Agglutinin response to bacterial infection in acute exacerbations of chronic bronchitis

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SYNOPSIS Agglutinin titres to *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus vulgaris* in the serum of patients with acute exacerbations of chronic bronchitis, patients producing mucoid sputum, and healthy controls were determined. Serological evidence of infection with *H. influenzae* was found in 38 of 57 patients with acute exacerbations, and *Str. pneumoniae* infection in 10 of the 57 patients, but was generally absent from healthy control subjects and from patients producing mucoid sputum. No serological evidence of infection with other organisms named above was found to be associated with exacerbations of chronic bronchitis. Ten patients with acute exacerbations were without serological evidence of infection by any of the bacteria tested.

Antibody response during chronic bronchitis was studied for the purpose of guidance in antibiotic therapy, since isolations of suspected micro-organisms do not produce rapid or reliable information. The problem has been investigated by several workers, and, despite the complex antigenic structure of *Haemophilus influenzae*, most of the earlier workers agreed that bronchitic patients had specific antibodies to this bacterium in their serum, whereas control subjects had not (Faunce, 1958; Murdoch, Leckie, Downie, Swain, and Gould, 1959; Glynn, 1959). Wilson, Dunn, and Blair (1924), studying agglutinins to *H. influenzae* in the sera of patients suffering from influenza and other respiratory diseases, found that 208 of 220 control sera gave negative results while infected patients had titres above 1/80. These titres were seen to subside rapidly in convalescence. May (1965) found that agglutinins at high titre were commonest in patients with purulent sputum and rare in normal subjects. Burns and May (1968) found precipitins to *Streptococcus pneumoniae*. May (1965) found inconsistencies in the occurrence of agglutinins and precipitins to *H. influenzae*, whereas Jenne, MacDonald, Lapinski, Bratberg, and Hall (1970) found that precipitin and passive haemagglutination titres reflected acute infections and exacerbations although

persisting antibodies were occasionally without apparent explanation. Several investigators have failed to demonstrate a change in titre of antibodies to *H. influenzae* during acute exacerbations of chronic bronchitis (Glynn, 1959; Morgan and Wood, 1965; Kohn, 1965; Ross, McMichael, Eadie, Lees, Murray, and Pinkerton, 1966). In this study the agglutinin titres to *H. influenzae*, *Str. pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus vulgaris* were assessed in chronic bronchitic patients with both mucopurulent and mucoid sputum, and in healthy controls.

Materials and Methods

SUBJECTS

The subjects under investigations were classified in three groups: 57 patients in exacerbation of chronic bronchitis with purulent sputum; 22 bronchitics with mucoid sputum; 15 healthy controls.

CULTURAL METHODS

Cultures were made on nutrient agar (Oxoid), horse-blood agar, and Fildes agar, prepared according to Cruickshank (1968), but with horse blood in place of sheep.

Sputum was digested with pancreatin buffer, by the method of Rawlins (1953), before plating, and cultures were incubated at 37°C, both aerobically and in an atmosphere with 10% added carbon

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dioxide. From healthy controls postnasal swabs were taken for culture.

SEROLOGY

Sera were stored at -20°C without preservative. The antigens, detailed in table I, were grown in broth culture at 37°C for 24 hours. Those from *H. influenzae* were prepared in accordance with the technique of May (1965). After incubation, sterility checks were made on all broth cultures by plating out a 0.1 ml sample; contaminated broths were discarded. After withdrawal of the test sample, phenol was added to the broth culture to give a final concentration of 1%, and the culture was allowed to stand overnight. Antigen was collected by centrifuging the broth at 6500 g for 40 minutes and discarding the supernatant. The deposits were collected and washed five times in 0.5% phenol saline. The final suspension in 0.5% phenol was stored at 4°C .

Before using the antigen in routine testing, a correlation between the number of organisms per ml and the opacity of Brown's tube no. 3 (Burrough's Wellcome) was derived for each antigen by a viable count technique. The optimal test concentration of each antigen was assessed by titrating each antigen against a reference rabbit serum. Each antigen was tested at five different concentrations (5×10^5 , 7.5×10^5 , 8×10^5 , 1×10^6 , and 2.5×10^6 organisms per ml) and the concentration selected for routine tests was that which represented the highest dilution of antigen suspension which gave agglutination in the presence of the highest dilution of antiserum. A known negative serum (anti-*Bacillus subtilis* serum) was employed in all cases as a control check for non-specific agglutination.

Agglutinin titres were assessed by a modification of the Widal test described by Cruickshank (1968) using disposable haemagglutination trays in place of Dreyer tubes.

Serum and sputum samples produced by this group were taken from each subject on days 1, 3, 7, 30, and 60 of study.

Results

Results of isolations are given in table II. By 'predominant', it is implied that the species in question was found in large numbers (about 50% of colonies on primary culture plates), and was not a normal member of the supposedly harmless respiratory flora. Two such bacteria did not, in our experience, occur simultaneously. *Haemophilus influenzae* was predominant in 27 of 57 subjects having exacerbations of chronic bronchitis, but in only six of 22 with mucoid sputum and four of 15 postnasal swabs from healthy persons, ie, approximately half of the first and one quarter of the two latter. *Streptococcus pneumoniae* was predominant in the sputum of eight in exacerbation, of three with mucoid sputum, and of seven, ie, half, of the controls. *Escherichia coli* and *Proteus* were predominant only in eight patients who had received ampicillin therapy before admission.

The results of titrations are given in table II, and these also demonstrate an association of *H. influenzae* with exacerbations. Regarding a significant titre as 1:125, rising titres to it, reaching a peak at about seven days after admission (figure), were found in all 27 cases in which the organism was predominant in the sputum, and also in eight exacerbations from which it could not be isolated, at that time. Of these last, four had *Str. pneumoniae*

Organism	Antigen Content	Culture Medium	Viable Count Equivalent to Brown's Tube No. 3 (Organisms per ml)	Optimal Test Concentration (Organisms per ml)
<i>Haemophilus influenzae</i>	Composite antigen containing rough strains of all six Pittman capsular types	X + V broth (May, 1968)	3.4×10^8	5×10^8
<i>Streptococcus pneumoniae</i>	Composite antigen containing types I, II, & III	Todd-Hewitt broth (Oxoid)	2.0×10^8	8×10^8
<i>Staphylococcus aureus</i>	<i>Staph. aureus</i> Oxford NCTC 6571	Nutrient broth (Oxoid)	1.3×10^8	7.5×10^8
<i>Klebsiella pneumoniae</i>	<i>K. pneumoniae</i> NCTC 9633	Nutrient broth (Oxoid)	1.0×10^8	5×10^8
<i>Pseudomonas aeruginosa</i>	Composite antigen containing Habs serotypes 3 and 6	Nutrient broth (Oxoid)	1.2×10^8	5×10^8
<i>Escherichia coli</i>	<i>E. coli</i> NCTC 8196	Nutrient broth (Oxoid)	1.0×10^8	5×10^8
<i>Proteus vulgaris</i>	<i>P. vulgaris</i> NCTC 10031	Nutrient broth (Oxoid)	1.0×10^8	5×10^8

Table I Composition of antigens

	Bronchitic Patients	Control	
		Group A	Group B
<i>H. influenzae</i>			
Isolated	27	6	4
Rising titre	24 ¹	0	0
Falling titre	9	1	1
High titre 1/125	5	0	0
<i>Str. pneumoniae</i>			
Isolated	8	3	7
Rising titre	4 ¹	0	0
Falling titre	4 ²	2	0
High titre 1/125	4	1	0
<i>Staph. aureus</i>			
Isolated	5	4	1
Rising titre	1	1	1
Falling titre	2 ³	2	3
High titre 1/125	0	8	3
<i>K. pneumoniae</i>			
Isolated	0	1	0
Rising titre	0	0	1
Falling titre	2 ⁴	2 ⁵	2
High titre 1/125	0	0	0
<i>Ps. aeruginosa</i>			
Isolated	1	1	0
Rising titre	0	0	0
Falling titre	0	0	0
High titre 1/125	0	2	0
<i>E. coli</i>			
Isolated	7	2	0
Rising titre	0	0	0
Falling titre	0	0	0
High titre 1/125	0	0	0
<i>P. vulgaris</i>			
Isolated	1	0	0
Rising titre	0	0	0
Falling titre	0	0	0
High titre 1/125	0	0	0
Patients without significant titre to any of above, from which <i>E. coli</i> , <i>Staph. aureus</i> , <i>Str. pneumoniae</i> and <i>Ps. aeruginosa</i> were isolated.		10	4

Table II Summary of predominant sputum isolates and agglutinin responses

¹Two patients had rising titres to both *H. influenzae* and *Str. pneumoniae*.²Two patients had falling titres to *Str. pneumoniae* and rising titres to *H. influenzae*.³Two patients had falling titres to *Staph. aureus* and rising titres to *H. influenzae*.⁴One patient had falling *K. pneumoniae* titre with rising *H. influenzae* titre. One patient had falling *K. pneumoniae* titre and *H. influenzae* titre. The *H. influenzae* titre was higher.⁵Both patients had high titres to *Staph. aureus*.

as the predominant species in the sputum. Rising titres to *H. influenzae* or *Str. pneumoniae* were not seen in either control group but both groups had members with falling titres, ie, from an initial level of 1:125 or above, to these organisms. In addition, one patient in control group A had a high static titre to *Str. pneumoniae*.

Fig Agglutinin titres against *Haemophilus influenzae* and *Streptococcus pneumoniae* in 38 cases of acute exacerbation of bronchitis. Interpolative graph, showing average figures, as taken on days 1, 3, 7, 30, and 60 after admission.

Discussion

It is well established that *Haemophilus influenzae* may be associated with exacerbations of chronic bronchitis (see Turk and May, 1967; Stuart-Harris, 1968, for references). The results of the present study suggest that the presence of *H. influenzae* in purulent sputum is indicative of infection, but failure to grow the organism cannot exclude its involvement. This has previously been emphasized by May (1968). Although there is a relationship between purulent sputum and infection with *H. influenzae*, little significance can be attached to the presence of this organism in mucoid sputum since it is isolated with the same frequency from the postnasal swabs of healthy persons.

The results of agglutinin titrations support the findings of sputum culture indicating that *H. influenzae* is the major pathogen in exacerbations of chronic bronchitis. Serological investigation indicated that 38 patients were suffering from exacerbations due to this infection. It is interesting that all of the 27 patients from whom *H. influenzae* was isolated were included in this total, but that in six

patients who had rising titres to it, the organism was not isolated from their sputum.

The role of *Str. pneumoniae* is less well established and its presence in the sputum of bronchitic patients may be derived from the nasopharynx during expectoration (Brumfitt, Willoughby, and Bromley, 1957; Lees and McNaught, 1959). *Str. pneumoniae* was the predominant organism in the sputum of eight patients in exacerbation and although it appears that this organism plays a secondary role to *H. influenzae* in chronic bronchitis, the frequency of its isolation from patients with mucoid sputum (three of 22) and from postnasal swabs of healthy persons (seven of 15) makes the distinction between pathogenic and commensal organisms, on the evidence of sputum culture alone, difficult. Serological evidence shows that this organism is not always responsible for an exacerbation even though it is present in the sputum, but failure to isolate it from sputum cannot exclude it as a possible pathogen, and its involvement can only be assessed by serological means. Serological evidence suggests that *Str. pneumoniae* was probably the pathogen in 12 patients. Two patients had rising titres to both *H. influenzae* and *Str. pneumoniae* and were obviously suffering an exacerbation from a mixed infection, while two patients with falling titres to *Str. pneumoniae* had rising titres to *H. influenzae* and were probably suffering sequential infection. It seems, therefore, that *Str. pneumoniae* was eliciting an active antibody response in 10 patients, five of whom yielded the organism from sputum culture.

The presence of *Escherichia coli* and *Proteus vulgaris* in the sputum of bronchitic patients is almost certainly a consequence of antibiotic therapy before admission (May, 1968). In this study all of the eight patients from whom these organisms were isolated had received ampicillin therapy before their admission to hospital. It seems probable that a similar explanation would account for the presence of increased numbers of *Staph. aureus* and *Ps. aeruginosa* in bronchitic patients with purulent or mucoid sputum. The lack of involvement of *Staph. aureus*, *K. pneumoniae*, *Ps. aeruginosa*, *E. coli*, and *P. vulgaris* in exacerbations of chronic bronchitis as judged by serological means was reported by Burns (1968) and Burns and May (1968).

The significance of agglutinin levels to *Staph. aureus* is difficult to explain. Staphylococcal infection is experienced by most of the population at some point in their lives, and raised agglutinin levels to *Staph. aureus* may be present in normal healthy persons (Towers and Gladstone, 1958; Quie and Wannamaker, 1964), although skin lesions and chronic staphylococcal infections may fail to produce a rise in antibody titre (Lack and Towers,

1962; Wilson and Miles, 1964), whereas acute illnesses, such as staphylococcal osteomyelitis, can give a sevenfold or greater increase in titre (Lack, 1957; Towers and Gladstone, 1958).

In this study 10 patients were without serological evidence of bacterial infection although they appeared to be in acute exacerbation of chronic bronchitis. These may have been caused by virus infection or purely physical disturbance, but a second possible explanation is that these patients have developed a degree of immunological tolerance towards infection by *H. influenzae* caused by constant exposure to the antigen, especially since these four patients had a symptomatic history of chronic bronchitis of not less than 18 years.

The validity of these serological diagnoses, from the viewpoint of antibiotic therapy, has been evaluated, and shows satisfactory results. This will be the subject of a separate study.

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