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Immunoglobulin G (IgG) serological response to *Branhamella catarrhalis* in patients with acute bronchopulmonary infections

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SUMMARY Over 12 months serum was collected from 45 inpatients, with acute bronchopulmonary infection, in whose sputum *Branhamella catarrhalis* predominated, or was the sole pathogen. Serum was examined for IgG against *B catarrhalis* using an immunofluorescence antibody test. Acute and convalescent sera were compared with sera of age and sex matched controls. The convalescent sera had significantly higher titres than the acute sera which in turn had higher titres than the controls. The findings confirm the role of *B catarrhalis* as a respiratory pathogen and suggest that patients with chronic pulmonary disease are more prone to infection with this organism than the general population.

Over the past 10 years *Branhamella catarrhalis* has been increasingly recognised as an important pathogen, primarily of the respiratory tract.[1,2] Although initially thought to be solely an upper respiratory tract commensal, it has subsequently been associated with a wide variety of disease—acute otitis media in children,[3] acute maxillary sinusitis, exacerbations of chronic obstructive airways disease (COAD)[4]—and less commonly with meningitis, endocarditis, conjunctivitis and bacteraemia.

Koch's postulates are as yet unfulfilled for *B catarrhalis* and definitive laboratory diagnosis must therefore depend largely on the serological response by the host to the invading pathogen. To date, serological data have been somewhat lacking concerning the role of this organism in pulmonary infection. Chapman *et al*, however, showed that most patients with pulmonary infections caused by *B catarrhalis* developed convalescent IgG antibody which had bactericidal activity, thought to be due to activation of the classical complement pathway.[5]

The purpose of this study was to determine the serological response of patients with *Branhamella* associated chest infections to *Branhamella* antigen and to compare them with an age and sex matched control group.

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Material and methods

Over 12 months macroscopically purulent sputum samples from 45 inpatients aged 28–90 years were examined for the presence of *B catarrhalis*, using a liquefaction/dilutional technique. This method consisted of liquefying purulent sputum samples by diluting them 1/5 with buffered n-acetyl cysteine. The resultant solution was shaken for 15 minutes, and then diluted to a final concentration of 1 in 5×10^5 with $\frac{1}{4}$ strength Ringer's solution. A density of $> 10^7$ colony forming units/ml, occurring as the dominant or sole pathogen, was taken to be significant. The organism was confirmed to be *B catarrhalis*, by typical Gram stain, oxidase and catalase reactions, carbohydrate fermentation and nitrate reduction, in accordance with the criteria suggested by Doern and Morse.[6] The patients with positive sputum cultures were then interviewed, clinical details recorded, and the first serum sample taken. A second serum sample was taken as near to 10 days after the first, as was practicable before the patient's discharge, and the patient's progress was then recorded.

Serum samples were examined for the presence of IgG against *Branhamella*, using an immunofluorescence antibody test (IFAT) technique. Antigen was prepared by emulsifying an equal quantity of an overnight culture of 10 different strains of *B catarrhalis* to a concentration of 10^5 cells/ml. This was heated to

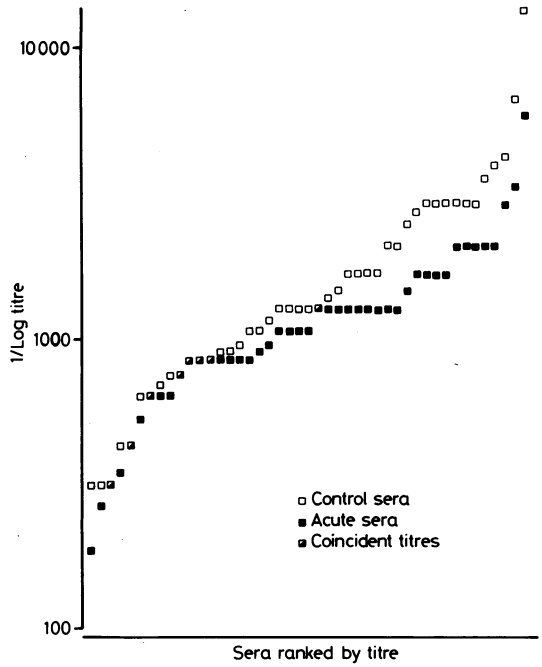

Fig 1 *Acute and control sera. Sera are not paired and are expressed as 1/log titre.*

Data for fig 1

Acute sera	*Control sera*
320	187
320	267
320	320
427	347
427	427
533	640
640	640
640	693
640	747
747	747
853	853
853	853
853	853
853	907
853	907
853	960
853	1067
907	1067
960	1173
1067	1280
1067	1280
1067	1280
1067	1280
1280	1280
1280	1387
1280	1493
1280	1707
1280	1707
1280	1707
1280	1707
1280	2133
1280	2133
1493	2560
1707	2773
1707	2987
1707	2987
1707	2987
2133	2987
2133	2987
2133	2987
2133	3627
2133	4053
2987	4267
3413	6827
5973	13653

60°C for 30 minutes and further diluted with an equal volume of phosphate buffered saline (PBS) before use. As a final pretreatment, the cells were vortex mixed for two minutes to reduce autoagglutination and to promote an even distribution of cells. Aliquots (0·01 μl) of killed cell suspension were placed on a polytetrafluoroethylene coated multislide (Hendley-Essex), which was then dried and fixed in acetone for 10 minutes. Test sera were diluted in a microtitre tray to give dilutions in the range 1/160 to 1/20480. These dilutions (0·01 μl aliquots) were then placed on the prepared slides and incubated for 30 minutes at 37°C. The slides were then washed for 10 minutes in PBS before being dried. A 1/40 dilution of sheep derived, fluorescein labelled antihuman IgG (0·01 μl aliquots) (Wellcome Diagnostics) was then placed on the slide and incubated for 30 minutes at 37°C. Treated slides were then mounted and viewed with the fluorescence microscope. Each pair of sera was prepared simultaneously, and each test carried out in triplicate, the final titre being a mean of the three readings.

A positive control serum was obtained by rabbit inoculation with *Branhamella* antigen, derived from study strains numbers 1 to 10. A killed cell suspension was prepared as above. The cells were then washed six times in PBS before being resuspended in 0·5% formalin (in PBS) and diluted to a density of 10^8 cells/ml. Antigen (0·1 ml) was inoculated intraperitoneally at weekly intervals for four weeks. Serum samples were taken before inoculation and one week after the final dose of *Branhamella* antigen. These samples were tested for IgG against *Branhamella* by the above IFAT method but using sheep derived, fluorescein labelled antirabbit immunoglobulin (Wellcome Diagnostics) in the final stage. Anti-*Branhamella* IgG titres increased from 1/5 to 1/20480.

The positive control rabbit serum was used to determine the specificity of the IgG being tested, being absorbed in turn with pneumococcal, haemophilus, and staphylococcal antigens, before retesting with *B catarrhalis* antigen. This did not result in a lowering of titre against *B catarrhalis*. We therefore concluded that there was no significant cross reactivity between *B catarrhalis* and other possible respiratory pathogens.

Results

B catarrhalis was isolated from about 5% of all

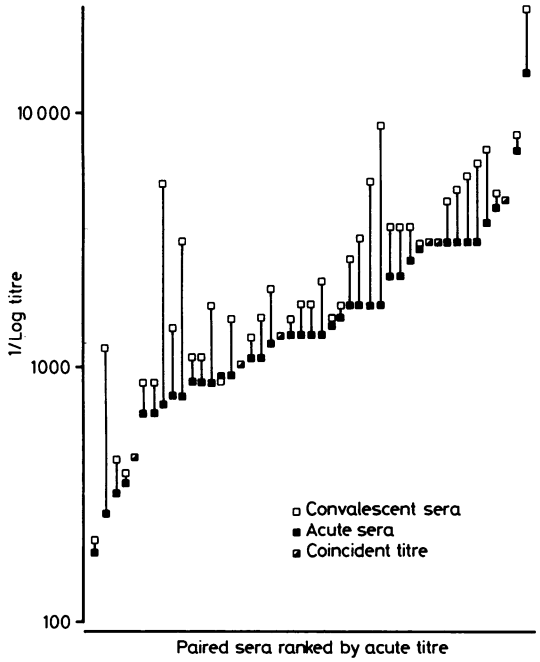

Fig 2 *Acute and convalescent sera. Sera are paired and expressed as 1/log titre.*

Data for fig 2

Acute sera	*Convalescent sera*
187	213
267	1173
320	427
347	373
427	427
640	853
640	853
693	5120
747	1387
747	2987
853	1707
853	1067
853	1067
907	853
907	1493
960	960
1067	1280
1067	1493
1173	1920
1280	1707
1280	2133
1280	1707
1280	1493
1280	1280
1387	1493
1493	1707
1707	2560
1707	8533
1707	5120
1707	2987
2133	3413
2133	3413
2560	3413
2773	2987
2987	2987
2987	2973
2987	5333
2987	4267
2987	4693
2987	5973
3627	6827
4053	4480
4267	4267
6827	7680
13653	23893

sputum samples received. A large number of patients were unsuitable, however, either because they had already been discharged from hospital or because a second serum sample was unobtainable. In total, 45 patients were entered into the study and of these, *Branhamella* occurred as the sole pathogen in 40 (89%). In four cases (9%) *Branhamella* was isolated along with *Haemophilus influenzae,* and in one case (2%) with *Streptococcus pneumoniae.*

The acute group of sera was taken from patients in the study within two days of them having developed purulent sputum, which subsequently grew *B catarrhalis.* The convalescent sera were taken between six and 30 days after the acute sera, and the control sera were drawn from patients with no history of acute or chronic pulmonary disease and matched for age and sex with each pair of test sera (figs 1 and 2). Fig 1 shows that the difference between the control and acute groups occurred at higher titres, there being little or no difference between them at lower values. Fig 2 shows that the immunological response was variable, with some patients having no increase in titre, while others had up to a seven-fold increase. Of the 45 patients in the study, four had a four-fold or greater rise in titre, and seven had a two-fold or greater rise.

The range of variance in each group was found to be too great for them to be considered as having a Gaussian distribution and therefore a non-parametric statistical analysis was used (Wilcoxon's rank sum test). Using this method, the acute sera had higher titres of IgG against *Branhamella* than the control sera ($p < 0.05$). The convalescent sera had higher titres than both acute and control sera ($p < 0.001$).

Of the 45 patients in the study, 32 (71%) had a

Table 1 *Patients immunocompromised due to debilitating disease or disease associated with specific immunological abnormalities*

Pathology	*n (%)*
Rheumatoid disease	2(4)
Diabetes	3(7)
Multiple myeloma	2(4)
Postoperative colostomy	1(2)
Chronic cardiac failure	2(4)
Renal transplant	1(2)
Carcinoma of colon	1(2)
Carcinoma of bone	2(4)
Lymphoma	1(2)

Table 2 *Patients receiving immunosuppressive treatment*

	n (%)
Not on treatment	30(67)
Oral steroids	12(27)
Cytotoxic drugs	2(4)
Radiotherapy	2(4)

history of chronic pulmonary disease, and of these, 29 had COAD. Two patients with COAD had pulmonary carcinoma, two bronchiectasis, and a further five asthma, one of whom also had pulmonary carcinoma. One patient had pulmonary fibrosis associated with dust inhalation. In total, 30 patients (67%) were smokers. Fifteen patients (29%) also had extrapulmonary disease (table 1), or were receiving treatment which may have reduced their immunocompetence (table 2).

Discussion

Since its separation from the genus *Neisseria*, numerous studies have implicated *Branhamella* as a pathogen in man.[7] Ninane *et al* described the organism as causing bronchopneumonia in coal miners.[8] More recently, there have been reports of it causing chest infections in the immunocompromised and in premature infants. *B catarrhalis* has also been increasingly recognised as a relatively common isolate in both childhood otitis media and in exacerbations of COAD.

The essential problem with *Branhamella*, however, is not in isolating the organism from sputum samples, but in determining its pathogenic potential. *B catarrhalis* has long been recognised as an upper airways commensal, and therefore its mere presence (unlike *Mycobacterium tuberculosis*) albeit in heavy, pure growth, is not an adequate criterion by which to gauge pathogenicity. Furthermore, sputum samples may become contaminated with oropharyngeal commensal organisms and may not therefore accurately reflect the lower respiratory tract flora. Thornley *et al* have shown however, that routine sputum samples are as accurate as transtracheal aspiration in the diagnosis of bronchopulmonary infection with *B catarrhalis*.[9] Further evidence pointing to the pathogenicity of this organism was produced by Leinonen *et al* when he demonstrated a rise of IgG and IgA in middle ear fluid and serum in children with otitis media.[10]

In this study, patients who were convalescent after an acute episode of pulmonary infection had a higher titre of IgG against *Branhamella* than matched controls, which was highly significant ($p < 0.001$). This would suggest recent immunological exposure to *Branhamella* antigen, and taken in conjunction with the clinical findings, would tend to confirm the ability of this organism to invade tissue and cause disease.

Of the 45 patients in the study, only four had a greater than four-fold rise in IgG titre, a further three having a two-fold or greater rise. This contrasts with the large statistical difference between the acute and convalescent groups when viewed as two entire populations. One possible explanation is that the acute and convalescent sera were taken too close together. The mean time difference for the seven patients with the highest increase in titre was 14 days, compared with a mean of 10 days for the remainder of the group. It may be that the peak IgG titre occurred after the second sample was taken.

This study also showed high titres of IgG in the acute group compared with controls, although as mentioned above there was considerable overlap between the two groups especially at lower titres. The first serum samples were taken on average within two days of the patient developing purulent sputum which grew *Branhamella*. It seems unlikely that given this time span, a significant rise in titre had occurred. These titres may therefore reflect the "background" levels, and would suggest that the study population had greater immunological exposure to *Branhamella* than the control group. Patients with chronic bronchopulmonary disease may therefore be more prone to infection with *Branhamella* than the general population. Debilitated patients, or those with specifically compromised immune systems, also seem to be at greater risk of infection with *B catarrhalis*. As can be seen from table 1, an appreciable number of patients in the study had extrapulmonary disease, including two with myeloma, which could potentially adversely affect their immunocompetence. Similarly, as can be seen from table 2, a large number of patients in the study (33%) were receiving treatment that could also have affected their immune status. Furthermore, pulmonary infection was uncommon in those with no previous history of pulmonary or extrapulmonary disease. The association between *B catarrhalis* infection and impaired immune response has previously been described. Diamond *et al* reported four cases of *B catarrhalis* pneumonia in patients with abnormalities of their humoral immune system, including one patient with multiple myeloma.[11] He concluded that normal immunoglobulins are an important host defence mechanism in protecting against infection with this pathogen. It is also worth noting that the first reported case of *B catarrhalis* pneumonia in the United States was in a patient with myeloma.[12] The patient subsequently died from fulminating pneumonia. The prognosis of *Branhamella* pneumonia in cases of myeloma is especially poor.

In conclusion, while bronchopulmonary infection from this organism is not as prevalent in Northern Ireland as in some other countries,[13] it is nevertheless an important respiratory pathogen, especially

when isolated from patients with COAD, or in the immuncompromised. This may have implications for treatment, as in this study 41 (91%) strains were β lactamase producers and were generally resistant to the commonly used oral penicillins (unpublished data).

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