FLUORESCENT ANTIBODY STAINING AND AGGLUTINATION REACTIONS IN *BORDETELLA PERTUSSIS* CULTURES[1]

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ABSTRACT

ELDERING, GRACE (Michigan Department of Health, Grand Rapids), WARREN C. EVELAND, AND PEARL L. KENDRICK. Fluorescent antibody staining and agglutination reactions in *Bordetella pertussis* cultures. J. Bacteriol. **83**:745–749. 1962—*Bordetella pertussis* antisera produced with smooth cultures gave positive results in agglutination and fluorescent antibody (FA) tests with smooth *B. pertussis* cultures. Similar results were obtained with antisera produced with the related species *B. parapertussis* and *B. bronchiseptica* when each was tested with the respective homologous antigen. Cross reactions occurred with some of the antisera and heterologous antigens but a positive agglutination test was not always correlated with a positive FA reaction, and the reverse was also observed. Adsorbed *B. pertussis* antisera specific for factors 2, 3, 4, and 5 agglutinated appropriate *B. pertussis* cultures, but gave negative FA reactions. Factor 1 antiserum prepared by adsorption with heated (100 C) antigen gave positive results in both FA and agglutination tests. Rough *B. pertussis* cultures were inagglutinable, but were FA positive when tested with unadsorbed antiserum produced with either smooth or rough cultures. Rough cultures were FA negative with factor 1 serum. The results suggest that for *B. pertussis* different serum components may be responsible for agglutination and FA staining. The implications with respect to the protection-inducing antigen are discussed.

While studying the application of the fluorescent antibody (FA) technique to the diagnosis of whooping cough (Kendrick, Eldering, and Eveland, 1961), we observed certain discrepancies in the results of *Bordetella pertussis* agglutination tests and FA reactions. When cultures isolated on Bordet-Gengou medium were tested with unadsorbed *B. pertussis* antiserum, the results were uniformly positive by both methods. However, adsorbed *B. pertussis* antiserum specific for particular serological antigens or factors gave negative FA results with all of the cultures tested, although the agglutination titers for the appropriate cultures were substantial. Such findings differed from those with other bacterial species. For example, adsorbed *Escherichia coli* antisera have been shown to react specifically with appropriate cultures in both agglutination and FA tests (Whitaker et al., 1958). The observations with *B. pertussis* led to the studies forming the basis for this report. The results suggest that the serum components responsible for the two antigen-antibody reactions may be different.

MATERIALS AND METHODS

Cultures. Cultures of *B. pertussis* and the related species, *B. parapertussis* and *B. bronchiseptica*, were from our stock collection, maintained in the lyophilized state and revived just before use.

Media. Bordet-Gengou medium, modified as described previously (Kendrick, Lawson, and Miller, 1950), was used for reconstituting cultures from the lyophilized state, and for the preparation of antigens for agglutination tests and slide preparations for FA staining. For the most part, cultures were grown in Cohen-Wheeler fluid medium for the preparation of antigens for antiserum production.

Antisera. Antisera were produced in rabbits according to the method of Kendrick et al. (1950), using killed culture suspensions as antigens. The procedure for adsorbing agglutinins has been described in an earlier report (Eldering, Hornbeck, and Baker, 1957). In most instances, repeated contact with the adsorbing antigen was required.

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TABLE 1. *Comparative results of fluorescent antibody (FA) staining and agglutination (Ag) tests with Bordetella pertussis antisera*

B. pertussis		*B. pertussis*		*B. parapertussis*		*B. bronchiseptica*	
Antiserum no.	Culture no.	FA*	Ag	FA	Ag	FA	Ag
R 573	5373	4+	7,000	−	200	−	500
R 550	5374	4+	5,000	−	300	3+	80
R 26	10536	4+	5,000	−	500	4+	100
R 583	10536	4+	5,000	−	1,000	−	500
NY 3996	40103	4+	5,000	−	500	4+	100

* FA results expressed as: −, negative; 2, 3, and 4+, relative fluorescence.

Agglutination tests. The procedure described by Kendrick et al. (1950) for agglutination tests for *B. pertussis* was followed, with slight modifications. The test materials were shaken in a mechanical shaker, instead of being rocked by hand. Preparations with unadsorbed antisera were incubated for 1 hr in a water bath (37 C); those with adsorbed antisera were allowed to stand overnight in the cold (10 C).

FA procedure. The whole serum or the adsorbed serum was conjugated with fluorescein isothiocyanate according to the method of Marshall, Eveland, and Smith (1958). In some instances, the globulin fraction was obtained by the method of Riggs, Loh, and Eveland (1960). As a check procedure, some sera were tested by the indirect FA method, i.e., the culture was treated with unlabeled antiserum followed by conjugated rabbit globulin antiserum prepared in the goat.

For examination of the slides, a Zeiss microscope with an Osram light source, a BG 12 exciter filter, and OG4 and GG4 barrier filters was used.

RESULTS

Reactions with unadsorbed Bordetella antisera. It has already been amply demonstrated by our own work (Kendrick et al., 1961), and by others (Repentigny and Frappier, 1956; Donaldson and Whitaker, 1960) that *B. pertussis* gives positive FA reactions with specific unadsorbed antiserum. Since cross-agglutination reactions are known to occur among the three *Bordetella* species, *pertussis*, *parapertussis*, and *bronchiseptica*, it was of interest to find out whether cross reaction could be demonstrated with the FA staining procedure. Table 1 shows the results with five unadsorbed *B. pertussis* antisera tested with each of the three species.

The *B. pertussis* antisera agglutinated *B. pertussis* cultures with titers of 1:5,000 or higher, and also gave strong FA reactions. The cross-agglutination titers with *B. parapertussis* ranged from 1:200 to 1:1,000, but FA reactions were consistently negative. With all but one serum, lower agglutination titers were observed for *B. bronchiseptica* than for *B. parapertussis*. The FA results with *B. bronchiseptica* varied, being positive with three of the antisera and negative with two.

Table 2 gives the results with two *B. parapertussis* and two *B. bronchiseptica* antisera. As would be expected, in each instance the antiserum reacted strongly with the homologous species in both agglutination and FA tests. One of the two *B. parapertussis* antisera agglutinated in low titer the two related species, and also caused fluorescent staining of both. The second serum was negative in both types of tests with *B. pertussis*. It was also negative with *B. bronchiseptica* by FA, but had a low agglutination titer (1:80) for this antigen. Both of the *B. bronchiseptica* antisera showed cross reactions against *B. pertussis* with both procedures. Both also agglutinated *B. parapertussis*, but one was positive and the other negative by the FA procedure. It is noted that in the cross reactions there was little correlation between agglutination reaction and FA staining. Some of the antisera with high titers for particular antigens failed to show fluorescent staining with those antigens, and the reverse was also true.

Reactions with adsorbed Bordetella antisera. In an earlier report (Eldering et al., 1957), the antigenic schema described by Andersen (1953) for the genus *Bordetella* was confirmed and expanded. Table 3 shows the various serological factors in relation to the cultures in which they have been demonstrated. According to the hypothetical schema, factor 7 is found in all *Bordetella* cultures, and explains in part the sero-

TABLE 2. *Comparative results of fluorescent antibody (FA) staining and agglutination (Ag) tests with Bordetella parapertussis and B. bronchiseptica antisera*

Antiserum	*B. pertussis*		*B. parapertussis*		*B. bronchiseptica*	
	FA	Ag	FA	Ag	FA	Ag
B. parapertussis R 457	2+	200	4+	9,000	2+	500
B. parapertussis R 616	—	—	3+	2,500	—	80
B. bronchiseptica R 3	2+	—	3+	2,000	4+	7,500
B. bronchiseptica R 558	4+	320	—	2,500	4+	1,200

TABLE 3. *Tentative analysis of 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

logical relationships among the species. All *B. pertussis* cultures have factor 1. Factor 12 is species specific for *B. bronchiseptica* and factor 14 for *B. parapertussis*. Factors, 2, 3, and 5 are major *B. pertussis* antigens, and serological differences among *B. pertussis* cultures may be explained by the presence or absence, in various combinations, of these factors. Factors 4 and 6 are minor *B. pertussis* antigens. Factors 8, 9, 10, and 11 distinguish different *B. bronchiseptica* cultures, and all of these except 11 are also possessed by all *B. parapertussis* cultures. A minor factor, 13, explains certain serological relationships between *B. pertussis* and B. *bronchiseptica*. This antigen is lacking in *B. parapertussis*.

The FA results with *B. pertussis* adsorbed antisera specific for various factors were negative, except with factor 1 serum, to be described later. The agglutination titers of these adsorbed antisera are shown in Table 4. It is noted that the titers of the adsorbed antisera range from 1:320 to 1:4,000 with the appropriate cultures. These sera had been adsorbed with various unheated *B. pertussis* cultures and also with *B. bronchiseptica*, the latter to remove the common factor 7. None of these sera agglutinated either *B. parapertussis* or *B. bronchiseptica*. FA reactions were uniformly negative with all *Bordetella* cultures.

TABLE 4. *Comparative results of fluorescent antibody (FA) staining and agglutination (Ag) tests with adsorbed Bordetella pertussis antiserum*

B. pertussis antiserum adsorbed		FA all cultures	Agglutination titers (cultures and major factors)		
Specific for factors	Prepared from serum no.		5373 (1, 3, 7)	5374 (1, 2, 5, 7)	5375 (1, 2, 4, 7)
3	R 547	—	4,000	—	—
3	R 421	—	2,000	—	—
2 + 4	R 526	—	—	3,000	3,000
2 + 5	R 532	—	—	1,000	1,000
2 + 5	R 551	—	—	1,000	320

TABLE 5. *Preparation of Bordetella pertussis factor 1 serum*

Procedure	Factors	
	Removed	Remaining
Serum produced with *B. pertussis* 5373	none	1, 3, 6, 7, 13
Serum adsorbed with heated *B. pertussis* 5373 (100 C, 2.5 hr)	3, 6	1, 7, 13
Serum adsorbed with *B. bronchiseptica* 22067 (7, 8, 9, 12, 13)	7, 13	1

The discrepancies just described between FA and agglutination reactions were observed with antisera adsorbed with unheated cultures. Factor 1 serum was prepared by adsorption of *B. pertussis* antiserum with heated *B. pertussis* antigen and unheated *B. bronchiseptica*. The steps in the preparation are outlined in Table 5.

When a *B. pertussis* 5373 saline suspension is heated at 100 C for 2.5 hr, its agglutinability is destroyed. However, the whole suspension, or the separated supernatant fluid obtained by centrifugation, but not the bacteria themselves, retain the ability to adsorb agglutinins for factors 3

TABLE 6. *Comparative results of fluorescent antibody (FA) staining and agglutination (Ag) tests with Bordetella pertussis factor 1 antiserum*

Antigen		Results with factor 1 antiserum	
Culture	Major factors	FA	Ag titer
B. pertussis 5373	1, 3, 7	4+	320
B. pertussis 5374	1, 2, 5, 7	4+	160
B. pertussis 5375	1, 2, 4, 7	4+	160
B. parapertussis	7, 8, 9, 10, 14	−	−
B. bronchiseptica	7, 8, 9, 10, 11, 12	−	−

and 6. After adsorption of an antiserum for *B. pertussis* 5373 with such a heated suspension, the agglutinins remaining are for factors 1, 7, and 13. Factors 7 and 13 may be removed by adsorption with *B. bronchiseptica* 22-067, leaving a serum with agglutinins for factor 1, the species-specific factor for *B. pertussis*.

The results with factor 1 serum are shown in Table 6. Although the agglutination titers of the factor 1 serum were relatively low with *B. pertussis* cultures, the FA reactions were positive. Cross reactions with the related species were not observed with either procedure. Similar results were obtained with three other factor 1 sera, prepared according to the procedure outlined.

Reactions with antisera produced with "rough" B. pertussis cultures. Rough or dissociated cultures of *B. pertussis* have been considered antigenically inert: they neither stimulate the production of agglutinins for rough or smooth *B. pertussis*, nor are they agglutinated by any *B. pertussis* antiserum with which they have been tested. However, FA tests with antisera produced with rough cultures were positive not only with the homologous culture and other rough *B. pertussis* cultures, but also with smooth strains (Table 7).

Antiserum produced with rough *B. pertussis* F-28 gave positive FA reactions with the homologous rough culture, two other rough *B. pertussis* cultures, and the smooth *B. pertussis* 10-536. The antiserum failed to agglutinate any of these cultures. Antiserum against the smooth *B. pertussis* 10-536 was also positive in FA tests with smooth and rough cultures, but did not agglutinate the dissociated cultures. The *B. pertussis* factor 1 antiserum described above gave negative results in FA and agglutination tests with rough

TABLE 7. *Comparative results of fluorescent antibody (FA) staining and agglutination (Ag) tests in relation to rough and smooth Bordetella pertussis cultures*

B. pertussis antiserum	Test	*B. pertussis* culture			
		F 28 (R)	25 (R)	26 (R)	10356 (S)
Culture for production					
F 28 (R)	FA	4+	3+	3+ to 4+	2+
	Ag	−	−	−	−
10536 (S)	FA	3+	2+	2+ to 3+	4+
	Ag	−	−	−	5,000
5373 (S)	FA	−	−	−	4+
(Factor 1)	Ag	−	−	−	200

B. pertussis cultures. Preimmunization serum samples from the rabbits used to produce these antisera gave negative FA results with smooth and rough *B. pertussis* cultures.

DISCUSSION

Of practical interest in considering the results of FA staining and agglutination tests with *Bordetella* species are the implications with respect to diagnostic procedures. It should be noted that none of the five *B. pertussis* antisera tested was positive by the FA procedure with *B. parapertussis*. Since this organism is occasionally found in cultures from patients with whooping cough-like disease, it would be necessary in the application of the FA method to include both *B. pertussis* and *B. parapertussis* antisera.

The second point to be emphasized concerns the results with adsorbed antisera. *B. pertussis* antisera specific for factors 2, 3, and 5 prepared by adsorption with unheated *B. pertussis* cultures were consistently negative in FA staining. A report by Andersen (1958), based on protection tests in mice, indicated that these antigenic factors were not concerned with protection. Unpublished data from our laboratory support this conclusion. The factor 1 antiserum was prepared by adsorption with heated *B. pertussis* and unheated *B. bronchiseptica*. This antiserum agglutinated in low titer all *B. pertussis* cultures tested and also gave positive FA reactions with these cultures.

The positive FA results with rough cultures of *B. pertussis* were surprising, since previous experience indicated such cultures to be antigenically

inert. It should be noted that the FA results with the factor 1 serum were negative with the rough cultures. It is well known, of course, that these cultures do not have any protective properties when tested in mice. One is immediately driven to the question: is factor 1 the antigen concerned with protection? The answer awaits the results of further work. Also unanswered is the question as to whether the serum components concerned with FA staining and the agglutination reaction are different.

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