Hemagglutination by *Pasteurella multocida* of Porcine Origin

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Received 22 September 1986/Accepted 19 January 1987

A total of 25 fresh isolates of *Pasteurella multocida* from pigs were tested for the ability to agglutinate erythrocytes of different origins. Of the 18 isolates from pigs with atrophic rhinitis (AR), 8 (44%) agglutinated human type O erythrocytes, whereas none of the 7 isolates from pigs without AR did so. Hemagglutination was mannose sensitive, and the activity was destroyed by heating and by trypsinization but was not affected by formaldehyde treatment or by homogenization of bacterial cells. The other 14 erythrocyte species tested, including human types A and B, were not agglutinated by *P. multocida*. The present study provides evidence that certain *P. multocida* isolates from pigs with AR possess a mannose-sensitive hemagglutinin.

Many bacteria can attach to and agglutinate erythrocytes in vitro. Correlations between piliation and hemagglutination (HA) among certain bacterial species have made the relatively simple and inexpensive HA test useful for studying bacterium-host cell surface interaction and mechanisms of attachment (5). HA is not, however, always caused by fimbriae (2).

It has been shown that strains of *Pasteurella multocida* type A isolated from rabbits and poultry can agglutinate human type O erythrocytes (6). Hemagglutinating properties of *P. multocida* isolated from pigs have not, to our knowledge, been thoroughly examined. In the present study, we investigated the hemagglutinating properties, with erythrocytes from different species, of *P. multocida* isolates from pigs with or without atrophic rhinitis (AR). Porcine AR is characterized by severe necrosis of the epithelia of the upper respiratory tract and by deformity and reduction both in volume and in size of the nasal turbinates and snout (8). *P. multocida* is recognized as one of the etiological agents of porcine AR (1), but the virulence factors involved in colonization of the upper respiratory tract remain unknown.

A total of 25 *P. multocida* isolates were retrieved from the nasal cavities of pigs affected (18 isolates) or not affected (7 isolates) with AR. The isolates were identified and characterized in our laboratory. They were grown overnight on blood agar plates or in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C. Dense bacterial suspensions were made in phosphate-buffered saline (0.01 M, pH 7.2) and adjusted to a density of approximately 10⁹ CFU/ml.

Blood was collected from the following species: sheep, calf, goat, pig, horse, rabbit, guinea pig, chicken, dog, cat, rat, mouse, and human (types A, B, and O). Erythrocytes were washed twice in phosphate-buffered saline and suspended to obtain a final concentration of 3%. Bacterial suspension (1 drop) was added to 1 drop of erythrocyte suspension and 1 drop of saline in the circular depression of a glass slide. The slide was rocked, at room temperature or at 4°C, for 10 min. *Escherichia coli* 8a (O115K⁻F1⁺) was used as a positive control. A parallel test, in which a drop of 1.5% (wt/vol) D-mannose was substituted for the drop of

saline in the mixture, was used to determine whether the activity was mannose sensitive (5).

Of the 18 isolates from pigs with AR, 8 (44%) agglutinated human type O erythrocytes; none of the isolates from pigs without AR showed this type of activity (Table 1). Erythrocytes from human types A and B, as well as erythrocytes from the 12 animal species, were not agglutinated by any of the isolates tested.

HA of human type O erythrocytes by *P. multocida* was observed with microorganisms grown either on blood agar or

TABLE 1. Agglutination of human type O erythrocytes by various isolates of *P. multocida* of porcine origin

Isolate	AR ^a	Antigen type		Dermonecrotic toxin ^b	HA
		Capsular	Somatic ^c		
23-4	+	A	4,7	—	+
2305-A	+	A	4,7	+	+
2305-B	+	A	4,7	+	+
28-1	+	A	3,16	—	+
28-6	+	A	3,16	—	+
28-7	+	A	1,15,7	—	—
1604	+	D	4,7	+	+
1703	+	D	4,7	+	—
23-8	+	D	1,7,15,16	—	—
23-3	+	D	4,7	—	+
24-5	+	D	NT ^d	—	+
24-6	+	D	3,16	—	—
27-1	+	D	NT	+	—
27-4	+	D	NT	+	—
28-9	+	D	15	—	—
28-12	+	D	15	—	—
3001	+	D	3,16	+	—
30-1	+	D	3,16	+	—
26-2	—	A	3,16	—	—
26-4	—	A	3,16	—	—
26-6	—	A	3,16	—	—
32-1	—	A	3,16	—	—
26-5	—	D	3,16	—	—
3601-1	—	D	3,16	—	—
3750-11	—	D	3,16	—	—

^a +, From a pig affected by AR; —, from a pig not affected by AR.

^b Determined by the guinea pig skin test (4).

^c Heddleston et al. typing system (3).

^d NT, Nontypable.

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in tryptic soy broth and was inhibited by the addition of D-mannose. In contrast, Pestana de Castro et al. (6) observed mannose-resistant HA. This discrepancy may be explained by the origin (rabbits and poultry) of the isolates in that experiment as well as by experimental conditions used, such as erythrocyte and D-mannose concentrations. Heat inactivation (1 h at 65°C or 10 min at 100°C) or treatment with trypsin (1 mg/ml for 30 min at 37°C; Sigma Chemical Co., St. Louis, Mo.) destroyed the hemagglutinating activity of the bacterium. However, formaldehyde-treated (2%; 1 h at room temperature) or homogenized (2 successive 5-min pulses, a treatment which did not disrupt the cells; Omni-Mixer; Ivan Sorvall, Inc., Norwalk, Conn.) bacterial cells and bacterial cells grown overnight at 16°C all agglutinated human type O erythrocytes.

HA was not associated with a particular capsular type or with the production of a dermonecrotic toxin. However, HA was frequently associated with strains bearing Heddlestone somatic antigens 4 and 7 ($P < 0.01$ by contingency table analysis).

We cannot, from our results, relate structure to hemagglutinating activity. Electron microscopic observation of negatively stained preparations of hemagglutinating and nonhemagglutinating isolates did not reveal the presence of fimbriae at the cell surface. Additional studies are needed in order to characterize the mannose-sensitive hemagglutinin of *P. multocida* isolated from pigs with AR. Recently, it was observed that purified lipopolysaccharide from *P. multocida* was able to agglutinate chicken and turkey erythrocytes but not sheep or horse erythrocytes (7).

The mannose-sensitive hemagglutinin of *P. multocida* may function as an adhesin and contribute to the virulence of the microorganism, but previous in vitro adherence experiments done in our laboratory (M. Jacques, Curr. Microbiol., in

press) did not seem to support this view. The exact role of this hemagglutinin in respiratory tract infections remains unclear.

This work was supported by a grant from the Conseil des Recherches et Services Agricoles du Québec (MMV-86-C-2022).

We thank Luc Héroux for technical assistance, Claudette Beaudin for secretarial work, and John L. Moore for reviewing the manuscript.

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