Antigenic Characterization of *Pasteurella pneumotropica* Isolated from Mice and Rats

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Antigenic characterization of *P. pneumotropica* derived from mice and rats were serologically investigated by use of hyperimmune rabbit sera against mouse strain M1 and rat strain R1 and absorbed sera m-1 and r-1 which were prepared by absorbing anti-M1 serum with strain R1 and anti-R1 serum with strain M1, respectively. All of 13 mouse strains employed were agglutinated with both anti-M1 and anti-R1 sera, but their agglutination titers with anti-M1 serum were usually higher than those with anti-R1 serum. Agglutination test of 13 rat strains with two antisera showed results converse to those of mouse strains. On the other hand, all the mouse strains were agglutinated with absorbed serum m-1 but not with absorbed serum r-1, while quite converse results were obtained with all the rat strains. Antigens reactive with the absorbed sera were remarkably destroyed by heating at 100°C for 1 hour and treating with 1 N HCl, suggesting to be capsular antigens of bacterial cells.

Pasteurella pneumotropica has been isolated from various animal species including mice, rats, Syrian hamsters, guinea pigs and dogs [1, 8, 10, 13], and regarded as a causative agent of pneumonia [1-3, 7, 8], subcutaneous abscess [10, 19, 20, 22], mastitis [6, 19], conjunctivitis [1, 11, 20], metritis [1], abortion [21] and urocystitis [1]. It is well known that mice and rats are often healthy carriers of the organism [4, 5, 7, 8, 13, 17, 18], however, host-specific parasitism of the organism has not yet been investigated except our previous study in which rat strains were shown to be infective to rats but rarely to mice, while mouse strains were highly infective to both animals [12].

In the present investigations, serological characterization of *P. pneumotropica* derived from mice and rats were made to get informations on host specificity of this organism.

Materials and Methods

Strains of *P. pneumotropica*: A total of 26 strains of *P. pneumotropica* derived from mice and rats were used in this study. Thirteen each of mouse and rat strains were isolated from different animal colonies which had no microbiological communication each other, and kept at -80°C before use without subculturing after isolation. The mouse strains were designated as M1 to 13 and the rat strains as R1 to 13, respectively.

Antigens for immunization: Forty-eight-hour horse blood agar cultures of strains M1 and R1 were harvested in 0.6% formalin saline and incubated at 37°C for 3 days. After washing 3 times, the killed bacteria were suspended in saline at a concentration of 200mg/ml.

Untreated antigens for agglutination

test: The growth from 48-hour cultures of 26 strains in nutrient broth were centrifuged at 3,000 rpm for 30 minutes and suspended in 0.6% formalin saline followed by incubating at 37°C for 3 days. After washing 3 times in phosphate buffered saline pH 7.2 (PBS), the pellets were suspended in PBS adjusting the turbidity to tube No. 3 of MacFarland's nephrometer series.

Heated antigens for agglutination test: Untreated antigens of strain M1 and R1 were heated at 100°C for 1 hour. After washing 3 times in PBS, the sediment was resuspended in PBS adjusting to the same turbidity as the original one.

HCl-treated antigens for agglutination test: HCl-treated antigens were prepared according to the method devised by Namiooka and Murata [15,16] for preparation of somatic antigen of *Pasteurella multocida*. Eleven-hour culture of strain M1 or R1 on a horse blood agar plate (9 cm diameter) was harvested in 10 ml of 1N HCl saline and incubated at 37°C overnight. The bacterial suspension was washed in saline adjusted the pH to 2.5, 3.5, 4.5, 5.5, 6.5 and 7.2 to shift gradually from strong acid to base, and finally suspended in PBS, adjusting the turbidity to tube No. 3 of MacFarland's nephrometer.

Antisera: Equal volumes of antigen for immunization and Freund's incomplete adjuvant (Iatron, Tokyo) were mixed thoroughly to make "water in oil" emulsion.

A female Japanese White rabbit weighing about 2.5 kg was injected into gluteal muscle with 0.5 ml of the emulsion, followed by intravenous injection of 0.2 ml of antigen alone four weeks later. One week after the second injection, rabbits were exsanguinated by heart puncture under anesthesia with chloroform. Sera were stored at -20°C.

Absorption procedure: To the washed sediment resulted from 1 ml of the antigen for immunization was added 1 ml of 1:5 dilution of immune serum, which had been heated at 56°C for 30 minutes. The mixture

was incubated at 37°C for 2 hours and placed at 4°C overnight. After centrifugation, the serum was checked for remaining antibodies against the strain used for absorption by agglutination test. When absorption was incomplete, the same procedures were repeated until the antibodies were completely absorbed.

Agglutination test: Agglutination test was performed by tube dilution method. Unabsorbed sera were diluted to 1:5 with saline and heated at 56°C for 30 minutes before use. To serial two-fold dilutions of serum, beginning at 1:10, equal amounts (0.5 ml) of antigen were added. After incubation at 37°C for 2 hours and then at 4°C overnight, the tests were read with the unaided eyes, and final titer was determined as the reciprocal of the highest serum dilution showing visible agglutination.

Results

1. Agglutination test with unabsorbed immune sera

Agglutinabilities of untreated antigens of all the mouse and rat strains were examined with unabsorbed immune sera against strain M1 of mouse origin and strain R1 of rat origin. Results are shown by agglutination titers in Table 1. Strains M1, M4 and M7 were agglutinated at 1:2,560 dilution of anti-M1 serum, whereas other strains of mouse origin showed lower titers ranging from 1:320 to 1:1,280. Agglutination titers of rat strains R1 to R13 to anti-M1 serum were significantly lower than those of mouse strains, being 1:320 at the highest and 1:20 at the lowest.

On the other hand, agglutinabilities of the same strains with anti-R1 serum were converse to those with anti-M1 serum as shown in Table 1. Nine of 13 rat strains including strain R1 showed the titer of 1:1,280 with antiserum against strain R1. Agglutination titers of the remaining 4 strains were 1:640, 1:320, 1:640 and 1:

Table 1. Agglutinabilities of mouse and rat strains of *P. pneumotropica* with unabsorbed anti-M 1 and anti-R 1 sera

Antigen strain		Agglutination titer with	
Origin	No.	anti-M 1	anti-R 1
Mouse	M 1	1 : 2560	1 : 640
	M 2	1 : 1280	1 : 320
	M 3	1 : 1280	1 : 160
	M 4	1 : 2560	1 : 640
	M 5	1 : 640	1 : 160
	M 6	1 : 640	1 : 160
	M 7	1 : 2560	1 : 640
	M 8	1 : 640	1 : 320
	M 9	1 : 640	1 : 320
	M 10	1 : 1280	1 : 320
	M 11	1 : 1280	1 : 160
	M 12	1 : 320	1 : 80
	M 13	1 : 1280	1 : 160
Rat	R 1	1 : 160	1 : 1280
	R 2	1 : 320	1 : 1280
	R 3	1 : 80	1 : 1280
	R 4	1 : 80	1 : 1280
	R 5	1 : 160	1 : 1280
	R 6	1 : 40	1 : 1280
	R 7	1 : 160	1 : 1280
	R 8	1 : 20	1 : 640
	R 9	1 : 80	1 : 320
	R 10	1 : 160	1 : 640
	R 11	1 : 40	1 : 160
	R 12	1 : 320	1 : 1280
	R 13	1 : 80	1 : 1280

160, but all higher than those with antiserum against mouse strain M1. Agglutination titers of all the mouse strains with antiserum against strain R1 were remarkably lower than those of rat strains, distributing between 1 : 20 and 1 : 320.

2. Agglutination test with absorbed sera

Anti-M1 serum was absorbed with strain R1 and anti-R1 serum with strain M1, and resulted sera were provisionally named as absorbed serum m-1 and absorbed serum r-1, respectively.

Agglutinabilities of untreated antigens of all the mouse and rat strains were

Table 2. Agglutinabilities of mouse and rat strains of *P. pneumotropica* with absorbed sera m-1 and r-1

Antigen strain		Agglutination titer with	
Origin	No.	m-1	r-1
Mouse	M 1	1 : 640	< 10
	M 2	1 : 160	< 10
	M 3	1 : 320	< 10
	M 4	1 : 40	< 10
	M 5	1 : 320	< 10
	M 6	1 : 40	< 10
	M 7	1 : 160	< 10
	M 8	1 : 160	< 10
	M 9	1 : 80	< 10
	M 10	1 : 160	< 10
	M 11	1 : 160	< 10
	M 12	1 : 160	< 10
	M 13	1 : 160	< 10
Rat	R 1	< 10	1 : 640
	R 2	< 10	1 : 80
	R 3	< 10	1 : 320
	R 4	< 10	1 : 80
	R 5	< 10	1 : 640
	R 6	< 10	1 : 320
	R 7	< 10	1 : 640
	R 8	< 10	1 : 320
	R 9	< 10	1 : 80
	R 10	< 10	1 : 160
	R 11	< 10	1 : 40
	R 12	< 10	1 : 80
	R 13	< 10	1 : 640

tested with the absorbed sera and results are summarized in Table 2. All of the mouse strains were agglutinated by absorbed serum m-1 at titers from 1 : 40 to 1 : 640, but none of the rat strains reacted with the serum even at 1 : 10 dilution. Conversely all the rat strains showed agglutination titers of 1 : 40 to 1 : 640 against absorbed serum r-1 but all the mouse strains were not agglutinated by the serum.

3. Agglutination test of heated and HCl-treated antigens with absorbed sera

In order to know characters of antigens

Table 3. Agglutinabilities of heated and HCl-treated antigens with absorbed sera m-1 and r-1

Absorbed sera	Antigen	Treatment of antigen	Agglutination titer
m-1	M 1	Formalin-killed	1 : 640
		Heated at 100°C for 1 hr	1 : 40
		1 N HCl	1 : 80
	R 1	Formalin-killed	< 10
		Heated at 100°C for 1 hr	< 10
		1 N HCl	< 10
r-1	M 1	Formalin-killed	< 10
		Heated at 100°C for 1 hr	< 10
		1 N HCl	< 10
	R 1	Formalin-killed	1 : 640
		Heated at 100°C for 1 hr	1 : 40
		1 N HCl	1 : 40

reactive with absorbed sera m-1 and r-1, heated and HCl-treated antigens of strains M1 and R1 were tested for their agglutinabilities against the absorbed sera. Results are shown in Table 3 in comparison with agglutinabilities of formalin-killed antigens of the same strains.

In both strains, agglutination titers of heated and HCl-treated antigens with absorbed homologous serum were remarkably reduced to 1 : 40 or 1 : 80 from 1 : 640 of formalin-killed antigens. On the other hand, all kinds of antigens of strains M1 and R1 were not agglutinated by absorbed heterologous sera r-1 and m-1, respectively.

Discussion

In the present study, antigenic differences were demonstrated between mouse and rat strains of *P. pneumotropica* by agglutination tests with absorbed immune sera, although common antigens were also shared between them as shown by agglutination tests with unabsorbed immune sera. Namely, an antigen or antigens reacted with absorbed serum m-1 seemed to be specific to mouse strains and another antigen or antigens reacted

with absorbed serum r-1 to rat strains, respectively. It was not known whether the specific antigens were of monovalent or polyvalent, but these results strongly suggested host-specific parasitism of *P. pneumotropica*.

It is well known that a part of capsular antigens of Gram-negative bacilli such as Enterobacteriaceae [9], *Pasteurella multocida* [16] and *Bordetella bronchiseptica* [14] is thermolabile. On the other hand, Namioka and Murata [16] reported that capsular antigens of *P. multocida* were completely destroyed by treating with 1N HCl. In the present study agglutination titers of antigens reacted with absorbed sera were remarkably decreased by heating at 100°C for 1 hour and treating with 1N HCl in comparison with those of formalin-killed whole cells. Thus the antigens specific to either mouse or rat strains were considered to be heat labile capsular antigens.

As far as we know, no report has been concerned on difference of biochemical properties between mouse and rat strains of *P. pneumotropica*. We also compared some characters of the organism such as H₂S production, growth on Simmons citrate medium, KNO₃ reduction, gelatin liquefac-

tion and fermentations of 20 kinds of carbohydrates between mouse and rat strains, but could not find any characters correlative to origins of the strains (unpublished data).

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マウス，ラット由来 *Pasteurella pneumotropica* の抗原的差異

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P. pneumotropica のマウスおよびラット由来株間の抗原的差異を高度免疫家兎血清に対する凝集反応によって検討した。実験には，マウス由来 M1 株とラット由来 R1 株の免疫血清および両動物種由来株各13株をそれぞれ使用した。

その結果，供試菌株はいずれも抗 M1 血清と抗 R1 血清で凝集したが，マウス株の抗 M1 血清に対する凝集価は抗 R1 血清のそれより高く，ラット株は抗 R1 血清でより高い凝集価を示した。一方，抗 M1 血清を R1 株で

吸収し，逆に抗 R1 血清を M1 株で吸収して，それぞれ m-1 および r-1 血清を作製し，これらを用いて凝集反応を行ったところ，マウス株は m-1 血清に反応したが，r-1 血清には反応せず，ラット株ではこれとまったく逆の成績を示した。そして，これら吸収血清に反応する抗原は100°C 1時間加熱と1N 塩酸処理によって凝集価が顕著に低下した。これらの成績から，吸収血清 m-1 と r-1 に反応する抗原はマウス株およびラット株にそれぞれ特異的な莢膜抗原であることが示唆された。