ASSOCIATION BETWEEN *BORDETELLA PERTUSSIS* AGGLUTINOGEN 2 AND FIMBRIAE

E. J. CARTER AND N. W. PRESTON

*Pertussis Reference Laboratory, Department of Bacteriology and Virology, University
Medical School, Manchester M13 9PT*

SUMMARY. Fimbriae have been demonstrated on strains of *Bordetella pertussis* that possess agglutinin 2 (types 1, 2, 3 and 1, 2), but not on those that lack it (types 1,3 and 1). This correlation between fimbriation and the presence of agglutinin 2 has been found with fresh isolates from children and with laboratory strains that are virulent for mice. If fimbriae enhance the attachment of bacteria to mucosal cells, these findings offer an explanation for the predominance of serotypes 1,2,3 and 1,2 in non-vaccinated communities. The findings also suggest that agglutinin 3 is not a fimbrial antigen, and because this is an essential component of fully effective whole-cell vaccine, a subcellular vaccine prepared from fimbriae alone may be inadequate.

INTRODUCTION

The role of fimbriae in the attachment of pathogenic bacteria to mucosal surfaces is well recognised (Sawyer and Rosenthal, 1978; Wilson, Miles and Parker, 1983). Moreover, there have been many reports of the presence of fimbriae on strains of *Bordetella pertussis*, but these have not included studies of the serotypes of the strains examined (e.g., Morse and Morse, 1976; Blom, Hansen and Poulsen, 1983).

We have investigated the relationship between fimbriation and serotype of strains isolated from patients with whooping cough, and also of the unusual laboratory strains that are virulent for mice.

MATERIALS AND METHODS

Strains of Bordetella pertussis. During the 1977–79 epidemic of whooping cough, isolates of *B. pertussis* were sent from many parts of Britain to our reference laboratory for serological typing. The present investigation included 17 of these cultures from patients aged 2 months to 11 years. Apart from one strain that came from Kettering General Hospital and one from Monsall Hospital, Manchester, all were received from Public Health Laboratories (see table I).

A culture that had been isolated in Coventry in 1967 was of particular interest because it had been found to contain all four *B. pertussis* serotypes (Stanbridge and Preston, 1974); these have since been preserved in our laboratory as freeze-dried cultures.

Six strains virulent for mice have been in our collection for many years. Strain GL353, and its derivative (353/Z) of enhanced mouse-virulence, are both of serotype 1 (Preston, 1966). Strain W.18-323 is the Kendrick challenge strain, which in our laboratory tests is of serotype 1.

Received 3 Jan. 1984; accepted 17 Jan. 1984.

TABLE I
Serotype, fimbriation and source of strains of B. pertussis

Bacterial strains	Serotype 1,2,3 Fimbriate		Serotype 1,2 Fimbriate		Serotype 1,3 Non-fimbriate		Serotype 1 Non-fimbriate	
	Place of isolation	Strain designation	Place of isolation	Strain designation	Place of isolation	Strain designation	Place of isolation	Strain designation
Strains isolated in 1978 from children aged 2 months to 11 years	Kettering	K19587	Leicester	39424	Guildford	36379	...	...
	Newcastle upon Tyne	57871/1*	Maidstone	48371	Leeds	A36752		
	Plymouth	83133	Manchester	71333	Newcastle upon Tyne	57871/8*		
	Preston	18335	Plymouth	85068	Nottingham	220822		
	Shrewsbury	183880	Portsmouth	8002	Plymouth	84455		
Strain isolated from child in 1967	Swansea	28771/6†	Preston	22314	Plymouth	85248		
					Swansea	28771/4†		
	Coventry	41633/S/8‡	Coventry	41633/11/9‡	Coventry	41633/11/10‡	Coventry	41633/S/2‡
Mouse-virulent strains	...	9797	...	CN1262	...	CN1262C	...	W.18-323
							...	GL353
							...	353/Z

* Two single-colony subcultures from the original isolate 57871.

† Two single-colony subcultures from the original isolate 28771.

‡ Four single-colony subcultures from the original isolate 41633.

Three other strains are probably derivatives of the Kendrick strain: 9797 which came to us from Switzerland (type 1,2,3), CN1262 from Wellcome Research Laboratories (type 1,2), and CN1262C from Copenhagen (type 1,3).

Serotyping of B. pertussis cultures. Slide agglutination with our own monospecific antisera (Preston, 1970) gave a roughly quantitative comparison of the agglutinability of bacterial suspensions: +++ = rapid formation of large agglutinates, complete within 3 min; ++ = agglutination visible within 3 min and complete within 5 min; + = slow formation of small agglutinates, incomplete within 5 min; – = no agglutination visible to the naked eye within 5 min.

Electron microscopy. Bacteria were grown for 2–3 days at 36°C on Charcoal Blood Agar (Oxoid) in Petri dishes. The organisms were then suspended in distilled water and washed three times by centrifugation and re-suspension. A drop of the washed bacterial suspension was transferred to a formvar-coated grid, allowed to dry, and then shadowed with platinum at an angle of 19° in a 300S coating unit (Nanotech Scientific Ltd, Prestwich, Manchester M25 8WD).

For negative staining, formvar-coated grids were coated with carbon which was ionised by the plasma glow technique in the 300S unit. A drop of the washed bacterial suspension was placed on the grid, and a drop of 1% methylamine tungstate (Emscope Laboratories Ltd, Ashford, Kent TN23 2LN) was added. After removal of excess liquid with filter paper, the specimen was allowed to dry; subsequent examination was confined to areas showing an even spread of stain.

Electron microscopy was performed at 100 kV with an Hitachi H600 microscope. Photographic negatives were obtained on 9 × 12 cm Ilford "Line" film.

RESULTS

Strains of B. pertussis isolated in 1978

Strains of each serotype of *B. pertussis* (1,2,3; 1,2; 1,3) were randomly selected from many hundreds of isolates received from different parts of the country. The usual patterns of agglutination with the three monospecific typing sera are shown in table II; most of the strains listed in table I reacted in that manner.

However, strain 28771 reacted in an unusual manner (+++, ++, +++, with antisera 1, 2 and 3 respectively). Single colony subcultures of this strain showed that it was a mixture of type 1,2,3 (designated strain 28771/6) and type 1,3 (designated strain 28771/4); these subcultures reacted in the characteristic manner (+++, ++, +++, and ++, –, +++, respectively). Such mixtures of serotype are not uncommon amongst fresh isolates from patients (Preston and Stanbridge, 1972).

TABLE II
Characteristic agglutination patterns of the serotypes of B. pertussis

Serotype	Degree* of agglutination with			
	monospecific serum for			no serum
	factor 1	factor 2	factor 3	
1,2,3	+++	+++	+++	–
1,2	+++	+++	–	–
1,3	+ or ++	–	+++	–
1	+ or ++	–	–	–

* See Materials and Methods.

FIG. 1.—Electron micrographs of three fresh isolates of *B. pertussis*, shadowed with platinum: **a**—strain 18335 (type 1,2,3), fimbriate; **b**—strain 85068 (type 1,2), fimbriate; **c**—strain 220822 (type 1,3), non-fimbriate. ($\times 26\,000$).

Electron microscopy showed that all strains that reacted with factor-2 serum (type 1,2,3 or type 1,2) possessed fimbriae, whereas those that did not react with factor-2 serum (type 1,3) were non-fimbriate (table I and fig. 1). In most of the fimbriate strains, $>90\%$ of the cells had fimbriae; but, with the mixed serotype culture (strain 28771), only about half of the cells had fimbriae. However, in its type 1,2,3 derivative (strain 28771/6) $>90\%$ of the cells had fimbriae, but in its type 1,3 derivative (strain 28771/4) no fimbriae were found.

Although strain 57871 agglutinated in the usual manner for a type 1,2,3 strain—strong reaction with all three monospecific antisera—little more than half of the cells exhibited fimbriae. Single colony subcultures revealed that this culture, too, was a mixture (table I): strain 57871/1 agglutinated as a type 1,2,3 culture and had a

FIG. 2.—Electron micrographs of two fresh isolates of *B. pertussis*, with negative staining: **a**—strain 85068 (type 1,2); fimbriate, **b**—strain A36752 (type 1,3), non-fimbriate. ($\times 96\ 000$). In **a** there are additional short fimbriae not seen in shadowed preparations (fig. 1).

very high proportion of fimbriate cells; strain 57871/8 agglutinated as a type 1,3 culture (table II) and possessed no fimbriae.

In shadowed preparations (fig. 1), fimbriate bacteria were found to have about 5–15 fimbriae per cell. With negative staining (fig. 2), about twice as many fimbriae were seen on fimbriate cells, though many of these were shorter than those seen by shadowing. It seemed likely that the fimbriae seen by shadowing corresponded to the longer ones seen by negative staining. With type 1,3 cells, no fimbriae were seen even with negative staining (fig. 2b).

Strain of B. pertussis isolated in 1967

Stanbridge and Preston (1974) gave a detailed account of an isolate in which an unusual range of serotypes was demonstrated by single colony subculture: the three serotypes described above (1,2,3; 1,2; 1,3), and also the degraded form of the organism (type 1) which is rarely recovered from the human nasopharynx and only then as a minority component of a mixed-serotype culture (Preston and Stanbridge, 1972).

We thought that these four serotypes would make an interesting comparative study in fimbriation, because they were likely to be variants of a single serotype with which the child was originally infected. The serotypes which were agglutinated with factor 2 serum (41633/S/8 and 41633/11/9) were found to possess fimbriae, whereas those which were not agglutinated with this serum (41633/11/10 and 41633/S/2) had no fimbriae (fig. 3).

FIG. 3.—Electron micrographs of four single-colony sub-cultures from one isolate of *B. pertussis* (strain 41633), shadowed with platinum: **a**—41633/S/8 (type 1,2,3), fimbriate; **b**—41633/11/9 (type 1,2), fimbriate; **c**—41633/11/10 (type 1,3), non-fimbriate; **d**—41633/S/2 (type 1), non-fimbriate. ($\times 26\ 000$).

Strains of B. pertussis virulent for mice

Strains of *B. pertussis* freshly isolated from children are not virulent for mice, but a few laboratory strains produce a lethal infection when injected intracerebrally (Preston, 1966). These include the Kendrick challenge strain, W.18-323, which in our laboratory tests is of serotype 1. Our collection also includes three other strains (9797, CN1262 and CN1262C) which were received from different laboratories, and which

are probably derivatives of the Kendrick strain (see Materials and Methods). They are of type 1,2,3, type 1,2 and type 1,3 respectively (table I).

However, strains 9797 and CN1262 did not give characteristic patterns of agglutination (table II), reacting only weakly with factor-2 serum. Moreover, the weak reactions were not caused by mixed populations in which some cells produced agglutinin 2 and others did not; single colony subcultures were all found to give the same weak reaction with factor-2 serum.

Consistent with this finding was the appearance of these two strains by electron microscopy: most cells were fimbriate, although they had less than five fimbriae per cell. The strains of type 1,3 and type 1 virulent for mice (CN1262C and W.18-323 respectively) had no fimbriae. Nor did two other strains of type 1 (GL353 and 353/Z) that were virulent for mice.

DISCUSSION

Strains of *B. pertussis* that are isolated from children with whooping cough possess a common agglutinin (factor 1) together with either, or both, of two other agglutinins (factors 2 and 3). They are designated type 1,2,3, type 1,2 or type 1,3, according to the combination of these antigens that they possess. Type-1 organisms, devoid of antigens 2 and 3, do not cause whooping cough, though they may be virulent for mice.

We have shown a complete correlation between fimbriation and the possession of agglutinin 2 for isolates from children and for strains virulent for mice: type 1,2,3 and type 1,2 strains were fimbriate, whereas type 1,3 and type 1 were not. We deduce from these observations that agglutinin 2 is a fimbrial antigen, but that agglutinin 3 is not—unless it is associated with fimbriae that were not revealed by our techniques. We intend to investigate the locations of these antigens more fully with labelled antibody. However, our repeated experience of mixed serotypes, even in fresh isolates from children, emphasises the need for such studies to be linked with a simultaneous demonstration of the serological purity of the cultures (Preston, Surapatana and Carter, 1982).

The role of fimbriae in the attachment of bacteria to mucosal epithelium may well explain the predominance of strains that possess factor 2 (types 1,2,3 and 1,2) in non-vaccinated communities (Preston, 1976). In the past, many countries have used vaccine that was rich in factor 2 but deficient in factor 3, and they have experienced a vast predominance of infections with type-1,3 strains (Preston, 1976). These organisms are normally at a disadvantage because of their lack of fimbriae, but they are sufficiently pathogenic to cause whooping cough in partially immunised children.

Britain was one such country in the 1960s. However, the recent low levels of vaccination have left a large population of children who are susceptible to fimbriate strains (types 1,2,3 and 1,2) and these have again replaced type-1,3 strains as the predominant serotypes (Preston, 1983).

Whole-cell vaccine containing all three agglutinins offers complete protection against whooping cough (Preston, 1983). However, if we wish to develop a subcellular vaccine (Preston, 1982), we must ensure that it includes agglutinin 3. The present study suggests that fimbriae alone may be inadequate.

We are grateful to the staff of the many laboratories throughout Britain from whom we received *B. pertussis* cultures for serotyping. We also thank Dr P. S. Handley and Mr L. M. Hill for their advice on the electron microscopy, and Mrs S. M. Craigen for secretarial assistance.

REFERENCES

- Blom J, Hansen G A, Poulsen F M 1983 Morphology of cells and hemagglutinogens of *Bordetella* species: resolution of substructural units in fimbriae of *Bordetella pertussis*. *Infection and Immunity* 42:308-317.
- Morse S I, Morse J H 1976 Isolation and properties of the leukocytosis- and lymphocytosis-promoting factor of *Bordetella pertussis*. *Journal of Experimental Medicine* 143:1483-1502.
- Preston N W 1966 Potency tests for pertussis vaccines: doubtful value of intracerebral challenge test in mice. *Journal of Pathology and Bacteriology* 91:173-179.
- Preston N W 1970 Technical problems in the laboratory diagnosis and prevention of whooping-cough. *Laboratory Practice* 19:482-486.
- Preston N W 1976 Prevalent serotypes of *Bordetella pertussis* in non-vaccinated communities. *Journal of Hygiene*, 77:85-91.
- Preston N W 1982 Toxicity of pertussis vaccine. *British Medical Journal* 284:1817-1818.
- Preston N W 1983 Whooping cough and pertussis vaccination. *British Medical Journal* 287:1881-1882.
- Preston N W, Stanbridge T N 1972 Efficacy of pertussis vaccines: a brighter horizon. *British Medical Journal* 3:448-451.
- Preston N W, Surapatana N, Carter E J 1982 A reappraisal of serotype factors 4, 5 and 6 of *Bordetella pertussis*. *Journal of Hygiene* 88:39-46.
- Sawyer W D, Rosenthal R S 1978 Pili and bacterial pathogenicity. In: Bradley D E, Raizen E, Fives-Taylor P, Ou J (eds) *Pili*. Robinson Blackmore, St John's, Newfoundland, pp 3-21.
- Stanbridge T N, Preston N W 1974 Variation of serotype in strains of *Bordetella pertussis*. *Journal of Hygiene* 73:305-310.
- Wilson G, Miles A, Parker M T (Eds) 1983 *Topley and Wilson's Principles of bacteriology, virology and immunity*, 7th edn. Edward Arnold, London. p 347.