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Somatic O Antigen Relationship of *Brucella* and *Vibrio cholerae*

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The antigenic relationship between *Brucella* species and *Vibrio cholerae* was examined by agglutinin and agglutinin-absorption tests by using rabbit antisera. Brucella antisera agglutinated only the Inaba serotype of *V. cholerae* and at low titer. Inaba-reactive antibody was absorbed by either heat-stable (100 C, 2 hr) Ogawa or Inaba O antigens. Cholera antisera from rabbits immunized with either O or HO antigens of either Ogawa or Inaba serotypes contained brucella agglutinins. This activity was absorbed completely from Ogawa antisera by either Ogawa or Inaba O antigens but only partially from Inaba antisera by Ogawa O antigen. These findings support the claim of Gallut that the cross-reaction is due to heat-stable O antigens of *V. cholerae* rather than heat-labile flagellar antigens as described in many text books. The cross-reactive component is more dominant in the Inaba than in the Ogawa serotype of *V. cholerae*.

The appearance of brucella agglutinins in sera from animals or man immunized with cholera vaccine or various cholera antigens has been reported on a number of occasions (3–6, 12, 17). Although the reason for appearance of these agglutinins, based on the studies of Eisele et al. (4) and McCullough et al. (11), is frequently attributed to a flagellar H antigen of *Vibrio cholerae* that is related to a somatic antigen in the three species of *Brucella*, Gallut (6, 7) ascribed the cross-reaction to heat-stable somatic O rather than H antigens of *V. cholerae*.

In view of the growing interest in serologic surveys for cholera antibody in susceptible populations (13–15) and the effect this cross-reaction might have on interpretations of the results, the antigenic relationship between *Brucella* and *V. cholerae* was re-examined by agglutinin and agglutinin-absorption tests.

MATERIALS AND METHODS

Antisera. Rabbits were immunized by repeated intravenous injections of living *V. cholerae* (Ogawa strain VC 12 and Inaba strain VC 13, respectively) to produce anti-HO (flagellar plus somatic) sera and with vibrios of the same strains boiled under a reflux condenser for 2 hr to destroy H antigen (1) for production of anti-O sera. The brucella antisera were obtained from rabbits that had been infected 30 days previously by intraperitoneal injection of approximately 10^8 cells of *Brucella suis* strain 3s 101. All sera were inactivated at 56 C for 30 min and were stored at −20 C without preservatives.

Agglutination tests. For O-agglutination tests, 0.5-ml volumes of living cultures (3 to 4 hr) of Ogawa strain VC-12 or Inaba strain VC-13 [in 1% Trypticase (BBL)-1% NaCl broth and having a turbidity corresponding to 1 unit of the U.S. Opacity Standard] were added to two-fold serum dilutions in 0.5-ml volumes in tubes (13 by 100 mm). Tubes were incubated at 37 C for 1 hr and held overnight at 4 C before reading. Overnight refrigeration facilitated reading and generally caused a twofold increase in end point. Titer was expressed as the highest final serum dilution showing definite agglutination. There was no agglutination in *V. cholerae* H antiserum under these conditions. Heiberg (10), Gallut and Brounst (8), and Goodner et al. (9) demonstrated that O-agglutination with *V. cholerae*, in contrast to *Salmonella*, is a rapidly developing reaction most readily demonstrated with living vibrios. Although boiled suspensions can be employed to measure O-agglutinins (1), their agglutinability is greatly reduced. The fact that live organisms are more satisfactory for this purpose has been substantiated by my own unpublished data.

HO antigens were prepared by suspending 18-hr heart infusion agar-grown cultures of the above listed strains of *V. cholerae* in 0.85% NaCl containing 0.1% Formalin and adjusting turbidity to 1 opacity unit. Agglutination tests were executed as stated above except that incubation was at 37 C for 18 hr. Such suspensions were agglutinated by both *V. cholerae* O and H antisera.

Brucella agglutination tests were performed, as described by Spink et al. (16), by using the standard *B. abortus* strain 1119 agglutinating antigen kindly supplied by the Animal Disease Eradication Division, Agricultural Research Service, U.S. Department of Agriculture.

Agglutinin absorption. Sera were diluted 1:5 and absorbed serially three times by suspending in them packed agar-grown vibrio cells that had been boiled for 2 hr under a reflux condenser to destroy H-antigen (1). During each absorption, the serum-cell mixtures were incubated at 37 C for 1 hr and overnight at 4 C.

Absorbed monospecific Ogawa and Inaba typing sera were prepared by reciprocally absorbing anti-Inaba O serum with living Ogawa cells, and vice versa, to remove cross-reacting antibody (2). Other monospecific Ogawa and Inaba typing sera were kindly donated by H. L. Smith, Jr., Jefferson Medical College of Philadelphia.

RESULTS

Reactivity of unabsorbed sera. Table 1 shows that low dilutions of antibrucella serum agglutinated both O and HO antigens of Inaba, but not of Ogawa, serotypes of *V. cholerae*. In contrast, both HO and O sera against Ogawa or Inaba serotypes had relatively higher titers against brucella antigen. The reactivity of the brucella antigen with anticholera O sera indicates that at least a substantial portion of the cross-reacting antigenic activity in *V. cholerae* is associated with heat-stable (presumably somatic) rather than heat-labile (flagellar) antigens.

Reactivity of absorbed sera. Absorption of brucella antiserum with either Ogawa or Inaba O antigens (Table 2) failed to lower the titer of the serum against *Brucella*, whereas these antigens completely removed agglutinins against the Inaba type that were present in unabsorbed serum. From these findings, one may infer that any activity directed against cholera antigens is a relatively minor component of the agglutinating antibody response to brucella antigen and that it was readily absorbed by heat-stable cholera O antigens.

Absorption of either HO or O anti-Ogawa sera with either Ogawa or Inaba O antigen (Table 3) completely removed cross-reactivity with brucella antigen. Absorption of Ogawa HO serum with Ogawa O antigen removed all antibody that reacted in the O antigen titration system, but failed to exhaust the serum of reactivity with either Ogawa or Inaba HO antigen. The residual titer presumably was due to H antibody which did not react with brucella antigen. As would be expected, absorption of each serum with Inaba O antigen failed to remove all antibody cross-reacting with Ogawa O or HO antigen.

Results of a similar absorption experiment performed with Inaba HO and O antisera are shown in Table 4. In contrast to experience with Ogawa antisera (Table 3), absorption with Ogawa O antigen greatly reduced but did not eliminate reactivity with brucella antigen, whereas Inaba O antigen absorbed all brucella-reactive antibody. This Inaba HO serum absorbed with Inaba O antigen retained reactivity with both Ogawa and Inaba HO antigens, again presumably due to presence of H antibody; nevertheless, it failed to agglutinate brucella. As expected, absorption with Ogawa O antigen did not remove all antibody cross-reacting with Inaba O or HO antigens.

Monospecific Ogawa and Inaba typing sera normally employed for identification of serotypes of *V. cholerae* were also examined (Table 5). Only the Inaba-specific sera reacted with brucella, an observation consistent with data reported above.

DISCUSSION

The results reported in the present paper support the findings of Gallut (6, 7) that cross-reactivity of *V. cholerae* and *Brucella* antigens is

TABLE 1. *Cross-reactions of unabsorbed sera*

Antiserum	Agglutinin titer against antigen				
	Ogawa		Inaba		Brucella 1119
	Living (O)	Formalinized (HO)	Living (O)	Formalinized (HO)	
Brucella no. 514	<20	<20	160	80	2,560
Brucella no. 117	<20	<20	40	20	1,280
Ogawa HO[a]	20,480	10,240	10,240	5,120	640
Ogawa O[b]	5,120	2,560	5,120	1,280	320
Inaba HO[a]	20,480	5,120	20,480	5,120	2,560
Inaba O[b]	5,120	2,560	5,120	2,560	640

[a] Serum from rabbits hyperimmunized with living *V. cholerae*.
[b] Serum from rabbits hyperimmunized with boiled (refluxed) *V. cholerae*.

TABLE 2. *Absorption of brucella antiserum (no. 514) with cholera O antigens*

Antigen	Titer unabsorbed	Titer after absorption with[a]	
		Ogawa O	Inaba O
Ogawa, living (O)	<20	<20	<20
Ogawa, formalinized (HO)	<20	<20	<20
Inaba, living (O)	160	<20	<20
Inaba, formalinized (HO)	80	<20	<20
Brucella	2,560	2,560	2,560

[a] Absorbing antigens were boiled for 2 hr.

associated with heat-stable (somatic) rather than heat-labile (flagellar) antigens as claimed by Eisele et al. (4) and McCullough et al. (11).

B. suis antiserum contained low-titer agglutinins only against the Inaba serotype which were readily absorbed by both Ogawa and Inaba O antigens without lowering the anti-Brucella titer. Therefore, the portion of the brucella-agglutinating antibody response which reacts with *V. cholerae* would seem to be a very minor component of the total response.

Anticholera sera raised against either O or HO immunizing antigens of either Ogawa or Inaba strains of *V. cholerae* contained brucella agglutinins, indicating that heat-stable cholera antigens stimulated brucella antibody.

Absorption of both O and HO antisera against both serotypes of *V. cholerae* indicated the following. (i) Absorption of anti-Ogawa serum (either

TABLE 3. *Absorption of Ogawa HO and O antisera with cholera O antigens*

Antiserum	Antigen	Titer unabsorbed	Titer after absorption with	
			Ogawa O	Inaba O
Ogawa (HO)[a]	Ogawa, living (O)	20,480	<20	5,120
	Ogawa, formalinized (HO)	10,240	80	2,560
	Inaba, living (O)	10,240	<20	<20
	Inaba, formalinized (HO)	5,120	80	80
	Brucella	640	<20	<20
Ogawa (O)[b]	Ogawa, living (O)	5,120	<20	2,560
	Ogawa, formalinized (HO)	2,560	<20	320
	Inaba, living (O)	5,120	<20	<20
	Inaba, formalinized (HO)	1,280	<20	<20
	Brucella	320	<20	<20

[a] Serum from rabbits hyperimmunized with living *V. cholerae*.
[b] Serum from rabbits hyperimmunized with boiled (refluxed) *V. cholerae*.

TABLE 4. *Absorption of Inaba HO and O antisera with cholera O antigens*

Antiserum	Antigen	Titer unabsorbed	Titer after absorption with	
			Ogawa O	Inaba O
Inaba (HO)[a]	Ogawa, living (O)	20,480	<20	<20
	Ogawa, formalinized (HO)	5,120	320	320
	Inaba, living (O)	20,480	2,560	<20
	Inaba, formalinized (HO)	5,120	1,280	320
	Brucella	2,560	80	<20
Inaba (O)[b]	Ogawa, living (O)	5,120	<20	<20
	Ogawa, formalinized (HO)	2,560	<20	<20
	Inaba, living (O)	5,120	1,280	<20
	Inaba, formalinized (HO)	2,560	320	<20
	Brucella	640	80	<20

[a] Serum from rabbits hyperimmunized with living *V. cholerae*.
[b] Serum from rabbits hyperimmunized with boiled (refluxed) *V. cholerae*.

TABLE 5. *Cross-reactions with monospecific absorbed V. cholerae typing sera*

Monospecific antiserum	Agglutinin titer against antigen				
	Ogawa		Inaba		Brucella 1119
	Living (O)	Formalinized (HO)	Living (O)	Formalinized (HO)	
Ogawa (DBS)[a]	2,560	1,280	<20	<20	<20
Ogawa (JMC)[b]	1,280	640	<20	<20	<20
Inaba (DBS)	<20	<20	640	320	160
Inaba (JMC)	<20	<20	640	640	80

[a] Division of Biologics Standards.
[b] Jefferson Medical College.

O or HO) with either Ogawa or Inaba O antigen depleted brucella agglutinating activity. (ii) Absorption of anti-Inaba serum (either O or HO) with Ogawa O antigen lowered the titer but failed to remove all brucella-agglutinating antibody, whereas absorption with Inaba O antigen removed all such activity.

Inasmuch as *V. cholerae* O antigens were capable of both stimulating and absorbing cross-reacting brucella antibody, the role of heat-stable antigens is affirmed and is in agreement with Gallut (6, 7). Furthermore, sera containing *V. cholerae* H antibody (e.g., anti-Ogawa HO or anti-Inaba HO sera absorbed with *V. cholerae* O antigen) failed to agglutinate brucella, except in the case of anti-Inaba HO serum absorbed with Ogawa O antigen. In the latter instance, however, the same phenomenon was observed with anti-Inaba O serum, ruling out the essential role of H antibody as the cause of this particular cross-reaction.

It is possible that the differences between the present findings and those of McCullough et al. (11) may be explained at least in part by (i) their use of only an Ogawa strain (NIH 41) as an agglutinating and absorbing antigen and (ii) their use of boiled suspensions of this strain to measure O-agglutinins, resulting in a less sensitive measurement of these antibodies (8–10).

Thus, it would appear that *Brucella* shares a heat-stable antigenic component with both serotypes of *V. cholerae*, but that the cross-reacting antigenic component is more dominant in the Inaba serotype. In view of the recent demonstration that immunity against cholera in both vaccinated and unvaccinated human subjects in East Pakistan (13–15) can be correlated with the level of circulating vibriocidal antibody (against somatic antigens of *V. cholerae*), the possible role of brucella infection in stimulating such antibody against cholera in the population should be investigated.

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