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Mouse-protective Properties of *Bordetella pertussis* Serotypes in Passive Tests

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In a passive protection procedure in which the ED_{50} values of *Bordetella pertussis* antisera were determined, groups of mice were given graded intraperitoneal doses of serum, followed the next day by intracerebral challenge with 100,000 organisms. Antiserum produced with *B. pertussis* culture 5373, serotype 1.3, protected mice against challenge with culture 18-323, serotype 1.2.3, as effectively as did an antiserum produced with a serotype 1.2.3 culture. When two groups of mice similarly treated with pertussis immune serum were challenged with culture 353Z (serotype 1) and 18-323, respectively, much lower ED_{50} values were obtained with the animals challenged with 353Z. Passive protection tests with adsorbed antiserum gave equivocal results, suggesting that some of the adsorbing antigen remained in the serum and interfered with the tests. There was no evidence that serotype is related to protection.

The antigenic analysis of *Bordetella pertussis* described by Andersen (1) in 1953 and confirmed and extended by Eldering, Hornbeck, and Baker (3) in 1957 has recently attracted greater interest because of the reports of Preston (4, 5, 6) and of Preston and Evans (7), in which serotype is linked to protection. A recent shift from serotypes 1.2 and 1.2.3 to 1.3 among cultures isolated from whooping cough patients in Britain was also reported by Preston (5). The evidence supporting the view that protection against infection with a culture of a particular serotype is dependent upon immunization with a vaccine of that serotype is mainly circumstantial. However, the possibility that such an association exists should be thoroughly investigated, since the practical implications with respect to the choice of cultures for vaccine are evident.

Recently, we (2) reported the results of mouse protection tests in which animals immunized with single-culture vaccines of *B. pertussis* representing various serotypes were challenged either with culture 18-323 or with 353Z. When the challenge culture was 18-323, the one generally used in potency tests of vaccine, the ED_{50} results were the same (within the limits of error of the test) no matter what serotype was used for immunization. This was true whether the vaccine was prepared with a culture like 18-323 having agglutinogens 1.2.3.4.5.7, or with the Preston strain 353Z with only agglutinogen 1. However, when immunized mice were challenged with culture 353Z, much lower ED_{50} results were observed (better protection), and this was true regardless of the serotypes represented in the vaccine. The LD_{50} values for the two challenge cultures were remarkably similar, so that the difference in ED_{50} end points could not be explained by a difference in virulence.

When unimmunized mice were inoculated intracerebrally with the two challenge cultures, certain differences were observed. Mice receiving culture 18-323 died earlier than those infected with 353Z, although by the end of the 14-day observation period these differences disappeared. Also, groups of mice given graded doses of the cultures were more likely to give a well-graded response to 18-323 than to 353Z. It seems unlikely that these differences can be attributed to the serotypes.

This report is concerned with passive mouse protection tests of antisera produced in rabbits against *B. pertussis* cultures representing various serotypes, and several challenge cultures.

MATERIALS AND METHODS

Methods for the production of antisera and for agglutinin adsorption have been described earlier (3). The antisera used in the experiments reported here were usually produced with a series of 8 to 12 injections, given at 3-day intervals. The sera were inactivated at 56 C for 30 min and were preserved with Merthiolate (1:10,000).

Passive protection tests in mice. The mice were white Swiss from the Michigan Department of Public Health colony. Mixed sexes of the same age were used, with a weight range of 20 to 24 g, a range chosen to

correspond with that of actively immunized mice at the time of challenge. Three groups of 14 mice each were injected intraperitoneally with graded doses of the test serum in a 0.5-ml volume. For example, for the reference serum used in these tests, a dosage schedule giving a graded response was 0.1, 0.01, and 0.001 ml. At 18 to 24 hr after the mice were injected with serum, they were challenged with an intracerebral inoculation of living organisms contained in a 0.03-ml volume, the dose standardized to 100,000 bacilli. At the same time, three groups of 10 mice each were given graded doses of the challenge culture for determination of its LD_{50}, and 10 mice received the challenge dose of 100,000 organisms, as a control.

The mice were observed daily for 14 days, and the deaths were recorded. Any deaths on the 1st or 2nd day after challenge were considered to be due to trauma and were omitted from the calculations. The ED_{50} for each test serum and the LD_{50} for the challenge culture were calculated by a regression curve method.

Preimmunization serum corresponding to each test serum was tested at least once by use of the largest test dose (either 0.1 or 0.2 ml). Examples of the results in terms of surviving mice per total number tested are as follows: 5/47, 3/32, 2/41, 2/42.

Serotypes. To conform with reports of Preston and others, the data are limited to those concerned with factors 1, 2, and 3. Factor 4 described by Andersen (1) is present in her type culture 5375, but has not been found in recently isolated cultures. Factor 5 is nearly always found with 2, and agglutinins for 2 and 5 are difficult to separate. Factor 6 is usually found with 3, although 3 may be present without 6. Factor 13 is a weak antigen which is of interest mainly because it is found in some strains of *B. pertussis* and also *B. bronchiseptica*.

RESULTS

A hyperimmune pertussis serum produced in rabbits with multiple-culture vaccine was used as a reference. In 19 consecutive tests of this serum, the mean ED_{50} was 0.017 ml and the median was 0.013. The range was 0.002 to 0.050, and one standard deviation was 0.012. With four extreme values omitted (the two highest and two lowest), the range was 0.004 to 0.029.

Three antisera produced with culture 5373, serotype 1.3, were tested in eight experiments, by use of culture 18-323, serotype 1.2.3, for challenge. All three sera showed good protection, with R651 perhaps slightly more effective than the other two (Table 1). The mean of the eight results was 0.024 ml. These results show that antisera produced with a 1.3 culture protect against challenge with a serotype 1.2.3 culture.

In further experiments, the protective property of the reference serum was tested with two challenge cultures. Since preliminary tests had shown that smaller doses were needed to protect against challenge culture 353Z than against culture 18-323, the dosage schedule 0.01, 0.001, and 0.0001 ml was used for mice to be challenged with this culture, and the usual amounts, 0.1, 0.01, and 0.001 ml, for animals challenged with culture 18-323. The results of five tests are given in Table 2. The mean ED_{50} for the tests in which 18-323 was used for challenge was 0.0249 ml, and for mice challenged with 353Z, 0.0009 ml, a ratio of 28:1. As in active protection tests, it was easier to protect against the factor 1 culture 353Z than against 18-323. Table 3 summarizes the results of an experiment in which groups of mice given antisera representing three different serotypes were challenged with cultures of serotype 1.2.3, 1.3, and 1.2, in a nine-way test. The tabulated results should be compared horizontally rather than vertically, since the three sera were not produced in exactly the same way. The reference serum lot 9 tested with culture 18-323 had an ED_{50} of 0.016; with the challenge culture 16-433, serotype 1.2, the ED_{50} was 0.003. The response with the 1.3 culture, 19-466, was invalid. Both sera R744, serotype 1, and R740, serotype 1.3, gave the highest ED_{50} values with challenge culture 18-323 and the lowest with the 1.3 culture, 19-466. In experiments not tabulated, mice given the various antisera were challenged with culture

TABLE 1. *Protection afforded by three antisera produced with Bordetella pertussis 5373, serotype 1.3*

Serum	Expt no.	ED_{50} (ml)	
		Test	Reference
R651	548	0.008	0.008
	550	0.003	0.006
R662	555	0.013	0.003
	557	0.008	0.003
	562	0.023	0.020
	592	0.032	no test
R711	611	0.074	0.029
	619	0.032	0.012

TABLE 2. *Tests of pertussis reference serum comparing two challenge cultures*

Expt no.	ED_{50} (ml)	
	18-323 challenge	353Z challenge
624	0.0035	0.0003
634	0.0130	0.0009
635	0.0500	0.0023
636	0.0320	0.0003
637	0.0260	0.0007
Mean	0.0249	0.0009

353Z. The ED_{50} for reference 9, serotype 1.2.3 (average of four tests), was 0.0012; for R744, serotype 1, 0.028; and for R740, serotype 1.3, 0.005. It is of interest that in all of these tests it appeared to be more difficult to protect against the 18-323 challenge than against any of the others.

Protection tests with antiserum adsorbed to specificity for a single serotype were attempted. An experiment is shown in Table 4 in which unadsorbed and adsorbed serum R651 produced with the 1.3 culture 5373 was tested. Surviving mice per total number tested are shown for the animals receiving each dose of the serum. The response with the unadsorbed serum was graded, with 6 of 18, 10 of 21, and 15 of 19 mice surviving in the respective dosage groups, or an ED_{50} of 0.007 ml. A specific factor 1 serum was obtained by adsorbing R651 with *B. bronchiseptica* to remove the genus agglutinins (factor 7), and with boiled homologous antigen 5373 to remove factor 3 agglutinins. The test with this adsorbed serum gave a poorly graded response, i.e., 0/20, 9/20, and 8/17 for the three doses of serum. The ED_{50} was 0.050 ml, which was much higher than the end point of the unadsorbed serum. However, the survivors in the groups receiving the mid-dose of both the unadsorbed and adsorbed serum were almost the same. Serum R651 adsorbed to retain only factor 3 agglutinins gave an ungraded response: the survivors per total number tested were: 2/20, 12/20, and 2/18 in the different doses.

In five other experiments testing 13 adsorbed sera, there were seven ungraded responses, with the largest proportion of survivors in the groups receiving the mid-dose of serum. In some tests, the largest amount of serum, 0.1 ml, killed many of the mice in the interval before challenge. There were instances in which the mid-dose of the adsorbed serum protected a larger proportion of the mice than did the same dose of the unadsorbed serum. These anomalous results suggested that some of the adsorbing culture remained in the serum, making it toxic for the mice. We plan to attempt to remove the antigen by passing the sera through a diethylaminoethyl column. Until we have the results of such studies, it is impossible to interpret the results in Table 4.

TABLE 3. *Bordetella pertussis passive protection tests using three different challenge cultures*[a]

Antiserum	ED_{50} (ml) with challenge culture		
	18-323 (1.2.3)	19-466 (1.3)	16-433 (1.2)
Reference 9 (1.2.3)..	0.016	not graded	0.003
R744 (serotype 1)...	0.065	0.006	0.047
R740 (serotype 1.3)..	0.087	0.004	0.017

[a] The LD_{50} of challenge culture 18-323 was 300 organisms; of 19-466, 3,400; and of 16-433, 950.

TABLE 4. *Protection tests with unadsorbed and adsorbed antiserum produced with culture 5373, serotype 1.3*

Serum R651	S/T[a] with doses			ED_{50}
	0.001	0.01	0.1	
				ml
Unadsorbed.	6/18	10/21	15/19	0.007
Adsorbed to factor 1...	0/20	9/20	8/17	Not graded
Adsorbed to factor 3...	2/20	12/20	2/18	Not graded
Preimmune..	0/17	1/15	4/15	Little protection
Reference no. 5.....	3/20	8/20	17/20	0.013

[a] Survivors per total number tested.

DISCUSSION

The results of passive protection tests in mice, like those of active tests presented earlier (2), give no indication that protection is associated with serotype. Antisera produced with cultures of serotype 1, 1.3, or 1.2.3 protected against challenge with *B. pertussis* culture 18-323, serotype 1.2.3. When challenge cultures 18-323 and the Preston serotype 1 culture 353Z were compared, lower ED_{50} results (better protection) were obtained with the 353Z culture. Challenge cultures representing serotypes 1.2 and 1.3 were also tested in comparison with the 1.2.3 culture, and here again no serotype specificity was observed.

The validity of the results is supported by a number of the conditions of the tests. In each instance, the challenge culture was used in a dosage of 100,000 organisms. For cultures 18-323 and 353Z, this is approximately 300 times the LD_{50}. The sera were given in graded doses with 10-fold increments, and the result occurring after challenge was accepted only if the response was graded.

Differences among the various challenge cultures, apart from their agglutinogens, are of interest. One can only speculate as to the effect on the whole pertussis immunization program had a challenge culture other than 18-323 been used in the official potency tests.

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