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Induction of cross-protection in mice against dolphin *Erysipelothrix rhusiopathiae* isolates with a swine commercial vaccine

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Abstract

Erysipelothrix rhusiopathiae is well known to cause disease in dolphins. This disease occurs either in an peracute way, leading to mortality even before clinical signs are observed or in a sub-acute way, characterized by rhomboidal skin lesions, that can be treated with penicillin or its derivatives. Commercial swine vaccines, containing inactivated serotype 2 strains, are currently used for vaccination but it is not known whether these vaccines induce protection against *E. rhusiopathiae* isolates from dolphins. In the present study, it was demonstrated in a mouse model that vaccination with a commercial swine vaccine (Eurovac Ery, Eurovet, Belgium) containing inactivated serotype 2 *E. rhusiopathiae* strains induced protection against challenge with three *E. rhusiopathiae* isolates from dolphins. The duration of the protection varied, depending on the challenging isolate, between 8 and >23 weeks. There was however no positive correlation between the amount of antibodies at the moment of challenge and the observed protection.

In conclusion, vaccination trials in mice indicate that commercial serotype 2 swine *Erysipelothrix* vaccines induce protection against erysipelas caused by dolphin pathogenic isolates. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Erysipelothrix rhusiopathiae*; Mouse cross-protection; Dolphin isolate

1. Introduction

Erysipelothrix rhusiopathiae, a gram-positive bacterium, is the causative agent of erysipelas in swine, and other animals, including cetaceans such as dolphins and belugas.

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In dolphins, the course of the disease can be peracute or sub-acute. Chronic infections with polyarthritis and/or endocarditis, as has been observed in swine, have not been described in dolphins (Sweeney and Ridgway, 1975). The disease is feared in marine mammal facilities because the acute septicemic infection mostly results in death even before clinical signs occur. However, recovery of the sub-acute form can be achieved by rapid treatment with antibiotics (usually penicillin derivatives), alone or combined with hyperimmune serum, immediately after the onset of the symptoms (Wood, 1992).

Therefore prevention of the disease, in particular by the use of vaccination, is important. The choice of the vaccine in dolphins and the vaccination protocol are not based on scientific data as little research has been done on vaccination in dolphins. Since dolphins are very sensitive to *E. rhusiopathiae* infections, attenuated live vaccines are not used as they remain too pathogenic (Gilmartin et al., 1971). Consequently, the few facilities that are currently vaccinating are using commercially available inactivated swine vaccines made from serotype 2.

Based on antigenic characteristics of soluble peptidoglycans, strains of *E. rhusiopathiae* are classified into 22 serotypes and group N (Wood, 1981). Only three dolphin isolates seem to have been serotyped and identified as serotypes 5 and 6, suggesting that other serotypes than those mostly isolated from swine could also be responsible for erysipelas infection in dolphins. Cross-protection between some serotypes, but not between all, has been demonstrated in mice and swine (Wood, 1979; Takahashi et al., 1984; Sawada and Takahashi, 1987). Although swine can be protected against erysipelas by vaccination with serotype 2, it is not known whether the latter protects against the serotypes and strains causing erysipelas in dolphins.

The aim of this study was to determine whether vaccination with serotype 2 strains protects against dolphin *Erysipelothrix* isolates. Since such protection studies can not be done in dolphins, experiments were performed in mice as described earlier (Gledhill, 1945; Sawada and Takahashi, 1987).

2. Material and methods

2.1. Erysipelas isolates and strains

The swine vaccine strain Br 12–92 of serotype 2 was obtained from Eurovet (Belgium). Isolate 266/2344, belonging to serotype 1a was recovered from the kidney of a swine that died from erysipelas in 1979. The two swine strains were used to control the validity of the mice model. Isolate 266/6611 was cultured from the liver of a 14-year-old female dolphin that died from erysipelas in 1987 at the Brugge dolphinarium, Belgium. This strain cannot be assigned to the classical serotypes (Lacave, G. and Schlater, L., unpublished data). Isolate 266/9085 was obtained from the lungs of an 11-year-old male dolphin that died from an intestinal torsion in 1995 at the Brugge dolphinarium, Belgium and has not been serotyped yet. Isolate Ery 5207 was detected in different organs of a 5-year-old male dolphin that died from erysipelas at the Zoo of Antwerp, Belgium, in 1990. This isolate was partially identical to serotype 5 (Lacave, G. and Schlater, L., unpublished data). Isolate 94/1650 was recovered from the mesenteric lymph nodes of a

Table 1
The mice LD_{50} of swine and dolphin *E. rhusiopathiae* isolates

Isolate or strain	LD_{50} dose in CFU	Species origin	Serotype
266/2344	2×10^5	Swine	Serotype 1a
Br 12–92	6×10^2	Swine	Serotype 2
266/6611	2.8×10^3	Dolphin	Serotype *X*[a]
Ery 5207	1×10^3	Dolphin	[Serotype 5][b]
94/1650	6×10^3	Dolphin	[Serotypes 20 and 21][b]
266/9085	$> 2 \times 10^{11}$[c]	Dolphin	–[d]

[a] None of the classical serotypes.
[b] Partially identical.
[c] No pathogenicity in the mice.
[d] Not serotyped.

1-week-old dolphin calf from the Brugge dolphinarium in Belgium, which died from a head trauma with no signs of septicemia. This isolate was partially identical to serotypes 15 and 21 (Lacave, G. and Schlater, L., unpublished data) (Table 1).

The lyophilized bacteria were resuspended in phosphate buffered saline (PBS) and grown in BHI broth at 37°C for 72 h under constant shaking (85 rpm). The bacteria were harvested by centrifugation (30 min at 3000*g*) and washed three times in PBS. Subsequently they were stored in different aliquots at −80°C in a 90% LYM medium (one volume brain heart infusion broth (BHI, Oxoid, Italy), three volumes inactivated horse serum (GIBCO, Great Britain) and 0.5% glucose (VEL, Belgium)). In order to determine the number of viable bacteria 10-fold dilutions in PBS of each aliquot were inoculated on BHI agar and incubated for 48 h at 37°C before counting the number of colony forming units (CFU).

2.2. Lethality testing and immunization studies

Experimental procedures and animal management procedures were undertaken in accordance with the requirements of the Animal Care and Ethics Committee of the University of Ghent, Belgium.

The mice LD_{50} dose of each isolate was determined, as described previously, by inoculating 0.1 ml of 10-fold dilutions of each isolate intraperitoneally in BALB/C mice of at least 12 weeks of age. The mice were housed in an isolation unit where they received food and water ad libitum. Mice were observed during 10 days for clinical signs and mortality. Subsequently, the LD_{50} titer was calculated following Kärber (1969).

Thirty-nine mice received one subcutaneous inoculation of 0.1 ml of the serotype 2 swine vaccine Eurovac Ery (Eurovet, Belgium). Per strain, a cross protection study was performed by challenging mice, in groups of 2–4 of same sex and age, 3–4, 8–10 and 20–23 weeks after vaccination. The challenge doses, given intraperitoneally in 0.1 ml PBS, were, respectively, 400 LD_{50} of strain Ery 5207, 200 LD_{50} of strain 266/2344, 700 LD_{50} of strain 266/6611, 350 LD_{50} of strain Br 12–92 and 450 LD_{50} of strain 94/1650. Non-vaccinated mice were used as controls. Clinical signs and mortality were daily observed for 50 days. Kidneys, spleen, liver and lungs were sampled from dead or

euthanized sick mice for isolation of *E. rhusiopathiae*. Isolation occurred on blood agar plates plus 5% (v/v) whole defibrinated sheep blood.

Mice were bled twice, just before the vaccination and the challenge infection, for the determination of their *Erysipelothrix*-specific antibody levels in an ELISA.

2.3. Erysipelothrix-specific antibody ELISA

E. rhusiopathiae isolates 266/6611 and Ery 5207 were cultured as described. The resulting pellet was washed three times with coating buffer (50 mM carbonate–bicarbonate buffer, pH 9.4) and finally resuspended to a turbidity equivalent of MacFarland index 2. The latter equals 1×10^7 *E. rhusiopathiae*/ml determined by viable count on BHI agar. One hundred microliters of a mixture (1/1) of both bacterial suspensions were used to coat 96-well ELISA plates (Polysorb (Nunc)) overnight at 4°C. Blocking occurred with 5% glycin in coating buffer (1 h at room temperature). Subsequently, the serum samples, previously heat-inactivated and pretreated with kaolin, were diluted in PBS supplemented with 0.05% Tween-20 and 5% non-fat dry milk powder to a final dilution of 1/50. Aliquots of 100 μl of test sera were transferred in antigen-coated and control (coated with plain coating buffer) wells (1 h at 37°C). One hundred microliters of rabbit anti-mouse immunoglobulins, conjugated to horseradish peroxydase, DAKO (Belgium), were used as antiserum at a dilution of 1/1000 (1 h at 37°C). In between each step wells were washed with saline supplemented with 0.05% Tween-20. The absorbance was measured at 405 nm after addition of 50 μl ABTS-solution (Boehringer-Mannheim, Belgium) (1 h at 37°C). The absorbance of a sample was calculated as the absorbance of the antigen-coated well minus the absorbance of the buffer-coated well.

3. Results

3.1. The mice LD_{50} of the Erysipelothrix isolates and strain

The swine reference strain Br 12–92 was 2–10 times more pathogenic for mice than the dolphin isolates Ery 5207, 266/6611 and 94/1650 and even more than 300 times more pathogenic than the swine strain 266/2344. The dolphin isolate 266/9085, unexpectedly isolated from the lungs of a dolphin that had died from an intestinal torsion, appeared to be apathogenic (Table 1).

3.2. Antibody response following vaccination

Before vaccination all mice were negative for antibodies against *Erysipelothrix*. Non-vaccinated mice remained negative until challenge infection. Vaccination with the commercial serotype 2 swine vaccine yielded specific anti-*Erysipelothrix* antibodies in all mice. Antibodies could be demonstrated in samples 3 weeks post-vaccination and were still present 23 weeks after vaccination. There was no positive correlation between the OD value at the moment of challenge and the observed protection.

3.3. Challenge infection

All control animals died between 1 and 7 days post-challenge. *E. rhusiopathiae* could be isolated from the kidneys, spleen, liver and lungs of all of them.

Vaccination with the commercial serotype 2 swine vaccine induced a protective immunity against challenge with the five pathogenic *Erysipelothrix* isolates. Total protection was observed from the third week post-vaccination until the 10-week post-vaccination against all challenge strains except against isolate 94/1650. In this case, the three challenged mice died after a mean of 23 days which is longer than the survival of the control animals, indicating an incomplete protection. Twenty-three weeks post vaccination, complete protection only occurred against isolate 266/6611 and the vaccine strain Br 12–92. At that moment, one of the two mice challenged with strain 266/2344 died after 2 days, one of the two mice challenged with strain Ery 5207 died after 5 days and one of the two mice challenged with strain 94/1650 died after 7 days. *Erysipelothrix* could be isolated from all the sick euthanized mice or the mice that died following challenge. Fifty days after challenge, none of the surviving mice showed any disease symptom.

4. Discussion

The results of this study define that the isolates 266/6611 and Ery 5207, isolated from dolphins that died from an *Erysipelothrix* septicemia, and isolate 94/1650, accidentally isolated from a dolphin calf, are all three pathogenic for BALB/C mice. On the other hand, 266/9085, that was incidentally isolated from the lungs of a dolphin, was not pathogenic for BALB/C mice. Since this isolate still has to be serotyped, it is not clear whether it is a non-pathogenic *E. rhusiopathiae* strain or an *E. tonsillarium* strain. Indeed Takahashi et al. (1984, 1992) demonstrated that some strains of the *E. rhusiopathiae* serotypes 4, 9 and 17 and of the *E. tonsillarium* serotypes 7 and 22 are non-pathogenic for mice ($LD_{50} > 10^6$).

Erysipelothrix strains have different pathogenicities for different host species. For example strains that are pathogenic for swine are also pathogenic for mice, but not vice versa. Strains that are highly pathogenic for mice are sometimes non-pathogenic for swine (dYY line, Sawada and Takahashi, 1987; Takahashi et al., 1984, 1987; BALB/C, inbred-NZB, Eames, 1988). A difference in pathogenicity was also described for different mice lines (Watari et al., 1993). Mice seem to be more susceptible to infection than swine (Takahashi et al., 1984, 1992) with the exception of serotype 1a, where strains have been described as more pathogenic for swine than for mice (Takahashi et al., 1985).

Three dolphin pathogenic erysipelas strains have been described as serotypes 5 and 6. These isolates are pathogenic for mice but non-pathogenic for swine (Takahashi et al., 1984, 1992). Mice appear therefore as a better model to test cross-protection of dolphin isolates than swine.

The isolate 94/1650 that was incidentally isolated from the mesenteric lymph nodes of a dolphin in the absence of septicemia symptoms induced mortality in BALB/C mice. Its LD_{50} was comparable to the one of the two other dolphin pathogenic isolates. It is thus

possible that the bacteria were isolated during the incubation period of the infection. This isolate has been serotyped as partially identical to serotypes 15 and 21. Isolates of serotypes 15 and 21 have been described to be weakly pathogenic for swine (Sawada and Takahashi, 1987; Takahashi et al., 1992). However, one cannot exclude that this dolphin was carrier of a for dolphins non-pathogenic *E. rhusiopathiae* strain, like occurs in pigs or turkeys and that dolphin might be a source of contamination for other animals (Wood, 1992; Takahashi et al., 1987).

Furthermore, even within serotypes, differences in protection have been described (Wood, 1979; Takahashi et al., 1984; Sawada and Takahashi, 1987) and the results in cross-protection obtained by the different researchers is not completely identical. Timoney and Groschup (1993) showed that protection is not serotype-specific. A result consistent with previous observations that polysaccharides, non-proteinaceous antigens are the type-specific antigen useful in serotyping and different from those inducing protective immune responses (Wood, 1979).

In conclusion, results from this study show that a commercial inactivated serotype 2 *Erysipelothrix* swine vaccine cross-protects BALB/C mice against different dolphin pathogenic *Erysipelothrix* strains, among which serotypes partially identical to 5, 15 and 22. However, the prolonged disease in vaccinated mice after challenge suggests that a booster vaccination is required for complete protection. These conclusions have led to the decision to booster dolphins belonging to an erysipelas vaccination test population (dolphinarium Brugge, Belgium and Zoomarine, Portugal) 3–4 weeks after their primo-vaccination.

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