Bacterial precipitins and their immunoglobulin class in atopic asthma, non-atopic asthma, and chronic bronchitis

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Davies, R. J., Holford-Strevens, V. C., Wells, I. D., and Pepys, J. (1976). Thorax, 31, 419-424. Bacterial precipitins and their immunoglobulin class in atopic asthma, non-atopic asthma, and chronic bronchitis. In a study of groups of patients with atopic (extrinsic) asthma, non-atopic (intrinsic) asthma, and chronic bronchitis, no difference could be detected in the numbers having precipitating antibodies against species specific antigens from *Staphylococcus aureus* or *Streptococcus pneumoniae* compared to suitably matched control subjects. Precipitating antibodies against species specific antigens from *Haemophilus influenzae*, demonstrated in this investigation by double diffusion in agar gel, were found much more frequently in patients with chronic mucopurulent or obstructive bronchitis (50%) than in either asthmatic subjects (6%) or normal controls (6%) ($P < 0.0005$). While the precipitating antibody demonstrated in these patients against the extracts of *Str. pneumoniae* and *Staph. aureus* was in the IgG class alone, IgM and IgA antibody were detected against the species specific but not the non-species specific antigens of *H. influenzae*. These results underline the importance of *H. influenzae* as an infecting agent in chronic bronchitis and suggest that the finding of precipitins against the species specific H₁ and H₂ antigens of this bacterium denotes infection either concurrently or in the recent past. There is no evidence to suggest from this study that infection with *Staph. aureus*, *Str. pneumoniae* or *H. influenzae* is any more common in asthmatics as a group compared to controls or between patients with the non-atopic (intrinsic) and atopic (extrinsic) form of the disease.

The interpretation of the pathogenic role played by bacteria isolated from sputum in respiratory disease is difficult and often misleading because specimens may be contaminated by bacteria resident in the pharynx and mouth. In an attempt to overcome this difficulty indirect methods such as the demonstration of agglutinins or precipitins against bacteria have been used to indicate present or past infection. Such methods have suggested that *Haemophilus influenzae* and *Streptococcus pneumoniae* are the common and important infecting organisms in chronic bronchitis and bronchiectasis while other bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus vulgaris* are rarely involved (Burns and May,

1967; Burns 1968a and b; Nicholls, Pease, and Green, 1975). Similarly precipitating antibodies against antigens from *Staphylococcus aureus* have been found with increased frequency in patients with cystic fibrosis as well as bronchiectasis (Burns and May, 1968). Bacterial infection is thought to play an important role in certain cases of asthma, particularly those described as intrinsic (Williams *et al.*, 1958; Hampton, 1965) though there is little evidence to support this claim. Knowledge of the particular immunoglobulin class of antibody against both bacterial and viral antigens is of importance in clinical practice since the finding of antibody in the IgM class has been taken as evidence favouring recent infection, while the finding of IgG antibody alone has been considered to imply past contact (Bürgin-Wolff, Hernandez, and Just, 1971; O'Neill and Nichol, 1972).

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The purpose of this investigation was to compare the distribution of precipitating antibody against species specific antigens from *H. influenzae*, *Str. pneumoniae* and *Staph. aureus* in patients with atopic (extrinsic) asthma, non-atopic (intrinsic) asthma, and chronic bronchitis and to define its immunoglobulin class. In this report comparison was made with carefully matched control groups, and a simplified method was used to demonstrate precipitins against species specific antigens from *H. influenzae*.

MATERIAL AND METHODS

PATIENTS The characteristics of the patients are shown in Table I.

Atopic and Non-atopic Asthma All the subjects were attending hospital outpatients at the time of the study, none having asthma of sufficient severity to necessitate inpatient therapy. All had evidence on at least one occasion of reversible airways obstruction, defined here as a 15% or more increase in forced expiratory volume in one second (FEV₁) or peak expiratory flow rate (PEFR) after the inhalation of 200 µg of salbutamol.

Asthma has been divided into extrinsic and intrinsic groups on a basis of whether or not a known external allergen provokes airway obstruction together with the presence or absence of positive immediate skin tests (Rackemann, 1931; Rackemann, 1947). Without the aid of bronchial provocation challenge testing it is difficult to be certain that a particular allergen causes the patient's asthma (Davies, 1974). For this reason the asthmatic subjects in this study were divided solely on a basis of skin prick testing with a battery of 23 common inhalant and food allergens. Atopic asthmatics were those showing one or

more positive immediate skin reactions to these allergens while the non-atopic asthmatics had no such positive skin tests (Pepys, 1973).

The proportions giving a positive immediate family history of asthma, rhinitis or eczema were similar in both groups, as were the numbers showing the presence of a blood eosinophilia (>400 eosinophils/mm³).

Chronic Bronchitis All the patients, who were attending their general practitioner or hospital outpatients at the time of this study, had productive cough and fulfilled the criteria for chronic bronchitis suggested by the Medical Research Council Committee on the Aetiology of Chronic Bronchitis (1965). Ten subjects had chronic or recurrent mucopurulent bronchitis with their FEV₁ or PEFR within the normal range, while the other 25 had an FEV₁ or PEFR two standard deviations below the expected for their height and age, indicating the presence of chronic obstructive bronchitis. Patients with reversible airways disease on testing with 200 µg of salbutamol, blood eosinophilia or the presence of positive skin prick tests to any of a battery of 23 common inhalant or food allergens were excluded.

CONTROL SUBJECTS Two control groups were studied in an attempt to match the different age ranges, sex predominance, and prevalence of cigarette smoking in the groups with chronic bronchitis and asthma.

Control group I consisted of healthy laboratory and medical personnel together with members of their families. None had chronic bronchitis, asthma, rhinitis or eczema.

Control group II consisted of adults over the age of 40 years who were attending a health screening programme in a general practice in

TABLE I
NUMBERS AND CHARACTERISTICS OF PATIENTS IN GROUPS WITH ATOPIC ASTHMA, NON-ATOPIC ASTHMA, AND CHRONIC BRONCHITIS, AND SUBJECTS IN TWO CONTROL GROUPS

Subjects	n	Mean Age and Range (years)	Sex Ratio M : F	Percentage			
				Family History Asthma, Rhinitis, Eczema	+ve Immediate Skin Tests to Common Allergens	Blood Eosinophilia $>400/\text{mm}^3$	Cigarette Smokers
Control I	50	40 14-65	1:1	18	24	2	56
Atopic asthma	50	35 9-63	1:1	56	100	48	46
Non-atopic asthma	50	43 14-71	1:1	48	0	56	48
Chronic bronchitis	35	57 43-79	2:1	23	0	0	97
Control II	30	51 41-68	2:1	15	17	3	73

south-east London. All were healthy and none gave a history of chronic bronchitis, asthma, rhinitis or eczema. All subjects in groups I and II were tested with a battery of 23 common allergens and were screened for the presence of blood eosinophilia.

BACTERIAL EXTRACTS The particular bacterial extracts used in this study were species specific and chosen to correspond with those employed by other investigators (Burns and May, 1967; Burns, 1972).

α Teichoic Acid from *Staph. aureus* (a polyribitol phosphate with α linked N acetyl glucosaminyl residues) Cell walls from *Staph. aureus* strain 3528, kindly provided by Dr. A. R. Archibald, were treated with water at 100°C for 15 min and with trypsin at 37°C for 18 h to remove protein. The walls were then extracted with 0.5 M sodium hydroxide for 30 min. The extract was passed through Dowex 50 and finally eluted from a DEAE cellulose column using a gradient of water to 1.0 M acetic acid adjusted to pH 5.0 with pyridine (Archibald and Stafford, 1972; Archibald, personal communication). After freeze drying the appropriate fractions, the α teichoic acid was reconstituted and gave a single precipitin arc against patients' sera on immunoelectrophoresis.

Extract from Str. pneumoniae The filtrate from culture of a rough strain of *Str. pneumoniae* (NCTC No. 10319) in 1% glucose broth was taken, Seitz filtered, dialysed, and passed through Sephadex G25 to remove low molecular weight constituents. After freeze drying, the reconstituted extract was autoclaved at 10 lb per square inch for 10 minutes to denature protein. Immunoelectrophoresis of this extract against patients' sera showed a single precipitin arc.

Extract from H. influenzae (a) Non-species specific H_1 to H_3 antigens: A suspension of non-capsulated *H. influenzae* was disrupted by ultrasound and centrifuged, and the supernatant was dialysed against 0.02 M ammonium bicarbonate and finally freeze dried. After reconstitution this extract showed precipitin lines on immunoelectrophoresis against patients' sera in the H_1 to H_3 positions (May, 1972).

(b) Species specific H_1 and H_2 antigens: The H_1 to H_3 extract of *H. influenzae* was fractionated by batch absorption with DEAE-Sephadex in acetate barbital buffer (Davies, Laughton, and May, 1974). The mixture was stirred continuously for 24 hours, the DEAE-Sephadex spun down, and the supernatant filtered, dialysed against 0.02 M

ammonium bicarbonate, and freeze dried. On reconstitution the extract showed precipitin lines on immunoelectrophoresis against patients' sera in the H_1 and H_2 positions only.

METHODS

DOUBLE DIFFUSION This was carried out in Ion agar No. 2 (Oxoid) in a citric acid-phosphate buffer.

IMMUNOELECTROPHORESIS This was performed using Ion agar No. 2 (Oxoid) in a 0.05 M veronal buffer pH 8.6 on 8×8 cm glass slides with a voltage gradient of 6 volts/cm.

RADIOIMMUNOELECTROPHORESIS The procedure was a modification of a technique previously described (Pepys *et al.*, 1973) in which the antigenic extracts were subjected to electrophoresis in agar gel followed by development of precipitin arcs using patients' sera. The immunoglobulin class present in the precipitin arcs was determined by the uptake of ^{125}I labelled IgG fraction of rabbit antihuman IgG, IgM or IgA sera (Behringwerke) and of normal rabbit serum as control.

RESULTS

The results of the double diffusion tests against the sera of the patients and controls are shown in Table II. The prevalence of precipitins to *Staph. aureus* teichoic acid was no higher in the patients than in either control group.

Although a higher percentage of the patients with chronic bronchitis showed precipitins to the *Str. pneumoniae* extract than in control group I, 53% compared with 36%, this difference did not reach significance and when the prevalence was compared with that in control group II, better matched for age and smoking habit, the percentages were identical.

A similar percentage of the chronic bronchitics and of control group II showed antibodies to the non-species specific H_1 to H_3 antigens of *H. influenzae*, but a much higher prevalence of antibodies to the species specific H_1 and H_2 antigens occurred in chronic bronchitics compared with all other groups ($P < 0.0005$).

The immunoglobulin class of the precipitating antibody in the sera of the patients with chronic bronchitis and asthma is illustrated in Table III. The majority of antibody was in the IgG class, except in patients with chronic bronchitis where antibodies of IgA and IgM classes were addition-

TABLE II

RESULTS OF PRECIPITIN TESTS BY DOUBLE GEL DIFFUSION WITH BACTERIAL EXTRACTS IN PATIENTS WITH ASTHMA AND CHRONIC BRONCHITIS COMPARED TO CONTROLS

Subjects	n	% with Precipitins			
		<i>Staph. aureus</i> α Teichoic Acid 1 mg/ml	<i>Str. pneumoniae</i> Extract 2 mg/ml	<i>H. influenzae</i> Non-specific 'H ₁ to H ₅ ' 20 mg/ml	<i>H. influenzae</i> Specific 'H ₁ + H ₂ ' 1 mg/ml
Control I	50	78	36	28	6
Atopic asthma	50	64	28	22	8
Non-atopic asthma	50	54	32	22	6
Chronic bronchitis	35	50	54	60	50
Control II	30	73	53	43	6

TABLE III

IMMUNOGLOBULIN CLASS OF PRECIPITATING ANTIBODY AGAINST BACTERIAL EXTRACTS IN PATIENTS WITH CHRONIC BRONCHITIS AND ASTHMA

Bacterial Antigens	Disease	Patients with Precipitating Antibody n	Immunoglobulin Class of Precipitating Antibody		
			IgG	IgA	IgM
<i>H. influenzae</i> H ₁ } Specific H ₂ } H ₃ } H ₄ } Non-Specific H ₅ }	Chronic bronchitis	7	7	5	5
		8	8	0	0
<i>Str. pneumoniae</i> extract	Chronic bronchitis	9	9	1	0
	Atopic and non-atopic asthma	10	10	0	0
<i>Staph. aureus</i> α Teichoic acid	Atopic asthma	4	4	0	0
	Non-atopic asthma	3	3	0	0

ally shown to the specific H₁ and H₂ antigens of *H. influenzae*.

DISCUSSION

In this study no differences were found in the prevalence of precipitating antibody against the antigens tested from *Staph. aureus*, *Str. pneumoniae*, and *H. influenzae* in patients with atopic asthma compared to controls. Further, no difference could be detected in the distribution of these precipitins in the group with non-atopic (intrinsic) asthma compared to those with the atopic (extrinsic) form of the disease. This makes it unlikely that infective episodes with these bacteria are any more common in asthma compared to normals, or indeed between the two major subdivisions of the disease. Although the antigens used in this investigation were very similar to those employed in other studies (Burns, 1972), it is certainly possible that differences might have been detected if other antigens from these bacteria had been used. Important differences both in the frequency and immunoglobulin class of antibody response might well result, depending on whether the anti-

gen comes from cell wall or cytoplasm. Similarly, although *Staph. aureus*, *Str. pneumoniae*, and *H. influenzae* are considered to be the major respiratory tract pathogens, other bacteria may be important infective agents in asthma. Despite the fact that there is no evidence favouring more frequent infection with these bacteria in groups of asthmatic subjects, this does not necessarily mean that they are not important in the development of particular episodes of reversible airway obstruction, possibly through hypersensitivity mechanisms. Extracts of killed bacteria such as *Neisseria catarrhalis* and *H. influenzae* have been shown to lead to airway narrowing on bronchial provocation testing (Hampton, 1965; van der Zwan, Orie, and de Vries, 1975).

Fifty per cent of the patients with chronic mucopurulent or obstructive bronchitis had precipitating antibody against species specific antigens from *H. influenzae*. This figure is considerably higher than that found in either of the control groups (6%) or the asthmatic groups (8% and 6%), and is in close agreement with previous findings in both this country and Australia (Burns, 1972). Precipitating antibody against species specific *H.*

influenzae antigens was demonstrated in these patients by the much simpler and more sensitive technique of double gel diffusion rather than the standard method of immunoelectrophoresis. In the present study all the sera from the patients with chronic bronchitis were tested by both double gel diffusion using the species specific H_1 and H_2 antigens as well as by immunoelectrophoresis; there was a close agreement between the two methods, confirming the work of Davies *et al.* (1974).

Previous investigations of the presence of precipitins against *Str. pneumoniae* extracts have indicated a higher prevalence of these antibodies in the serum of patients with chronic bronchitis compared to control subjects (Burns, 1968a, b, 1972). Although in the present study 53% of the patients with chronic bronchitis showed precipitins compared to 36% of the subjects in control group I, comparison with a control group better matched for age and smoking habit showed identical results. Of the subjects in control group II, 53% had precipitins against the extract from *Str. pneumoniae*, the same antigen that was used in the original study by Burns (1968a, b). This difference can probably be explained by the fact that in the previous studies blood bank sera were used as controls, rather than sera taken from carefully matched and analysed control subjects. The role played by *Str. pneumoniae* in chronic bronchitis is not clear, though it is generally considered to be a pathogen in acute exacerbation of bronchitis (May, 1972) where rising titres of agglutinating antibody against pneumococcal bacteria have been found in some patients (Nicholls *et al.*, 1975). The evidence from this study, at least in terms of precipitating antibody against a pneumococcal extract, suggests that infections with *Str. pneumoniae* are no more common among chronic bronchitics than control subjects of similar age and smoking habits.

Precipitating antibody against α teichoic acid from *Staph. aureus* was found in 78% of the 50 control subjects in group I and in 73% of the subjects in control group II, agreeing very closely with previous studies which have shown that precipitins were present in 79% of normals but increased to almost 100% in infected subjects (Martin, Daugherty, and White, 1965). The patients with asthma and chronic bronchitis in this study had a similar prevalence of precipitins against this antigen compared to controls, casting doubts as to whether *Staph. aureus* is an important infecting agent in these particular respiratory diseases.

Only antibody of IgG class could be demonstrated by the technique of radioimmuno-electrophoresis against teichoic acid from *Staph. aureus* and the extract from *Str. pneumoniae*, except in one patient where IgA antibody to *Str. pneumoniae* was shown. The presence of IgG antibody provides evidence of previous contact with these bacteria. Although levels of IgG antibody may rise during acute infection (Martin *et al.*, 1965) the absence of IgM antibody suggests that it is unlikely that recent infection with *Staph. aureus* or *Str. pneumoniae* had occurred in these patients. IgM antibody, however, was demonstrable by this technique against species specific antigens from *H. influenzae* in the patients with chronic bronchitis, underlining the pathogenicity of this bacterium in this disease. Of considerable interest was the fact that in this study IgM antibody could be detected only against the species specific H_1 and H_2 antigens of *H. influenzae* and not against the non-specific H_3 , H_4 , and H_5 antigens. Rising titres of agglutinating antibody almost certainly in the IgM class have been demonstrated against *H. influenzae* organisms during exacerbation of chronic bronchitis (Nichols, 1975, personal communication). These findings help to explain the preliminary observations by Gregg and co-workers that specific precipitins were demonstrable for only a few weeks in the serum of patients with mild chronic bronchitis following transient Haemophilus infections (May *et al.*, 1973). Further, it has been noted that precipitating antibody against the H_1 antigen disappears after a few months from the serum of patients with advanced chronic bronchitis where infection has been eradicated by chemotherapy (Jenne *et al.*, 1970). The fact that some of the antibody against the species specific H_1 and H_2 antigens of *H. influenzae* is in the IgM class adds weight to the statement by May *et al.* (1973) that detection of precipitating antibody against H_1 antigen in a patient's serum is indicative either of continuing Haemophilus infection or infection in the recent past.

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