importance of rapid diagnosis of viral respiratory disease and of measures to reduce cross-infections. In contrast to other studies [9, 10], the rate of proven viral infections in association with an admission to hospital due to pulmonary deterioration was not significantly different in CF-patients whether not colonized (41%; 19/46); intermittently colonized (38%; 6/16) or chronically colonized with *P. aeruginosa* (29%; 53/181).

A viral-bacterial synergism may be responsible for the onset of colonization with *P. aeruginosa*. Nearly two thirds of patients who became colonized with *P. aeruginosa* had a serologically proven single or mixed viral respiratory tract infection associated with the first isolation of *P. aeruginosa*. These illnesses were caused by adenoviruses (53%; 9/17), parainfluenza types 1 to 3 (47%; 8/17) and RSV (35%; 6/17). The point-prevalence for *P. aeruginosa* increases with age in the first two decades of life in our CF-population: 21% (0–2 years), 58% (3–5 years), 49% (6–9 years), 68% (10–15 years), and 89% (16–19 years). These data show that many patients become colonized with *P. aeruginosa* after the age of 10 years, corresponding to the mean age of CF-patients becoming initially colonized with *P. aeruginosa* in association with a proven viral infection. The high frequency of respiratory

tract infections as documented in young non-CF infants and preschool-children and the still increasing prevalence of *P. aeruginosa* at school age in CF leads to the hypothesis that the extent of lung damage prior to the viral infection predisposes to initial colonization with *P. aeruginosa*. The seasonal variation of the onset of initial and chronic colonization is pronounced, confirming the data from Denmark [11].

CF-patients already intermittently or chronically infected with *P. aeruginosa* at the time of the proven viral infection developed a significant rise of *P. aeruginosa* CFUs per millilitre of sputum in 52% of illnesses (57/109 episodes microbiologically evaluable). Considering all significant rises in CFUs/ml of *P. aeruginosa* in patients chronically or intermittently colonized, 46% (57/123) of episodes were associated with a serologically proven respiratory infection. Therefore, even in patients intermittently or chronically infected with *P. aeruginosa*, a viral–bacterial synergism may be assumed.

Evaluating the role of other bacterial species recovered from respiratory secretions during viral illnesses, *S. aureus* plays a dominant role in CF-patients still not colonized by *P. aeruginosa* (26%; 9/35 episodes microbiologically evaluable) in contrast to CF-patients already colonized with this species (11%; 12/109 episodes microbiologically evaluable; $\chi^2 = 4.599$; $p = 0.05$). This may be explained by a bacterial antagonism between *P. aeruginosa* and *S. aureus*.

The high isolation rate and persistence of *C. albicans* in patients with cystic fibrosis as well as the pronounced seroprevalence and the frequency of significant rises of specific IgG concurrently to the isolation of this fungus reflects the clinical role of *C. albicans* in cystic fibrosis, underestimated in the past.

The inhalation of *A. fumigatus*, can lead to bronchial asthma, allergic bronchopulmonary aspergillosis (ABPA), invasive aspergillosis and aspergilloma, alone or in combination with ABPA. CF-patients with *A. fumigatus* in their respiratory tree showed significantly higher specific IgG to *A. fumigatus* than CF-patients of comparable age without this fungus. 86% (24/28) CF-patients with repeatedly positive cultures (≥ 3 isolations in consecutive samples) were seropositive in contrast to 59% (16/27) with less frequent isolations ($\chi^2 = 4.850$, $p < 0.05$). The highest specific IgG to *A. fumigatus* was seen in a female patient with ABPA and aspergilloma. *A. fumigatus* is an ubiquitous fungus to which all lungs are constantly exposed. The increase of prevalence with age may be explained by progressive pulmonary damage leading to impaired mucosal defence and ineffective removal of the spores from the airways. The consequence in CF-patients is a pronounced immune response to *A. fumigatus* which may lead to further lung damage eg. by antibody-dependent cellular cytotoxicity. In agreement with previous studies [12], no correlation was found between specific Aspergillus IgG and either specific Aspergillus IgE or total serum IgE level. By multiple step

regression analysis we found a negative effect of the level of specific IgE to *A. fumigatus* on FVC, FEV₁, MEF₅₀, MEF₂₅, and sGAW, and an additional effect of the specific IgG to *A. fumigatus* on MEF₅₀ and MEF₂₅. In contrast to the effect of specific IgE, the consequences of specific IgG on lung function have been confirmed [12]. An additional fungal species *Exophiala/Wangiella dermatidis* can be isolated from 9% of German CF-patients, using long-term culture [13]; with repeated isolation this leads to elevated specific serum antibodies in 89%, indicating an invasive process rather than superficial colonization.

Only limited data are available on atypical bacteria in cystic fibrosis. Thomassen *et al.* [14] found that two of ten CF-patients had anaerobes in sputum as well as in thoracotomy specimens. However, the level of *P. aeruginosa* was comparable with that of the anaerobes suggesting that the anaerobes played only a minor role in these patients.

The culture of mycobacteria from respiratory secretions of CF-patients may be challenged by frequent contamination with *P. aeruginosa*. A decontamination technique of 0.25% N-acetyl-L-cysteine and 1% sodium hydroxide (NALC–NaOH) followed by 5% oxalic acid treatment reduces the overgrowth with *P. aeruginosa* from 74% to only 5% [15]. 1.3 to 1.8% of CF-patients seem to be infected with atypical mycobacteria and 0.3 to 1.4% with typical mycobacteria [16]. Recently a prevalence of about 20% of atypical mycobacteria in respiratory secretions from adult CF-patients has been reported [17].

Our study proves the clinical and microbial relevance of respiratory viral infections in young CF-patients not colonized with *P. aeruginosa* as well as in older CF-patients already colonized with this species. Therefore vaccination against influenza A and B should be strictly recommended to CF-patients, and all efforts made for rapid diagnosis of viral illness as well as the development of effective vaccines against adenoviruses, parainfluenza and RSV. In addition the roles of *C. albicans* and of *A. fumigatus*, which become more and more prevalent with increasing age, were estimated.

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