SEROLOGICAL STUDY OF *BORDETELLA PERTUSSIS* AND RELATED SPECIES[1]

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It has become increasingly evident during recent years that cultures of *Bordetella pertussis* (Moreno-Lopéz, 1952) (*Haemophilus pertussis*), even when first isolated, differ serologically. The basis for the differences has been studied by various workers including Andersen (1953), Lacey (1953) and the group in our own laboratory, but no generally accepted antigenic analysis has been worked out. Neither is it known whether the differences among cultures are related in any way to differences in protective properties among vaccines prepared from particular cultures.

Andersen's studies based on the methods of Kauffmann led her to suggest a schema for the three species we now consider under the genus *Bordetella*. According to her, the relationships among the species are the result of a common heat stable "O" antigen and differences within species to heat labile K antigens, some of which are common to more than one species. Lacey's approach was to induce as many antigenic changes or "modulations" as possible by varying the incubation temperature and the salts in the medium.

In the present study, the *B. pertussis* cultures of Andersen were included as a basis for comparison and reference, and the differentiation of heat labile and heat stable antigens was attempted. Antigens were prepared from cultures of *B. pertussis*, *Bordetella parapertussis* and *Bordetella bronchiseptica* grown on Bordet-Gengou medium and killed either with Merthiolate, or with heat at 120 C for 1 hr. Antisera against the various antigens were produced in rabbits, and the pooled serum from two rabbits was used for agglutination and agglutinin adsorption tests.

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MATERIALS AND METHODS

Antigens. Cultures for antigens were grown on a modified Bordet-Gengou medium containing 16 per cent sheep blood. The growth was harvested into salt solution, and sedimented by centrifugation. The cells were resuspended, and the suspension standardized to the desired density with the aid of a photometer. The organisms were killed with Merthiolate 1:5,000, or with heat at 120 C for 1 hr.

Antisera. Antisera were produced in rabbits by the intravenous injection of the antigens described above. Rabbits with a preimmunization titer of 1:4 or greater for *B. pertussis*, *B. parapertussis*, or *B. bronchiseptica*, were rejected. The immunization schedule included graded doses of antigen increasing from 0.1 to 0.8 ml per kg of body weight, given at 3 day intervals over a 4 to 6 week period. Antisera were inactivated at 56 C for 30 min and preserved with Merthiolate 1:10,000.

Agglutination tests. A series of appropriate serum dilutions, usually ranging from 1:10 to 1:5,000, was prepared, and 0.1 ml amounts transferred to 10 by 75 mm tubes. The antigen, a suspension standardized to contain 20 billion organisms per ml, was added, 0.1 ml per tube. The tubes were shaken for 3 min in a mechanical shaker making 75 rpm, with a 4 inch stroke. The tubes were incubated for an hour in a 37 C water bath, followed by the addition of 0.5 ml salt solution to each tube to facilitate reading. The agglutination results were read against a light with the aid of a hand lens, and recorded as negative, 1, 2, 3, or 4, for each tube. The titer was taken as the highest dilution in which a 2 plus reaction occurred.

Agglutination-adsorption tests. Serum diluted 1:10 was added to a measured quantity of packed bacteria. After thorough mixing, the suspension was incubated for 4 hr in a 37 C water bath, and then placed in the cold room (10 C) overnight.

The cleared serum was tested for agglutinins, and the adsorption procedure repeated if indicated.

RESULTS

The results of agglutination tests using heated and unheated culture suspensions of each of the three species and the antisera produced against them are shown in table 1.

Antisera against unheated cultures had high agglutination titers for the homologous antigen, and low titers for heated antigens. However, antisera against heated cultures had low titers for heated antigens, and did not agglutinate unheated suspensions at all. The titers of the sera against heated cultures were the same for heterologous and homologous heated antigens. It is of interest that heated rough cultures of *B. pertussis* and *B. bronchiseptica* were also agglutinated by these antisera. The titers were not reduced by adsorption with unheated antigens. Reciprocal agglutinin adsorption tests indicated that the heat stable antigens of the three species were identical, although a quantitative difference was suggested by the observation that adsorption of the agglutinins from the *B. bronchiseptica* antiserum required twice the quantity of packed cells of *B. parapertussis*, and four times the quantity of *B. pertussis*, compared with the volume of *B. bronchiseptica* cells needed.

Attempts to show that *B. bronchiseptica* has a different heat stable antigen have so far been unsuccessful. These findings are in agreement with those of Andersen.

Heat labile antigens. At this time we are principally concerned with some of the results with the heat labile antigens, the K or envelope antigens of Kauffmann. Based on the results of agglutinin adsorption tests we have postulated 14 antigenic factors to explain the serological relationships. The results with Andersen's three type cultures of *B. pertussis*, strains 5373, 5374 and 5375, are shown in table 2.

The titers of the three antisera before adsorption are not shown in the table. Except in one instance each antiserum had an agglutination titer of 1:7,000 or higher for each of the three cultures. The exception was the 5373 antiserum with a titer of 1:2,000 for culture 5375. Serological differences were more apparent when the adsorbed antisera were tested, as shown in the table. The antigenic factors 1 through 5 listed under the culture numbers correspond with those

TABLE 1

Agglutination tests: Results with unheated and heated antigens and their homologous antisera*

Bordetella Test Antigens	Antiserum Against:	
	Unheated antigen	Heated antigen
B. pertussis #5375		
Unheated	5,000	Negative
Heated	20	200
B. bronchiseptica #5376		
Unheated	5,000	Negative
Heated	500	500
B. parapertussis #17-903		
Unheated	7,000	Negative
Heated	100	500

* Titers expressed as reciprocal of dilution.

TABLE 2

Heat labile antigens and their antisera: Agglutination tests with Bordetella pertussis antisera after adsorption with different cultures

Antiserum for Culture Number	Culture Used for Adsorption	Factors Left in Serum after Adsorption	Titers Reciprocal with Cultures		
			5373 1, 3, 6	5374 1, 2, 5, 6	5375 1, 2, 4
5373. Factors 1, 3, 6	5374	3	1,500	0*	0
	5375	3, 6	9,000	2,000	0
5374. Factors 1, 2, 5, 6	5373	2, 5	0	2,000	1,500
	5375	5, 6	1,000	100	0
	5373 + 5375	5	0	100	0
5375. Factors 1, 2, 4	5373	2, 4	0	500	1,500
	5374	4	0	0	200

* 0 = negative at 1:20.

assigned by Andersen. Factor 6 was added to explain a relationship between cultures 5373 and 5374 which is lacking between either of them and 5375. Differentially, antigenic factor 3 is found only in culture 5373, factor 5 in 5374, and factor 4 in 5375. The 5373 antiserum adsorbed with 5374 is specific for factor 3; adsorbed with 5375 it still has agglutinins for factors 3 and 6. In similar manner, by adsorbing 5374 and 5375 antisera, specific sera are obtained for factors 5 and 4, respectively. The factor 5 serum has a low titer with 5374. It is of interest that this serum has a higher titer for certain other cultures of *B. pertussis*. Attempts are in progress to produce an adsorbed serum with greater activity.

TABLE 3

Tentative analysis of the heat labile antigenic factors of Bordetella cultures

Culture	Factors
B. pertussis	
#5373	7, 1, 3, 6, 13
#5374	7, 1, 2, 5, 6, 13
#5375	7, 1, 2, 4, 13
B. parapertussis	
#17-903	7, 8, 9, 10, 14
B. bronchiseptica	
#5376	7, 8, 9, 12, 13
#214	7, 9, 12, 13
#899	7, 8, 10, 11, 12

Hypothetical antigenic factors for cultures of the three *Bordetella* species are listed in table 3.

Some of the data on which the *B. pertussis* factors are based are given in table 2. In studying *B. parapertussis*, the results of reciprocal agglutinin adsorption tests, using four cultures and their antisera, indicated that the cultures were serologically identical, confirming our observations over many years that this species is homogeneous.

Of six cultures of *B. bronchiseptica* studied, four were shown to be serologically identical. These are represented in the table by culture 5376. Culture 214 lacked an antigen present in 5376, factor 8 in the table. Culture 899 lacked a different antigen, factor 9, and contained an antigen, factor 10, not found in the other *B. bronchiseptica* cultures.

Information on the relationships among the three species was obtained by reciprocal agglutinin adsorption tests, using the cultures listed and their antisera.

Factors 1 through 6 are found in cultures of *B. pertussis* only, and factor 7 in all Bordetella cultures. Factor 14 is specific for *B. parapertussis*, and 12 for *B. bronchiseptica*. Factors 8, 9 and 10 account for relationships between *B. parapertussis* and different cultures of *B. bronchiseptica*. These factors do not explain all relationships, and changes will almost certainly have to be made as further cultures are studied.

As an example of the method used to check the

TABLE 4

Results with Bordetella bronchiseptica antiserum against culture 899, factors 7, 8, 10, 11, 12, tested before and after adsorption, using unheated antigens, and antiserum against unheated culture

Test Antigens		Titers before Adsorption	Titers (Reciprocal) after Adsorption				
			Adsorbing cultures and factors				
			#5373 *Bordetella pertussis* 7, 1, 3, 6, 13	#17-903 *Bordetella parapertussis* 7, 8, 9, 10, 14	#899	#214	#22-067
					Bordetella bronchiseptica		
					7, 8, 10, 11, 12	7, 9, 12, 13	7, 8, 9, 12 13
Culture	Factors		Factors remaining in adsorbed serum				
			8, 10, 11, 12	11, 12	0	8, 10, 11	10, 11
B. pertussis #5373	7, 1, 3, 6, 13	5,000	0	200	0	0	0
B. parapertussis #17-903	7, 8, 9, 10, 14	16,000	14,000	0	0	7,000	3,000
B. bronchiseptica							
#899	7, 8, 10, 11, 12	12,000	14,000	2,000	0	4,000	2,000
#214	7, 9, 12, 13	6,000	5,000	2,000	0	0	0
#22-067	7, 8, 9, 12, 13	8,000	5,000	3,000	0	3,000	0

validity of the hypothetical scheme, the results with one antiserum are given in table 4.

The antiserum used in these tests was produced with *B. bronchiseptica* culture 899, to which has been assigned antigenic factors 7, 8, 10, 11 and 12. The antigens used in agglutination tests and their factors are listed at the left, with the titers of the unadsorbed serum in the next column. The adsorbing cultures with their factors, and the hypothetical agglutinins remaining after adsorption, head the columns at the right. Since *B. pertussis* is related to culture 899 only through factor 7, which is present in all Bordetella cultures, all *B. pertussis* agglutinins should be removed by any of the adsorbing cultures. The 1:200 titer for *B. pertussis* remaining after adsorption with *B. parapertussis* is inconsistent with the theoretical scheme, and is unexplained. *B. pertussis*, which removes only factor 7, does not materially reduce the titer for the other cultures. *B. parapertussis*, which removes factors 7, 8 and 10, leaves only 11 and 12. The titers of 1:2,000 or 1:3,000 for *B. bronchiseptica* are consistent with the hypothetical factors. Removal of agglutinins for *B. bronchiseptica* strain 214 leaves agglutinins for factors 8, 10 and 11, reactive with 899, 22–067, and *B. parapertussis*. *B. bronchiseptica* strain 22-067 removes all agglutinins except those for factors 10 and 11, found in *B. parapertussis* and *B. bronchiseptica* strain 899.

It is obviously impossible to present here all the data upon which the factors as presented are based. Twelve other antisera were adsorbed and tested in a similar manner to the one shown in table 4. In general the results were those that would be expected if the antigenic factors as listed are correct. Work in progress includes testing of many cultures with adsorbed sera, and investigation of the significance of serological groups in relation to protection. We are also investigating these antigens by means of agar diffusion procedures.

SUMMARY

Results of agglutination and agglutinin-adsorption tests indicate the presence of a heat stable antigen common to *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica*. Rough strains of *B. pertussis* and *B. bronchiseptica* also contain this heat stable antigen.

An antigenic schema for the heat labile antigens of *Bordetella* has been postulated, using numbers 1 through 14. Specific antisera for various antigens have been prepared for use in the study of many cultures of each species. The relationship of the various antigens to protection is being investigated.

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