Infective respiratory exacerbations in young adults with cystic fibrosis: role of viruses and atypical microorganisms

E L C ONG, M E ELLIS, A K WEBB, K R NEAL, MARY DODD, E O CAUL, S BURGESS

From the Regional Department of Infectious Diseases and Tropical Medicine and the Adult Cystic Fibrosis Unit, Monsall Hospital, Manchester, and the Public Health Laboratory, Bristol

ABSTRACT Thirty six adults with cystic fibrosis were studied over one year to determine the incidence of infection with respiratory viruses and atypical organisms. Nineteen patients entered the study during an acute exacerbation of respiratory symptoms with an increase in purulent sputum production, cough, or breathlessness accompanied by a fall in FEV₁ (group 1); 17 patients entered when they were stable both clinically and in terms of lung function values (group 2). Group 1 patients had a mean of 2.6 (range 1-4) infective exacerbations during the year and group 2 patients a mean of 1.1 (0-2) exacerbations. Eleven patients developed serological evidence of viral (influenza virus A and B, cytomegalovirus, human rhinovirus 2, adenovirus) or *Mycoplasma pneumoniae* infection. There was no difference in seroconversion rates between group 1 (five patients) and group 2 (six patients). There was a weak association between viral seroconversion and the isolation of *Pseudomonas aeruginosa* from sputum, though this was not significant.

Introduction

Bacteria, most notably *Pseudomonas aeruginosa*, are considered to be the most important cause of respiratory exacerbations in patients with cystic fibrosis.^{1,2} The role of infection with viruses, *Mycoplasma pneumoniae*, *Coxiella burnetii*, *Chlamydia psittaci*, and *Legionella pneumophila* in the natural history of cystic fibrosis lung disease is unknown. The incidence of such infections has not been shown to be higher in patients with cystic fibrosis than in healthy controls.^{3,4} The consequences of these infections may be serious, however,^{4,5} and patients with cystic fibrosis are recommended to be immunised against influenza.^{6,7} Some 20% of respiratory exacerbations in cystic fibrosis may not be related to any of the common bacteria or viruses.⁸ Knowing the importance of these infective agents in causing clinical deterioration in patients with cystic fibrosis may contribute to the rational use of antibacterial treatment. Antiviral drugs such as acyclovir, amantadine, ribavirin, and ganciclovir

may be appropriate adjuncts to treatment if a specific virus were identified. A prospective study was undertaken to determine the incidence of infections with viruses and atypical microorganisms in a group of patients with cystic fibrosis with an acute respiratory exacerbation and a group who had stable respiratory disease.

Methods

We studied 36 patients with cystic fibrosis (18 of them male), mean age 23.6 (range 17-32) years, for evidence of infection with viruses, *C. psittaci*, *C. burnetii*, *L. pneumophila*, and *M. pneumoniae*. Patients who presented with symptoms of an influenza like illness were studied. Nineteen patients had an increase in purulent sputum production, cough, or breathlessness over a 48 hour period accompanied by a fall in FEV₁ of at least 15% from the value obtained when they were well one month earlier (group 1). The other 17 patients (group 2) were stable both clinically and in terms of lung function values when they entered the study. Both groups of patients were asked to contact the unit if they thought they had an infective exacerbation. We also studied 18 healthy hospital volunteers with no underlying pulmonary disease. The study started in

Address for reprint requests: Dr A K Webb, University of Manchester School of Medicine, Monsall Hospital, Manchester M10 8WR.

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October 1985 and finished in October 1986. Previous influenza vaccination was documented.

Nasopharyngeal aspirates, throat swabs, and samples of sputum and serum were collected from patients in group 1 when they presented with an exacerbation. A second serum sample was obtained two weeks later. Throat swabs and paired sera with a two week interval were tested when symptoms of an influenza like illness were reported subsequently by either group. The healthy volunteers provided a throat swab and a single serum specimen at the beginning of the study. Antibodies against influenza viruses A and B, respiratory syncytial virus, adenovirus, cytomegalovirus, measles virus, mumps virus S and V, *C psittaci*, *C burnetii*, and *M pneumoniae* were determined by complement fixation. An indirect fluorescent antibody test was used to detect antibodies to *L pneumophila*. Significant seroconversion was considered to have occurred when there was a fourfold increase in titre between paired serum samples. IgA reactive human rhinovirus 2 was determined by an enzyme linked immunosorbent assay (ELISA). Where appropriate, *M pneumoniae* specific IgM was measured.

Four cell lines (Hep-2 cells, MRC-5 cells, rolled monkey cells, primary human embryonic kidney cells) were used for isolation of viruses. Nasopharyngeal aspirates were processed and stained with monoclonal antibodies against influenza viruses A and B and respiratory syncytial virus.⁹ Adenovirus was detected by the direct immunofluorescence technique. In addition, aspirates were examined for parainfluenza viruses 1 and 3, cytomegalovirus, and measles virus by the indirect immunofluorescence technique.

The χ^2 test was used for statistical analysis.

Results

Patients in group 1 attended on 73 (mean 3.8, range 2-9) occasions compared with 19 for group 2 patients (mean 1.1, range 1-2). Group 1 patients had a mean 2.6 (range 1-4) exacerbations and group 2 a mean 1.1 (0-2). The mean (SEM) FEV₁ was 1.18 (0.12) l for group 1 and 2.13 (0.18) l for group 2 ($p < 0.001$); the FVC was 2.46 (0.24) l for group 1 and 3.10 (0.19) l in group 2 ($p < 0.01$).

Seroconversion occurred in five patients of group 1 and in six of group 2. A single serum sample from the 18 healthy volunteers had a measles antibody titre (1/512) suggesting recent infection. Human rhinovirus 2, influenza virus A and B, cytomegalovirus, *M pneumoniae*, and adenovirus were identified by serology in both groups of patients with cystic fibrosis (table 1). Coxsackie virus B5 was isolated from the throat swab of one patient in group 2 and the herpes simplex virus from a healthy volunteer. There was no significant difference in viral seroconversion rates

Table 1 Infective agents identified

	n	Method of identification		
		Complement fixation	ELISA	Cell culture
GROUP 1				
Influenza virus B	1	<1/8-1/192		
Cytomegalovirus	1	<1/20-1/512		
<i>Mycoplasma pneumoniae</i>	1	<1/16-1/64		
Influenza virus A	1	<1/16-1/512		
Rhinovirus 2	1		Positive	
Total	5			
GROUP 2				
Adenovirus	1	<1/16-1/64		
Influenza virus A	1	<1/20-1/192		
Rhinovirus 2	4*		Positive	
Coxsackie virus B5	1			Positive
Total	7			
HEALTHY VOLUNTEERS				
Measles virus	1†	1/512		
Herpes simplex virus	1			Positive
Total	2			

*Two patients had a single serum tested.

†A single serum was tested.

between groups 1 and 2. The principal bacterial pathogens in sputum from both groups were *P aeruginosa*, *P cepacia*, *Haemophilus influenzae*, and *Staphylococcus aureus* (table 2). Ten of the 28 patients with *Pseudomonas* species isolated from their sputum had evidence of viral seroconversion, compared with only one of the patients with *S aureus*, suggesting a weak association between viral seroconversion rate and pseudomonas infection ($p < 0.10$).

No demonstrable titres of *L pneumophila* antibody were found and immunofluorescent studies of the nasopharyngeal secretions were negative both in patients with cystic fibrosis and in controls.

Three patients with cystic fibrosis had influenza vaccination less than a year before entry to the study period but none had detectable antibodies by the

Table 2 Seroconversion rates and principal bacterial pathogens in sputum of patients with cystic fibrosis in the patients of groups 1 and 2

Bacterial pathogens	Number with pathogen		Number with seroconversion	
	Group 1	Group 2	Group 1	Group 2
<i>Pseudomonas aeruginosa</i>	10	3	1	2
<i>P cepacia</i>	4	1	2	1
<i>Haemophilus influenzae</i>	0	1	0	0
<i>Staphylococcus aureus</i>	0	1	0	1
<i>P aeruginosa</i> and <i>S aureus</i>	2	3	1	1
<i>P aeruginosa</i> and <i>H influenzae</i>	2	1	1	1
<i>P aeruginosa</i> , <i>S aureus</i> and <i>H influenzae</i>	0	2	0	0
<i>S aureus</i> and <i>H influenzae</i>	1	5	0	0
Total	19	17	5	6

complement fixation test. The two patients with significant seroconversion for influenza virus A and B had never been vaccinated.

Discussion

The number of infections with respiratory viruses and atypical microorganisms did not differ significantly in patients with cystic fibrosis between those with and those without an acute exacerbation of respiratory symptoms. This contrasts with previous studies,¹⁰⁻¹³ where significant associations between viral and atypical infections and deterioration in the pulmonary function and clinical condition were documented. The paucity of isolations of viruses in our study, however, confirms the findings of other investigators.^{11,12} The higher age group of our subjects may have contributed as virus isolation and incidence rates are inversely related to age.¹⁴ Another possibility is that an unidentified substance in the secretions in cystic fibrosis interferes with virus culture.

Eleven of the 36 patients had evidence of seroconversion for influenza virus A and B, cytomegalovirus, adenovirus, and human rhinovirus 2 and this is in line with the incidence in previous reports,^{4,8} which has ranged from 20% to 39%. Our study included human rhinovirus 2 whereas other studies did not. Four patients in group 2 and one in group 1 had a positive IgA ELISA result for human rhinovirus 2, indicating infection within the previous two months. It may reflect a lack of effect of rhinoviruses, a cause of respiratory deterioration in asthma and chronic bronchitis, on pulmonary deterioration in cystic fibrosis.

Absence of *L pneumophila* antibody titres contrasts with the findings of a previous study,¹² where 17% of patients with cystic fibrosis studied over four months had demonstrable titres of 1/32 or more.

Twenty eight patients had *Pseudomonas* species isolated from their sputum during the study. The incidence of pseudomonas isolations was higher in group 1 and only one patient without pseudomonas infection had evidence of seroconversion. This apparent relation between viral and atypical infections and pseudomonas related exacerbations supports the results of other studies.⁸

The absence of detectable antibodies to influenza virus in the three previously immunised patients may be due to the relatively insensitive complement fixation test.¹⁵ In a study of reactogenicity, immunogenicity, and the protective efficacy of influenza vaccine¹⁶ in children, including 83 with cystic fibrosis, there were five vaccine failures (less than a fourfold increase in antibody titre) in those with cystic fibrosis compared with only two in those without cystic fibrosis. More studies are needed to investigate the protective efficacy of vaccines currently used against respiratory viruses in patients with cystic fibrosis.

Development of techniques for rapid diagnosis of viral respiratory infections in patients with cystic fibrosis deserves a high priority. The serological methods used in our study provided the highest diagnostic yield but there is a delay of two weeks before significant seroconversion takes place. Immunofluorescence staining techniques with monoclonal antibodies using nasopharyngeal secretions is a method that could give rapid diagnosis of viral infections,⁹ but no positive results emerged from our study.

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