Infective agents and chronic bronchitis

C. A. C. ROSS, S. McMICHAEL, M. B. EADIE,
A. W. LEES, E. A. MURRAY, AND I. PINKERTON

From the Regional Virus Laboratory and the University Department of Infectious Diseases,
Ruchill Hospital, Glasgow, N.W.

Several claims have been made for the association of infective agents with acute exacerbations of chronic bronchitis. These include influenza A (Tyrrell, 1952; Walker, Douglas, Leckie, Pines, and Grant, 1958), influenza B (Stark, Heath, and Curwen, 1965), respiratory syncytial virus (Sommerville, 1963; Carilli, Gohd, and Gordon, 1964), and *Haemophilus influenzae* (Mulder, 1956; Brumfitt, Willoughby, and Bromley, 1957; Lees and McNaught, 1959; Glynn, 1959; Morgan and Wood, 1965). The multiplicity of these reported agents suggests that acute exacerbations of chronic bronchitis may be associated with many infections prevalent in the community at the time. In an attempt to confirm this suggestion we have studied the role of a wide range of micro-organisms in chronic bronchitis during the period 1961-5. Because of the technical difficulties of isolating respiratory viruses this was limited to a small group of 15 patients who were intensively observed over a period of two and a half years. The results reported elsewhere by Eadie, Stott, and Grist (1966) showed that rhinoviruses were isolated significantly more often from colds associated with acute exacerbations of chronic bronchitis than from colds not so associated. The present report describes the results of serological tests in these patients (group 2, below) together with those obtained in three larger groups. By complement fixation tests it was found practicable to test the sera not only against a considerable number of viruses (excluding rhinoviruses) but also against several other agents.

In our first study, paired sera collected from patients during acute exacerbations were tested for antibody responses to influenza A, B, and C, adenovirus group, parainfluenza type 1, respiratory syncytial (RS) virus, psittacosis group, Q fever, *Mycoplasma pneumoniae*, and *H. influenzae*. In the second study, a comparison was made of the persistence of high antibody levels in chronic bronchitis and in matched controls.

MATERIALS AND METHODS

The term 'chronic bronchitis' in the present studies was used in accordance with the definition of Scadding (1959), namely 'chronic or persistent productive cough, not attributable to localized disease of the lungs, bronchi, or upper respiratory tract'.

Four separate groups of patients with chronic bronchitis, two from general practice and two from hospital practice, were investigated. Table I gives particulars of each group in relation to the number of patients, age and sex distribution, and period of collection of the sera. For study no. 1, paired sera were collected during acute exacerbations from patients in groups 1, 2, and 3, the first specimen of serum being collected early in the illness and the second specimen generally 10 to 14 days later. For study no. 2, 'autumn-spring' sera (a specimen of serum in September-October of one year and again in March-April of the following year) were collected from cases of chronic bronchitis in groups 1 and 4, and from a similar number of controls not suffering from chronic bronchitis. Each case and its corresponding control were matched for sex and age ($\pm$ five years), and sera were collected from both within the

TABLE I
CHRONIC BRONCHITIS PATIENTS

Group	No. of Patients	Sex		Age-groups (yrs.)		Period of Collection	
		M	F	<40	$\geq$ 40	Paired Sera from Acute Exacerbations (Study 1)	'Autumn-Spring' Sera (Study 2)
1 (Dumfries, general practice)	60	40	20	8	52	Oct. 1961-March 1962	1961-1962
2 (Glasgow, general practice)	15	8	7	3	12	Jan. 1962-March 1964	Not done
3 (Glasgow hospital in-patients) ..	71	51	20	0	71	Oct. 1964-Nov. 1965	Not done
4 (Glasgow hospital out-patients) ..	26	20	6	2	24	Not done	1963-1964

same week. Serum from each case was tested at the same time as the corresponding control serum.

COMPLEMENT FIXATION TESTS Antigens for influenza A, B, and C, adenovirus group, and RS virus were prepared as described by Grist, Ross, Bell, and Stott (1966). For *M. pneumoniae* the method of Chanock, James, Fox, Turner, Mufson, and Hayflick (1962) was employed. *H. influenzae* antigen was prepared from cultures of a freshly isolated untypable respiratory strain of *H. influenzae* by the method of Glynn (1959), as modified by Tunevall (1953); the latter showed that antigen prepared by this method was species-specific to all strains of *H. influenzae*. Q fever and parainfluenza type 1 antigens were obtained from the Central Public Health Laboratory, Colindale, London; parainfluenza type 1 was chosen because, of the three parainfluenza type strains, it is most frequently associated with respiratory infections and shows heterotypic reactions with parainfluenza types 2 and 3 (Banatvala, Anderson, and Reiss, 1964). The optimal titre of each antigen was determined by two-dimensional titrations against human convalescent serum. Control antigens prepared from uninfected culture material were used at the same dilutions as the specific antigens.

Complement fixation tests were carried out in plastic plates with 0.1 ml. volumes each of serum dilution, complement (four 50% haemolytic doses), and antigen dilution. Dilutions of all reagents were made in barbitone-buffered saline (Bradstreet and Taylor, 1962). After standing at 4° C. overnight, the plates were kept at 37° C. for 20 minutes, and then 0.1 ml. of 2% optimally sensitized sheep cells was added to each cup. The plates were left for a further 40 minutes at 37° C. and were shaken at 15, 30, and 40 minutes and then allowed to settle at room temperature before being read. The serum titre was the dilution of serum giving 75% or greater fixation. All titres in this paper are expressed as reciprocals.

RESULTS

STUDY 1: ACUTE EXACERBATIONS OF CHRONIC BRONCHITIS The serological findings obtained in 125 acute exacerbations in patients from groups 1, 2, and 3 are shown in Table II. From some of the patients the quantity of serum obtained was inadequate for all the tests; this inadequacy was most obvious for *H. influenzae*, which was the last

test of the study. Evidence of infection as demonstrated by a fourfold or greater rising titre was obtained in 20 (16%) of the 125 exacerbations: influenza A, 7; influenza C, 4; RS virus, 4; influenza B, 2; parainfluenza 1, 2; and adenovirus group, 1. Although the greatest number of rising titres was obtained for influenza A, six of these seven patients were in group 3, and the onset of illness in all six was in February to March 1965, when there was a mild epidemic of influenza A in the Glasgow area. Similarly, the four cases with rising titres for RS infection (all in group 3) became ill between January and March 1965, when RS infection was epidemic in young children. No rising titres were obtained for psittacosis group, Q fever, *M. pneumoniae*, and *H. influenzae*.

Although a considerable number of high but not rising antibody levels (≥ 64) was obtained to one or more of the agents, these were not accepted as evidence of infection, since in some of the patients under observation for approximately two years (group 2) antibody levels remained consistently high for many months. This suggested that high titres might persist for a long time in patients with chronic bronchitis. However, as there were no controls in this study, the purpose of the next study was to compare the persistence of antibodies in cases of chronic bronchitis and in controls.

STUDY 2: PERSISTENCE OF ANTIBODIES The serological findings in the 'autumn-spring' sera of cases and controls in groups 1 and 4 combined are shown in Table III. The presence and persistence of high titres (≥ 64 in both sera) showed a significant difference between cases and controls only for *H. influenzae* (χ^2 , 0.01 $>$ $P >$ 0.001). No fourfold or greater fall in titre for *H. influenzae* was observed in any of the cases or controls during this six-month period. Although persisting high titres for influenza A and for influenza B were obtained in a considerable number of bronchitics, similar findings were also obtained in the controls.

TABLE II

SEROLOGICAL FINDINGS IN ACUTE EXACERBATIONS (GROUPS 1, 2, AND 3 COMBINED)

	Influenza A	Influenza B	Influenza C	Adeno- virus Group	Psitta- cosis Group	Q Fever	RS Virus	Para- influenza 1	<i>M. pneu- moniae</i>	<i>H. influ- enzae</i>
Paired sera examined	125	125	125	125	113	113	116	100	100	74
Rising titres . . .	7	2	4	1	0	0	4	2	0	0
Falling titres . . .	0	0	5	0	1	0	0	1	0	0
High titres . . .	18	2	5	2	1	0	13	0	2	18

TABLE III
SEROLOGICAL FINDINGS IN 'AUTUMN-SPRING' SERA

Antigen	No. of Cases or Controls Examined	High Titres (≥ 64)		Rising Titres		Falling Titres		Titres ≥ 8	
		Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Influenza A	80	12	10	3	0	1	4	62 (77.5)	56 (70.0)
B	80	3	0	5	1	0	2	50 (62.5)	46 (57.5)
C	80	7	6	4	0	0	2	66 (82.5)	57 (71.2)
Adenovirus group ..	54	0	1	1	2	0	0	30 (55.5)	28 (51.8)
Psittacosis group ..	54	0	0	0	0	1	1	10 (18.5)	8 (14.8)
Q fever	54	0	0	0	0	1	1	4 (7.4)	5 (9.2)
<i>H. influenzae</i>	46	15	3	1	1	0	0	45 (97.9)	44 (95.6)
RS virus	26	1	1	1	3	2	0	20 (76.9)	21 (80.7)

Figures in parentheses represent percentages.

Our findings that antibody for *H. influenzae* was detected (titres ≥ 8) in all but two of the controls and in all but one of the cases indicated that infection with this organism had occurred at some time in the past in the majority of both controls and cases; indeed, antibody for *H. influenzae* was much more commonly present than for any of the other agents.

DISCUSSION

In both our studies patients with chronic bronchitis showed a striking contrast in antibody responses to most of the viral agents and to *H. influenzae*.

In the first study of paired sera from patients with acute exacerbations, evidence of infection, as assessed by rising antibody titres, was obtained for several viral agents. However, no one virus was specially associated with acute exacerbations, the agents mainly involved being those responsible for the current respiratory infections in the general community. Thus it seems likely that in chronic bronchitis a wide range of viral respiratory infections can produce acute exacerbations of bronchitis in place of the more usual respiratory illnesses associated with these viruses. The absence of any rising titres to *H. influenzae* lends no support to the generally accepted view that this organism plays an important part in acute exacerbations, although it does not disprove it. It is possible that where there is chronic infection with this organism the antigenic stimulus produced by an acute exacerbation may be insufficient to produce a rise in antibody titre.

The second study, which included control cases, again showed a contrast between viral agents and *H. influenzae* in that a significantly greater number of both the 'autumn' and the 'spring' sera from the bronchitics than from the controls had high titres for *H. influenzae* but not for any of the

viral agents. This finding for *H. influenzae* is in keeping with the reports of Glynn (1959) and of Morgan and Wood (1965). Although we found that high titres for *H. influenzae* persisted for at least six months in all 15 bronchitics, the high titres in the three controls also remained unchanged. Moreover the detection of *H. influenzae* antibody (titres ≥ 8) in 96% of the controls indicates that the majority of adults over 40 years of age have been infected with this organism. This supports the general view that *H. influenzae* is not the essential initiating agent of chronic bronchitis. However, it seems possible that the damaged bronchial mucosa of bronchitics may encourage proliferation and extension of *H. influenzae* from the nasopharynx. Rosher (1939) and Straker, Bradford Hill, and Lovell (1939) have shown that in individuals both with and without respiratory disease there is at certain seasons of the year, generally the first quarter, extension of *H. influenzae* from the nasopharynx into the trachea and up into the nose. It seems likely that where there is damaged bronchial mucosa, as in patients with chronic bronchitis, infection with *H. influenzae* may be more extensive and persistent than in individuals without respiratory disease, and may result in higher antibody levels.

High but not rising titres of antibody to viruses is usually accepted as evidence of recent infection. Our observation that high titres (≥ 64) for myxoviruses such as influenza A and influenza C persisted in many control cases as well as in cases of chronic bronchitis for many months indicates that, at least in patients over 40 years of age, caution must be employed in interpreting high but not rising titres to myxoviruses as evidence of recent infection. The persistence of high antibody levels in such individuals may be a reflection of decreased efficiency of the bronchial mucosa in overcoming infection even in the absence of clinical features of chronic bronchitis.

SUMMARY

Sera collected during the period 1961-5 from four groups of patients with chronic bronchitis were examined by complement fixation for antibodies to a wide range of infective agents.

In the first study, paired sera collected during 125 acute exacerbations were examined for antibody responses to influenza A, B, and C, adenovirus group, parainfluenza type 1, respiratory syncytial virus, psittacosis group, Q fever, *M. pneumoniae*, and *H. influenzae*. Evidence of infection, as assessed by a fourfold or greater rise in antibody titre, was obtained in 16% of the exacerbations; the agents involved were all viruses and seemed to reflect the current incidence of respiratory viral infections in the general population.

In the second study, sera were collected at the beginning of the winter and again in the spring ('autumn-winter' sera) from chronic bronchitics and from control cases. High titres (≥ 64) for *H. influenzae* but not for any of the other agents were found in both 'autumn' and 'spring' sera in a significantly greater number of bronchitics than controls. Antibody for *H. influenzae* was, however, detected (titres ≥ 8) in the majority of controls as well as in bronchitics.

These studies suggest that a wide range of viral agents can produce acute exacerbations of chronic bronchitis. No serological evidence was found for the association of *H. influenzae* with either the initiation of chronic bronchitis or acute exacerbations. It seems possible that the damaged bronchial mucosa of bronchitics encourages proliferation and persistence of *H. influenzae*, resulting in high antibody titres to this organism.

We are grateful to the junior medical staff at Ruchill Hospital for the faithful collection of sera, and to Mrs. B. Thomson for technical assistance. We wish to thank Dr. G. Tunevall, Stockholm, for advice on the preparation of *H. influenzae* antigen. Dr. R. J. Fallon and Mr. Walter Brown for cultures of *H. influenzae*,

and Standards Laboratory, Central Public Health Laboratory, Colindale, for Q fever and parainfluenza type 1 antigens. The investigation was assisted by a grant from the Secretary of State for Scotland made on the recommendation of the Advisory Committee for Medical Research.

REFERENCES

- Banatvala, J. E., Anderson, T. B., and Reiss, B. B. (1964). Parainfluenza infections in the community. *Brit. med. J.*, **1**, 537.
- Bradstreet, C. M. P., and Taylor, C. E. D. (1962). Technique of complement-fixation test applicable to the diagnosis of virus diseases. *Mth. Bull. Minist. Hlth Lab. Serv.*, **21**, 96.
- Brumfitt, W., Willoughby, M. L. N., and Bromley, L. L. (1957). An evaluation of sputum examination in chronic bronchitis. *Lancet*, **2**, 1306.
- Carilli, A. D., Gohd, R. S., and Gordon, W. (1964). A virologic study of chronic bronchitis. *New Engl. J. Med.*, **270**, 123.
- Chanock, R. M., James, W. D., Fox, H. H., Turner, H. C., Mufson, M. A., and Hayflick, L. (1962). Growth of Eaton PPLO in broth and preparation of complement fixing antigen. *Proc. Soc. exp. Biol. (N.Y.)*, **110**, 884.
- Eadie, M. B., Stott, E. J., and Grist, N. R. (1966). Virological studies in chronic bronchitis. *Brit. med. J.*, in press.
- Glynn, A. A. (1959). Antibodies to *Haemophilus influenzae* in chronic bronchitis. *Ibid.*, **2**, 911.
- Grist, N. R., Ross, C. A. C., Bell, E. J., and Stott, E. J. (1966). *Diagnostic Methods in Clinical Virology*. Blackwell, Oxford.
- Lees, A. W., and McNaught, W. (1959). Bacteriology of lower respiratory-tract secretions, sputum, and upper-respiratory-tract secretions in "normals" and chronic bronchitics. *Lancet*, **2**, 1112.
- Morgan, W. K. C., and Wood, W. H. (1965). Antibody response to *Haemophilus influenzae* in obstructive airway disease. *Brit. med. J.*, **1**, 1128.
- Mulder, J. (1956). Bacteriology of bronchitis. *Proc. roy. Soc. Med.*, **49**, 773.
- Rosher, A. B. (1939). Some observations on the occurrence of *Haemophilus influenzae* in the trachea. *Reports on Public Health and Medical Subjects*, No. 90, p. 107. (Ministry of Health.)
- Scadding, J. G. (1959). Principles of definition in medicine with special reference to chronic bronchitis and emphysema. *Lancet*, **1**, 323.
- Sommerville, R. G. (1963). Respiratory syncytial virus in acute exacerbations of chronic bronchitis. *Ibid.*, **2**, 1247.
- Stark, J. E., Heath, R. B., and Curwen, M. P. (1965). Infection with influenza and parainfluenza viruses in chronic bronchitis. *Thorax*, **20**, 124.
- Straker, E., Bradford Hill, A., and Lovell, R. (1939). *H. influenzae* Nasopharyngeal carrier rates in different populations. *Reports on Public Health and Medical Subjects*, No. 90, p. 61.
- Tunevall, G. (1953). Studies on *Haemophilus influenzae*. *Acta path. scand.*, **32**, 258.
- Tyrrell, D. A. J. (1952). The pulmonary complications of influenza as seen in Sheffield in 1949. *Quart. J. Med.*, **21**, 291.
- Walker, W. C., Douglas, A. C., Leckie, W. J. H., Pines, A., and Grant, I. W. B. (1958). Respiratory complications of influenza. *Lancet*, **1**, 449.