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Bordetella pertussis Culture Having Only Species Factor 1

GRACE ELDERING, JACK HOLWERDA, AND JULIA BAKER

Division of Western Michigan, Bureau of Laboratories, Michigan Department of Public Health, Grand Rapids, Michigan

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ABSTRACT

ELDERING, GRACE (Michigan Department of Public Health, Grand Rapids), JACK HOLWERDA, AND JULIA BAKER. *Bordetella pertussis* culture having only species factor 1. J. Bacteriol. **91**:1759–1762. 1966.—Preston's description of *Bordetella pertussis* 353-Z as a culture possessing only factor 1 was confirmed by agglutination and agglutinin-adsorption tests. The LD_{50} values of this culture for mice inoculated by the intracerebral route were approximately the same as that of *B. pertussis* 18-323, the challenge culture in mouse potency tests of pertussis vaccine. In mouse protection tests, vaccine prepared with the factor 1 culture protected as well against challenge with *B. pertussis* 18-323, which has a broad antigenic pattern, as did vaccines made with cultures possessing factors 2, 3, 4, and 5 in addition to factor 1. When two groups of similarly immunized mice were challenged with 353-Z and 18-323, respectively, much lower ED_{50} values were obtained with the animals challenged with 353-Z. This was true whether a factor 1 vaccine or a vaccine with factors 1, 2, 3, 4, and 5 was tested.

In 1963, Preston and Evans (8) described a culture of *Bordetella pertussis*, designated 353-Z, that was unique in possessing only the common species-specific antigen, factor 1. From the results of passive protection tests in mice with the use of adsorbed antisera and challenging with either culture 353-Z or cultures having broader antigenic patterns, these authors concluded that protection was due to antibody specific for particular antigenic factors or agglutinogens. Later, Preston (7) reported that cultures isolated from whooping cough patients had changed in antigenic composition in recent years, and that the cultures used for the preparation of pertussis vaccine in Great Britain had not reflected the change. He suggested that the increased incidence of whooping cough in his country was due to a deficiency in the vaccine of a particular antigen, specifically factor 3. Chalvardjian (3) found that 56 of 58 *B. pertussis* cultures isolated in Toronto were of the 1.3 serotype and lacked factor 2. She reported also that 7 of 10 pertussis vaccines in use in the community were deficient in factor 3 antigen.

The antigenic schema for the genus *Bordetella* was described by Andersen (1) and expanded by Eldering, Hornbeck, and Baker (4). Eight heat-labile factors are postulated for *B. pertussis*, two (factors 1 and 7) of which are found in all *B. pertussis* cultures. Factor 7 is also common to the other members of the genus, *B. parapertussis* and *B. bronchiseptica*. The other six factors, 2, 3, 4, 5, 6, and 13, are found in various combinations, and their presence or absence may be used to separate cultures into serological groups. It is obvious that there are possible practical applications of the antigenic analysis in addition to its use as an epidemiological tool in following outbreaks of whooping cough. For example, if the serotype were related to protection, the selection of cultures for vaccine production should be based at least in part on the antigenic pattern of the cultures causing pertussis in the community.

A culture having only factor 1, such as the one described by Preston, is of particular interest, since it makes possible the preparation of a species-specific antiserum for *B. pertussis* without resorting to adsorption procedures. It also points the way to a new attack on the old problem of whether agglutinins and protective antibodies are the same. In 1963, we obtained a subculture of 353-Z from Preston, and the results of our work on this culture form the basis for this report.

MATERIALS AND METHODS

The procedures for the preparation of antisera against the various factors were described in an earlier report (4). It is of interest to recall that, before the Preston culture was available, factor 1 antisera had been prepared by adsorbing a 1.3 antiserum, produced with a culture like 5373, with the homologous culture that had been heated at 100 C for 2 hr. Heating at this temperature destroys the factor 1 antigen, but does not destroy the factor 3 adsorbing property of the culture.

RESULTS

B. pertussis 353-Z conforms to the criteria for smooth cultures, including typical colony appearance with a hemolytic zone on Bordet-Gengou medium and a typical morphology and arrangement of the organisms. Unlike most other *B. pertussis* cultures, it grows poorly in Cohen-Wheeler medium, and in only one of many trials was growth obtained in fluid medium that would protect mice against intracerebral challenge. Growth on Bordet-Gengou medium yields a protective antigen.

Agglutinability and agglutinin-producing property. Preston's description of 353-Z was confirmed by use of adsorbed antisera prepared in this laboratory. The culture was not agglutinated by sera specific for factors 2, 3, 5, and 6, and was agglutinated by factor 1 serum to the titer of the serum.

Antisera for 353-Z were produced in five rabbits. Agglutinin titers before and after adsorption of one of these antisera with each of three other strains were determined by use of 353-Z and the other three strains as antigens (Table 1). The unadsorbed serum had a high titer for each of the type cultures. Adsorption with culture 5373 to remove factors 1 and 3 left no agglutinins for 5374 and 5375, indicating that the 353-Z serum had no agglutinins for 2, 4, and 5. If agglutinins for factor 3 were present, adsorption with 5374 and 5375 would not remove these, and the serum so adsorbed would still react with 5373. However, agglutinins for 5373 were completely removed. The conclusion was that 353-Z was indeed a factor 1 culture. It should be recalled that all *Bordetella* cultures possessed factor 7, common to the genus. Apparently this was present as a weak antigen in 353-Z, since the antiserum described, R-708, had titers of only 1:20 and 1:100 for *B. parapertussis* and *B. bronchiseptica*, respectively. Further experiments relating to the agglutinin-adsorbing property of 353-Z only added to the evidence indicating that it was a factor 1 culture, and the details are omitted here.

TABLE 1. *Agglutination titers of unadsorbed and adsorbed antiserum produced with Bordetella pertussis 353-Z*

Antigen for agglutination test		Antiserum R-708			
Culture	Factors present	Not adsorbed	Adsorbed with culture		
			5373	5374	5375
353-Z	1	+5,000	+20	+20	+20
5373	1, 3	+4,000	Neg*	Neg	Neg
5374	1, 2, 5	+4,000	Neg	Neg	Neg
5375	1, 2, 4	+5,000	Neg	Neg	Neg

* Neg indicates no agglutination with 1:10 dilution of serum.

Virulence for mice. Virulence of the 353-Z culture for mice inoculated by the intracerebral route was compared with that of culture 18-323, the one routinely used for challenge in mouse potency tests of pertussis vaccine. The usual procedure was followed: the 24-hr growth on Bordet-Gengou medium was harvested in 1% Casamino Acids solution, and groups of 10 mice each were inoculated with graded doses of the culture suspension of 80, 400, and 2,000 organisms contained in 0.03 ml. The deaths occurring from the 3rd through the 14th day formed the basis for calculating the LD_{50} for the particular culture. The mean LD_{50} values for the six tests were 188 for 353-Z and 224 for 18-323, while the median LD_{50} values were 165 and 235, respectively (Table 2). It is evident that the two cultures were similar with respect to mouse virulence. For each culture, the challenge dose of 100,000 organisms used in the mouse potency test represented from 400 to 500 times the LD_{50}.

Mouse protection tests. Thirteen mouse protection tests were carried out over a 2-year period, in which mice immunized with either 353-Z or 10-536 antigen were challenged with *B. pertussis* 18-323 (Table 3). The ED_{50} results are expressed in milliliters of a vaccine containing 10 billion organisms per milliliter. The mean ED_{50} for the 353-Z vaccine was 0.031 ml and the median was 0.032 ml, compared with a mean of 0.035 ml and a median of 0.033 ml for the 10-536 vaccine. The two vaccines protected equally well against challenge with culture 18-323. It should be noted that cultures 10-536 and 18-323 have broad antigenic patterns; they possess factors 2, 3, 4, and 5 in addition to 1 and 7. If antigens 2, 3, 4, or 5 have anything to do with protection, the 353-Z vaccine with none of these antigens could not be expected to protect, particularly against challenge with a culture like 18-323.

The protective units were calculated for the two antigens in comparison with the National Institutes of Health Reference Antigen, as is done

TABLE 2. *Virulence for mice of Bordetella pertussis cultures 353-Z and 18-323*

Date of test	LD_{50} (no. of organisms)	
	353-Z	18-223
May 1963	300	230
August 1963	45	36
October 1963	105	240
December 1963	150	250
January 1964	180	380
August 1965	350	210
Mean	188	224
Median	165	235

for distribution lots of vaccine. Based on the mean ED_{50} values, the protective units for the 353-Z antigen were 7.1, and for 10-536, 6.4. There was no significant difference between the two vaccines and the National Institutes of Health Standard vaccine (analysis of variance, $P > 0.5$).

Five experiments were performed in which mice immunized with vaccines made from either 353-Z or 10-536 were challenged with 353-Z and 18-323 cultures (Table 4). Both vaccines were more effective in protecting mice against challenge with 353-Z than with 18-323. The ED_{50} doses were much lower when challenged with 353-Z. The difference, however, cannot be assessed quantitatively, because the ED_{50} was so low with 353-Z that it was below the lowest vaccine dose and could not be measured accurately. To detect whether there was a difference between the two vaccines in the response to the two challenge organisms, a different dosage schedule should be used for mice to be challenged with 353-Z, since in the ideal test the ED_{50} should fall somewhere around the mid-dose.

TABLE 3. *Bordetella pertussis mouse protection tests in which 353-Z, 10-536, and National Institutes of Health (NIH) reference vaccines were tested with challenge culture 18-323*

Expt no.	ED_{50}*		
	353-Z	10-536	NIH reference
585	0.032	0.066	0.020
587	0.025	—†	0.013
590	0.067	0.067	0.029
593	0.056	—	0.056
594	0.030	0.055	0.021
596	0.036	0.033	0.045
597	0.012	0.021	0.037
598	0.034	0.025	—
599	0.004	0.004	0.009
606	0.045	0.035	0.032
608	0.014	0.023	0.013
609	0.017	0.017	0.034
614	0.036	0.040	0.025
Mean ED_{50}	0.031	0.035	0.028
Standard error of mean	0.0049	0.0061	0.0040
Protective units based on mean	7.1	6.4	8.0‡
Median ED_{50}	0.032	0.033	0.027
No. of tests	13	11	12

* Expressed in milliliters of a vaccine containing 10 billion organisms per milliliter.
† No test.
‡ By definition.

DISCUSSION

The selection of cultures for pertussis vaccine is a matter of concern for manufacturing laboratory workers, epidemiologists, and immunologists. Thirty-five years ago, following Leslie and Gard-

TABLE 4. *Comparison of results with two challenge cultures in Bordetella pertussis mouse protection tests*

Expt no.	ED_{50} *					
	353-Z vaccine challenged by		10-536 vaccine challenged by		NIH reference challenged by	
	353-Z	18-323	353-Z	18-323	353-Z	18-323
587	(0.001)†	0.025	—	—	0.007	0.013
590	0.010	0.067	0.020	0.067	0.017	0.029
593	(0.005)	0.056	—	—	0.010	0.056
594	(0.003)	0.030	(0.005)	0.055	(0.004)	0.021
620	0.013	0.130	0.021	0.060	0.011	0.063
Mean	(0.006)	0.062	(0.015)	0.061	(0.010)	0.036

* Expressed in milliliters of a vaccine containing 10 billion organisms per milliliter.
† Numbers in parentheses indicate estimated values due to tests having ED_{50} below lowest dose.

ner's (5) description of smooth-to-rough changes among cultures of *B. pertussis*, recently isolated cultures which conformed to the description of phase I or smooth cultures were chosen. When methods for the preservation of cultures in the dried state were developed, it was generally agreed that lyophilized cultures could be used if they were smooth and were used within a limited number of subcultures from the dried state. With the development of the mouse potency test it was possible to test single culture suspensions, and the mouse protective property of a culture became a major criterion for selection. The general practice has always been, and still is, to use several cultures, usually at least four, for each lot of vaccine on the chance that some elusive property not yet defined may be possessed by certain cultures and not by others, and make the difference between a "good" vaccine and a "poor" one. The possibility that serotyping of cultures of *B. pertussis* according to the antigenic analysis based on agglutination reactions might prove to be a sound basis for selection of cultures is indeed an attractive one.

Convincing supportive evidence, however, is still lacking. Andersen and Bentzon (2) reported that they could find no correlation between type specificity and protection in the mouse, using intranasal challenge with a sublethal dose. Munoz (6) describes the *B. pertussis* agglutinogen as the most external of the many antigens possessed by the bacterial cell, and locates the protective antigen in the cell wall. He has separated an antigen which protects mice against intracerebral challenge, and which appears to be free from agglutinogen. The data presented here show no difference in protective property between vaccines prepared with a factor 1 culture and those prepared with cultures having broad antigenic patterns, when tested in mice challenged with the usual culture, 18-323.

On the other hand, it is difficult to explain the wide difference in protection which we observed in vaccinated mice, as measured by the two different challenge cultures, 353-Z and 18-323. At present we have no explanation, but we hope to explore the matter further, starting with passive protection tests, using various unadsorbed and adsorbed antisera and challenging with 353-Z and 18-323.

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