Precipitating antibody in the sputum in chronic respiratory disease*

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Summary

Precipitating antibody against bacterial antigens obtained from known pathogens has been looked for in sputum of patients with chronic respiratory disease. Preliminary results suggest that detection of precipitating antibody to a cell wall extract of the organism may be important in determining its pathogenicity in a particular patient. Local antibody production appears to be important in these conditions. It is proposed that this locally produced precipitating antibody may be contributing to the underlying lung pathology.

Introduction

Interpretation of the pathogenic significance of organisms isolated from the sputum of patients with chronic respiratory disease is notoriously difficult. It has been simplified by the serum precipitin test described by Burns and May (1967, 1968), but difficulties still exist. For example, serum precipitins usually belong to the immunoglobulin class IgG. They are, thus, long-lasting antibodies and their detection in serum does not necessarily indicate continuing infection with the organism at that time. Further, as May (1974) has pointed out, their occurrence does not correlate with protection against infection by the homologous organism.

The present study was undertaken with the object of determining the relationship between infection and local antibody detected in the sputum. In particular, the possibility was borne in mind that such antibody might relate more closely than serum antibody to the patient's present state.

Methods

Antigen extracts

1. Standard antigen extracts of *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were prepared by the method described by May (1972). In addition, antigens of *H. influenzae*

were prepared by the method of Davies, Laughton and May (1974).

2. Cell-wall antigens of *H. influenzae* and *P. aeruginosa* were prepared by hot phenol extraction (Branefors-Helander, 1973) of the washed deposit of bacterial suspensions disrupted by ultrasonic vibration.

3. A preparation of *Aspergillus fumigatus* (Bencard 27) was used as antigen in tests for antibody to this fungus. This is derived from a filtrate of a surface culture and contains cell-wall and cytoplasmic antigens.

Detection of antibody in sputum

Tests were normally carried out on 18- to 24-hr samples.

After many tests on specimens homogenized ultrasonically or separated into sol and gel phases by high-speed centrifugation, it was concluded that the most satisfactory procedure for preparation of sputum for precipitin testing was one based on dialysis of antibodies into water, which formed the test material (Longbottom, 1973). Details will be published elsewhere.

Tests for precipitating antibody were carried out by the double-diffusion and immunoelectrophoresis methods described by May (1972).

Immunoglobulins in sputum

Assays of IgG, IgA and IgM were performed on the sol phase of sputum samples subjected to high-speed centrifugation. The assays were carried out by the standard radial diffusion method on immunoassay plates (Behringwerke A.G.).

The relationship of *H. influenzae* antibody to the different immunoglobulin classes was investigated by radioimmuno-electrophoresis, using a technique devised by Davies, Holford-Strevens and Wells (1974). Sputum dialysate was electrophoresed in a standard immunoelectrophoresis agar preparation, and anti-IgG, anti-IgA, anti-IgM or antiselectivity piece was placed in the trough causing precipitation of the homologous immunoglobulin. Subsequently, the trough was filled with a solution of *Haemophilus* antigen labelled with I^{131} . After incubation the preparations were washed to remove excess antigen

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TABLE 1. Occurrence of serum and sputum precipitins against standard bacterial extracts and the *Aspergillus* extract

Clinical diagnosis	No.	Number of patients with antibody detected in	
		Sputum	Serum
Bronchiectasis	11	4 (All <i>H. influenzae</i>)	10 { 9 <i>H. influenzae</i> 2 <i>Streptococcus pneumoniae</i> 2 <i>P. aeruginosa</i> 3 <i>Klebsiella</i> spp.
Cystic fibrosis	10	5 { 4 <i>H. influenzae</i> 1 <i>P. aeruginosa</i> 1 <i>A. fumigatus</i>	10 { 7 <i>H. influenzae</i> 1 <i>S. pneumoniae</i> 7 <i>P. aeruginosa</i> 3 <i>Klebsiella</i> spp. 1 <i>Staphylococcus aureus</i> 1 <i>A. fumigatus</i>
Chronic bronchitis	6	3 (All <i>H. influenzae</i>)	5 { 3 <i>H. influenzae</i> 1 <i>P. aeruginosa</i> 1 <i>Klebsiella</i> spp. 1 <i>Staph. aureus</i>
Asthma	3	2 { 1 <i>H. influenzae</i> 1 <i>A. fumigatus</i>	3 (All <i>H. influenzae</i>)
Allergic aspergillosis	1	1 { <i>H. influenzae</i> and <i>A. fumigatus</i>	1 { <i>H. influenzae</i> and <i>A. fumigatus</i>
Aspergilloma	1	1 (<i>A. fumigatus</i>)	1 (<i>A. fumigatus</i>)
Recent chest infection	1	0	0

and autoradiographs were prepared. Demonstrable radio-activity in the precipitin arc formed by an immunoglobulin and its antiserum indicated that the *Haemophilus* antibody belonged to that immunoglobulin class.

Results

Occurrence of serum and sputum precipitins against standard bacterial extracts and the Aspergillus extract

The overall findings are shown in Table 1. One rather striking observation was the relatively large number of patients whose sputum apparently contained no antibody although this was present in the serum and bacterial pathogens were isolated from the sputum. This lack of antibody could not be accounted for by absence of immunoglobulins from the sputum, since these were demonstrated in samples from seventeen of eighteen patients studied.

Another finding of note was that in two patients antibody was demonstrated in sputum but not in serum, implying that the antibody was produced locally and had not entered the sputum by transudation from serum.

Occurrence of precipitins against cell-wall extracts

Preliminary studies of the sputum of twelve patients with no demonstrable antibody against cytoplasmic antigens of *P. aeruginosa* revealed antibody against its cell-wall antigen in four instances. Three of these patients were suffering from cystic fibrosis and the fourth from allergic bronchopulmonary aspergillosis.

Table 2 shows the findings of a more detailed study of the occurrence in sputum and serum of precipitins against both cell-wall and cytoplasmic antigens of *H. influenzae* and *P. aeruginosa*. Of particular interest is the contrast between the occurrence in sputum of antibodies to *Pseudomonas* cell-wall in patients with mucoid and non-mucoid strains in the sputum. It is quite widely believed that mucoid, but not non-mucoid, strains of *P. aeruginosa* can cause chronic infection of the respiratory tract, and these findings suggest that search for anti-cell-wall precipitins in sputum might provide a better guide to pathogenicity than the current serum precipitin test in which cytoplasmic antigens are used.

Immunoglobulins in sputum

Table 3 shows the levels of IgG, IgA (7S) and IgM found in the sputum of ten patients and the serum of five of them. IgM was not detected in the sputum of eight and was present in only very low concentration in that of the remaining two, confirming the belief that most of the sputum immunoglobulins are locally produced and are not the product of serum transudation into the bronchi.

Radio-immuno-electrophoresis of sputum

The immunoglobulin class to which *Haemophilus* antibodies in sputum belong has been investigated in eight patients. In every instance, radio-activity was demonstrable in IgG and IgA precipitates, but not IgM. In three instances secretory piece (presumably attached to IgA), precipitated by the appropriate

TABLE 2. Occurrence of serum and sputum precipitins against cell-wall and cytoplasmic antigens of *H. influenzae* and *P. aeruginosa*

Organisms isolated from sputum	No. of patients	Number of patients with antibody detected in			
		Sputum against		Serum against	
		Cell-wall antigen*	Cytoplasmic antigen*	Cell-wall antigen*	Cytoplasmic antigen*
<i>H. influenzae</i>	4	4	4	1	4
Mucoid <i>P. aeruginosa</i>	4	4	0	4	4
Non-mucoid <i>P. aeruginosa</i>	3	0	0	1	3
None found	4	2 (to both extracts)	0	2 (<i>P. aeruginosa</i> only)	3 { 2 <i>H. influenzae</i> 1 <i>P. aeruginosa</i>

* In each patient from whose sputum *H. influenzae* or *P. aeruginosa* was isolated, precipitin tests were carried out with extracts of the relevant species. In patients from whose sputum no pathogens were isolated, tests were carried out with extracts of both species.

TABLE 3. Levels of IgG, IgM and IgA (7S) in sputum and serum of ten patients with purulent sputum

Patient no.	Concentration (mg/100 ml) of					
	IgG in		IgA (7S) in		IgM in	
	Sputum	Serum ^a	Sputum	Serum ^b	Sputum	Serum ^c
1	720	2490	27	350	0	163
2	> 2000		100		0	
3	472		43		0	
4	< 160	1205	35	350	0	252
5	656		27		34	
6	1826		< 25		< 30	
7	< 160	1285	< 25	205	0	121
8	388	1325	< 25	332	0	279
9	1875	3180	< 25	350	0	186
10	472		< 25		0	

0 = non detected; a = normal range 500–1500; b = normal range 125–450; c = normal range 50–170.

antiserum, on subsequent addition of I^{131} -labelled *Haemophilus* antigen gave rise to radio-activity of the precipitate.

Discussion

The findings reported here are of a very preliminary nature and much work remains to be done. Even so, two conclusions seem to be valid. First, sputum antibody against cell-wall antigen seems likely to provide a better index of the pathogenicity of an organism in the respiratory tract than serum antibody against either cell-wall or cytoplasmic antigens. Secondly, the amount of serum antibody passing by transudation into sputum is remarkably low, and by far the bulk of antibody detected in the sputum, even of the IgG class, seems to be locally produced, at any rate in diseased states.

The full significance of these observations is not clear. The evidence presented suggests that other

local factors in addition to locally produced antibodies are required to keep the bronchial tree free of bacterial infection; it is also possible that local antibody may play a part in the mechanism whereby an organism causes tissue damage in the bronchial tree. It is well known that the interaction in the bronchial wall of *Aspergillus* antigen and homologous antibody can give rise to tissue damage leading to a form of cylindrical bronchiectasis and, in the light of the demonstration that local antibacterial antibody is common, there seems to be no obvious reason why a similar mechanism should not occur in chronic bacterial infections. In support of this possibility, Gordon *et al.* (1973) described a patient with pulmonary allergic disease in whom it was suggested by skin testing, demonstration of circulating antibody, and by a good response to steroid therapy, that the responsible allergen was derived from a mucoid strain of *P. aeruginosa* isolated from the patient's sputum.

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