Estimation of Protective Immunity of Pigs Inoculated with Swine Erysipelas Live Vaccine by Passive Mouse Protection Test

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Abstract. An experiment was conducted to determine whether the protective immunity of pigs inoculated subcutaneously with live erysipelas vaccine can be estimated from the results of passive mouse protection tests. There was a positive correlation ($P < 0.01$) between the survival rates of mice (SRM) and the growth agglutination (GA) titers on 171 serum samples tested. The SRM obtained with swine sera collected 10 or 15 days after vaccination had a good correlation with protective immunity of the pigs to subsequent intradermal challenge exposure made 2, 3 and 4 months after vaccination. Therefore, it was concluded that the degree of mouse protection obtained with sera of the pigs in the early stage after vaccination indicates the duration of protective immunity in the vaccinated pigs. None of the vaccinated pigs exhibited any skin reaction (erythema) to challenge exposure made within 1 month after vaccination, in spite of low or negative SRM with their sera collected just before the challenge. On the other hand, most of the vaccinated pigs that were challenge-exposed 3 or 4 months after vaccination manifested various intensities of skin reaction, in spite of positive SRM with their sera collected just before the challenge. Therefore, the protective immunity of the vaccinated pigs could not be estimated from the mouse protectivity of their sera collected just before the challenge.

We reported [3] a good correlation between serologic response in the growth agglutination (GA) test and the protective immunity to subsequent challenge exposure in pigs vaccinated with live erysipelas vaccine. It is also well known that antiserum to *Erysipelothrix rhusiopathiae* protects mice and swine from infection with the organism [1, 5, 6, 9, 11]. There are several reports on the relationship between the GA titer and the mouse protectivity of hyper-immune sera [1, 8, 10]. In addition, Nishimura et al. [2] attempted to correlate the serologic response (including passive mouse protection) and the susceptibility of un-

vaccinated pigs to a virulent strain of the organism. However, there are no reports correlating the mouse protection with the sera of the pigs vaccinated with live erysipelas vaccine and the protection of the pigs from subsequent challenge exposure to *E. rhusiopathiae*.

The purpose of this experiment was to determine whether the protective immunity of the pigs vaccinated with live erysipelas vaccine can be estimated from the results of the passive mouse protection test with the sera obtained from the pigs between vaccination and challenge exposure.

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Materials and Methods

Porcine serum: Sera collected from 29 Yorkshire pigs, which had been vaccinated with swine erysipelas live vaccine prepared from the acriflavine-fast attenuated Koganei strain [7] of *E. rhusiopathiae* and challenge-exposed subsequently with the virulent Fujisawa strain of *E. rhusiopathiae* in a previous experiment [3], were used. The sera were collected periodically and kept at -20°C until use. Serologic data (GA test) and clinical data for these pigs have been reported elsewhere [3]. Protective immunity of the pigs was estimated from the general systemic signs and the intensities of the skin reaction (erythema) in response to challenge exposure.

Passive mouse protection test: A total of 171 serum samples were subjected to the passive mouse protection test. Of 171 samples, 18 were collected before vaccination, 115 at various times between vaccination and challenge, and 38 at various times within 40 days after challenge. Each serum sample was tested in seven to ten (usually ten) 4-week-old female mice of the ddY strain. Each mouse was given 0.2 ml of a serum sample by intraperitoneal injection. The mice were challenge-exposed by subcutaneous injection of 100 MLD (110–380 viable organisms) of the virulent Fujisawa strain 4 hr after administration of the serum. An equal number of non-treated control mice was similarly challenge-exposed. They were observed for at least 10 days after challenge, and the percentage survival was recorded for each group. The mouse protectivity of the porcine sera was expressed as the survival rate of mice (SRM).

Results

Relationship between SRM and GA titer.

The relationship between SRM and GA titer [3] of porcine sera is shown in Fig. 1. There were significant positive correlations ($P < 0.01$) between SRM and GA titer of sera collected in the post-vaccination ($r = 0.461$) and post-challenge ($r = 0.611$) stages, respectively, and through the entire period of the experiment ($r = 0.613$).

Relationship between SRM and responses of pigs to challenge exposure.

Low or negative SRM were observed with most of the sera obtained from the pigs before they were challenge-exposed

Fig. 1. Correlation between survival rate of mice (SRM) and growth agglutination (GA) titer
 ○ Pre-vaccination ($n=18$)
 ● Post-vaccination ($n=115$, $r=0.461$)
 △ Post-challenge ($n=38$, $r=0.611$)
 Total ($n=171$, $r=0.613$)

(Fig. 1 and Table 1). On the other hand, high SRM was observed with sera obtained from the pigs after they were challenge-exposed (Fig. 1 and Table 1).

The SRM observed with the serum samples obtained from the pigs of groups 1 and 2 (Table 1) in early stages after vaccination and before challenge exposure were low or negative. The sera from only three pigs showed positive SRM (Pig Nos. 4, 5 and 8 in the early stage, No. 5 before challenge). However, none of the vaccinated pigs exhibited any skin reaction (erythema) to challenge exposure. Most of the sera obtained from the pigs after challenge exposure gave higher protection in mice than did those obtained before challenge exposure.

The SRM with the sera obtained from the pigs of group 3 in the early stage after vaccination was high as compared with that of the sera from group 1 or 2. One pig (No. 11) whose serum obtained in the

Table 1. Relationship between SRM[†] and responses of pigs to challenge exposure

Group*	Pig No.	SRM after vaccination		Response after challenge	
		Early stage**	Before challenge	Erythema in skin (cm)§	SRM‡
1 (1/2)	1	0	0	—	10
	2	0	0	—	40
	3	0	0	—	20
	4	30	0	—	40
	5	20	10	—	20
2 (1)	6	0	0	—	80
	7	0	0	—	0
	8	30	0	—	30
	9	0	0	—	20
	10	0	0	—	70
3 (2)	11	0	0	1×2	90
	12	90	40	—	100
	13	40	40	—	100
	14	90	70	—	40
	15	30	70	—	10
	16	70	10	—	60
4 (3)	17	0	10	8×8	100
	18	10	10	10×10	100
	19	0	20	10×10	80
	20	10	20	8×8	100
	21	90	40	—	100
	22	100	37	—	100
5 (4)	23	10	60	20×20	100
	24	0	40	15×20	100
	25	20	Not done	15×15	100
	26	50	37	1×1	100
	27	20	10	2×2	100
	28	30	60	1×1	100
	29	80	25	—	100

† Survival rate (%) of mice in passive mouse protection test with porcine serum.

‡ Maximum values of mouse protective activity of porcine sera obtained during 40 days after challenge exposure.

* The pigs of groups 1 to 5 were challenge-exposed 1/2, 1, 2, 3 and 4 months (shown in parenthesis) after vaccination, respectively.

** 10th or 15th day after vaccination (10th day in group 1).

§ Maximum size of local skin lesion observed at the site of inoculation in each pig during 2 weeks after challenge exposure, as reported previously [3]. — = No skin reaction.

early stage after vaccination did not protect mice had a mild local skin reaction in response to challenge exposure. Sera from the other pigs of group 3 showed positive

SRM varying from 30 to 90% in the early stage after vaccination and 10 to 70% before challenge. Those pigs had no skin reaction in response to challenge exposure.

The sera obtained from the pigs of group 3 after challenge exposure had a higher SRM than did sera from pigs of group 1 or 2.

In groups 4 and 5, the pigs whose sera had negative or low SRM in the early stage after vaccination developed strong local skin reactions in response to challenge exposure, regardless of positive SRM with sera obtained before challenge (Nos. 17–20 and 23–25). On the other hand, the pigs whose sera had high SRM in the early stage after vaccination were completely immune to challenge exposure (Nos. 21, 22 and 29). Three pigs of group 5, whose sera had 20 to 50% SRM in the early stage after vaccination, (Nos. 26–28) developed mild local skin reactions. Sera obtained from the pigs of groups 4 and 5 after challenge completely protected mice with only one exception (No. 19).

Discussion

It has been shown that there is a good correlation between the GA titer and the mouse protectivity of the sera from hyper-immunized or unvaccinated pigs [1, 2, 8]. Our present experiment demonstrated also that the mouse protectivity of the sera of vaccinated and challenge-exposed pigs correlated positively with the GA titer, but the correlation coefficients were not high. Yokomizo and Isayama [10, 11] reported that the agglutinating antibody was present in the IgM and IgG fractions of the rabbit antiserum, but the protective activity for mice was associated with only the IgG fraction. Tanaka et al. [8] reported similar results with equine and swine antisera. In the present study, the serum samples tested were collected from pigs at various stages after vaccination. Therefore, the amount of the IgG fraction may have differed from serum to serum even though the sera had the same GA titers. High SRM obtained

with sera from pigs after challenge exposure probably indicates that the level of IgG fraction in the sera was increased by the challenge exposure. We observed recently [4] that the GA titer of the IgG fraction of the sera of the pigs immunized with the acriflavine-fast attenuated Koganei strain developed remarkably after challenge exposure with the virulent Fujisawa strain 3 months after immunization.

In our previous experiment [3], a close correlation was observed between the GA titer in the serum of each pig in the early stage after vaccination or just before challenge and the intensity of skin reaction in the pig to challenge exposure at various times after vaccination. In the present experiment, the SRM obtained with the sera of the vaccinated pigs of groups 3, 4 and 5 in the early stage after vaccination also correlated with the intensities of the skin reaction in response to challenge exposure. Therefore, it was considered that the amount of the mouse protective antibody 10 or 15 days after vaccination also indicates the duration of protective immunity in the vaccinated pig.

None of the vaccinated pigs of groups 1 and 2 exhibited any skin reaction in response to challenge exposure, in spite of low or negative SRM with their sera collected in the early stage or just before challenge. On the other hand, most of the vaccinated pigs of groups 4 and 5 presented various intensities of the local skin reaction in response to challenge exposure, in spite of positive SRM with their sera collected just before challenge. These results suggest that the degree of protective immunity of vaccinated pigs could not be estimated from the mouse protectivity of their sera collected just before challenge exposure. Nishimura et al. [2] reported that some unvaccinated pigs showed no skin reaction

in spite of low mouse protectivity of their sera. This agrees with the results obtained with the vaccinated pigs of groups 1 and 2 in our present experiment. Such inconsistencies between the protective immunity of pigs to challenge exposure and the mouse protectivity of their sera collected just before challenge exposure suggest that there may be a possible role of another protective mechanism in addition to the serum antibody against *E. rhusiopathiae* infection in pigs.

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要 約

受身マウス感染防御試験による豚丹毒生菌ワクチン接種豚の感染防御能の推測: 沢田 拓士・村松 昌武・瀬戸健次 (農林水産省動物医薬品検査所)——豚丹毒生菌ワクチンを皮下接種した豚 29 頭の感染防御能と血清の受身マウス感染防御能の関係を調べた。ワクチン接種前 (18 例), ワクチン接種後攻撃直前まで (115 例) および攻撃後 (38 例) の血清, 合計 171 例についての受身マウス感染防御試験の結果, マウス生存率 (SRM) と生菌発育凝集抗体価 (GA 価) の間には正の相関関係 ($P < 0.01$) が認められた。ワクチン接種後 10 あるいは 15 日目の血清の SRM とワクチン接種 2, 3, 4 カ月後の強毒菌による攻撃に対する豚の感染防御能とは関連性が認められた。このことから, ワクチン接種後早期の豚血清のマウス感染防御能は接種豚の免疫の持続性を示唆すると思われた。ワクチン接種後 1 カ月以内に攻撃された豚は, 攻撃前血清での SRM が低いか, 多くは陰性であったにもかかわらず, 何ら接種部位における限局性皮膚反応 (発疹) を示さなかった。一方, ワクチン接種後 3 あるいは 4 カ月目で攻撃された豚の多くは, 攻撃前血清での SRM が陽性であったにもかかわらず, 種々の強さの発疹を呈した。これらの結果から, ワクチン接種豚血清のマウス感染防御能のみからその免疫状態を推測することは困難であると思われた。