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Experimental Bacteriophage Protection against *Staphylococcus aureus* Abscesses in a Rabbit Model

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In a rabbit model of wound infection caused by *Staphylococcus aureus*, 2×10^9 PFU of staphylococcal phage prevented abscess formation in rabbits when it was injected simultaneously with *S. aureus* (8×10^7 CFU) into the same subcutaneous site. Phage multiplied in the tissues. Phages might be a valuable prophylaxis against staphylococcal infection.

Studies (2, 4, 5, 10–14) have shown substantial efficacy of bacteriophage therapy for experimental infections by gram-negative bacteria, but for gram-positive bacteria the efficacy of phage has been limited and has been demonstrated only in a few recent studies (3, 7, 8). *Staphylococcus aureus*, a cause of wound and soft-tissue infection, is often resistant to all β-lactam antibiotics, and strains resistant to vancomycin occur (15): surgical infections may become untreatable. We describe a model for wound infection in rabbits by a strain of *S. aureus* that caused infection on a rabbit farm (9) and protection by a phage.

S. aureus strain 2698 from a rabbit was kindly provided by E. Espuña of Laboritorios HIPRA, Girona, Spain. Phage LS2a was isolated from sewage. Nutrient broth (no. 2), agar (no. 1), brain heart infusion, and nutrient agar were obtained from Oxoid.

Adult New Zealand White rabbits of 1.5 to 2.5 kg in weight were housed in separate cages. *S. aureus*, phage, and control suspensions (0.1 ml in each case) were injected subcutaneously into a shaved area on the flank of each rabbit. Where more than one injection was given, they were given at the same site, and when given together, bacteria were injected first followed by a phage or control suspension. The model was established, and then pilot protection studies were done with up to four rabbits per group. In three main studies, three groups of rabbits received the following: bacteria and phage, bacteria and control suspension, and phage-only controls (i.e., no bacteria), which also received a liquid produced by subjecting brain heart infusion to the procedure that had been used to purify the bacterial suspensions. Rabbits were examined daily for 4 to 6 days and then killed. Skin and fascia (2 by 2 cm) were removed from around the injection site, including any abscess present.

Bacterial innocula were prepared by culturing *S. aureus* in brain heart infusion for 14 h overnight and centrifuging it at $2{,}000 \times g$ for 5 min, washing, recentrifuging it twice, and then resuspending it in saline.

Phage and control suspensions were prepared as follows. 2698 (2×10^9 CFU) and 2×10^8 PFU of LS2a were shaken together in 20 ml of nutrient broth at 37°C for 3.5 h. Phage was purified by filtration (pore size, 0.45 μm), ultracentrifugation, resuspension in saline, and refiltration. The control suspension was prepared by lysing 14-h broth cultures of 2698 containing 10^8 CFU/ml by agitating them with 0.1-mm silica beads for 3 min, using the Fastprep system (www.qbiogene.com). The resulting suspensions were then processed in the same way as the phage lysates.

Bacteria were counted by incubating 0.1-ml portions of serial 10-fold dilutions of homogenates on nutrient agar. Phages were counted on nutrient agar using the overlay method (1), with the overlays consisting of 0.5% agar. Low numbers of phages in homogenates of livers and spleens were sought by incorporating 10-h broth enrichment cultures (with *S. aureus*) into agar overlays seeded with *S aureus*.

Data were analyzed by Fisher's exact and Mann Whitney U tests.

In a prophylaxis study, rabbits each received 8×10^7 CFU of *S. aureus* 2698 and either control suspension or 2×10^9 PFU of LS2a. After 4 days, one of the eight phage-treated rabbits had an abscess (area, 64 mm^2), whereas all eight of the untreated rabbits had abscesses (median area, 106; range, 32 to 144 mm^2) ($P = 0.001$). Bacterial CFU from the injection sites were lower ($P < 0.003$) for treated rabbits (median, 330; range, 0 to 11,000) than for the untreated controls (median, 2.3×10^4; range, 1,800 to 100,000). Numbers of PFU of phage from injection sites of infected rabbits that had received phage ranged from 240 to 600,000 (median, 6,800), while 1,200 and 340 phage PFU were cultured from two rabbits that had received 2×10^9 PFU of LS2a and no bacteria. No bacteria were isolated from the livers or spleens of any of the rabbits, and no phages were isolated from the spleens of the untreated rabbits. Phage (60 PFU) was isolated from the liver of only one treated rabbit. Phages were isolated from the spleens of all but one of the phage-treated rabbits and from five of the rabbits treated by enrichment only. In the remaining two, the counts were 20 and 1,400 PFU, while counts in the spleens of the phage-only controls were 700 and 5,700.

In a dose response study, rabbits each received 8×10^7 CFU of *S. aureus* 2698 and either 6×10^7, 6×10^6, or 6×10^5 PFU of LS2a or control suspension. One phage-only control was included for each dose group, receiving the same dose of phage but no bacteria. After 4 days, all but one of the 12 rabbits that had received bacteria produced abscesses; the exception had received the highest dose of phage. The median areas and bacterial counts in the abscesses (Table 1) increased consistently as the dose of phage decreased, with the largest abscesses being those of the control group, which contained higher bacte-

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TABLE 1. Abscess area and bacterial and phage counts[a]

No. of rabbits	Dose (10^3 PFU)	Abscess area (mm^2)		Count of 2698 in abscess (10^3 CFU)		Count of LS2a in abscess (10^3 PFU)	
		Median	Range	Median	Range	Median	Range
4	60,000	58	0–120	6	3–10	1,600	700–8,900
4	6,000	64	36–120	20	2–42	1,500	300–4,400
4	600	80	80–168	66	28–1,600	840	160–7,600
5	0[b]	120	64–168	600	54–840	0	0–0

[a] Counts were made 4 days after 21 rabbits received (subcutaneously) 8×10^7 CFU of *S. aureus* 2698 and either control suspension or one of three doses of phage LS2a. Where 0 is quoted as a count, this means <10 CFU or PFU.
[b] Control suspension.

rial counts than the abscesses of the groups receiving the two higher doses ($P < 0.01$ in each case). Phages were not cultured from the injection sites of the phage-only controls but were cultured from those of all rabbits that received bacteria and phage. In two of the rabbits receiving the lowest dose, 600,000 PFU, more phage was recovered than was administered (7,600,000 and 1,400,000 PFU). No bacteria were isolated from the livers or spleens of any of the rabbits, and no phages were isolated from the livers or spleens of the untreated rabbits. Phage was isolated from the spleens of two of three phage-only controls, those that had received 10^7 and 10^5 PFU, and from four of four, three of four, and one of four infected rabbits that had received 10^7, 10^6, and 10^5 PFU, respectively.

In a delayed-treatment study, 21 rabbits each received 5×10^7 CFU of *S. aureus* and either control suspension given immediately after the bacteria or 3×10^9 PFU of phage given 6, 12, or 24 h after the bacterial injection and at each time also to one phage-only control. Four days after the first injections, all the rabbits had abscesses, the sizes and bacterial counts of which did not differ significantly from those for the controls (Table 2). Phage counts from the injection sites of the phage-only controls were 500 PFU from the 6-h control and 2,000 PFU from each of the 12- and 24-h controls, lower than all those from the infected rabbits. No bacteria were isolated from the livers or spleens of any of the rabbits, and no phages were isolated from any liver or spleen of the untreated rabbits.

We demonstrated phage prophylaxis against experimental *S. aureus* infections similar to those that are common in humans, i.e., local and without foreign bodies. There was no evidence of general sepsis, and the rabbits, including controls, appeared remarkably well. The protection achieved in this and another (7) study of *S. aureus* was much less striking than that achieved against gram-negative bacteria, in which protection has been demonstrated at phage PFU/bacterial CFU ratios of 1/1,000,000 (14) and 1/100,000 (12). Perhaps phages are not as effective in the treatment of infections by gram-positive infections, though one study by Nakai et al. (8) has yielded slightly more convincing results, protecting fish from lactococcal infection with a phage PFU/bacterial CFU ratio of 1/40.

TABLE 2. Abscess area and bacterial and phage counts[a]

No. of rabbits	Time before dose (h)	Abscess area (mm^2)		Count of 2698 in abscess (10^3 CFU)		Count of LS2a in abscess (10^3 PFU)	
		Median	Range	Median	Range	Median	Range
4	6	290	256–416	475	190–4,000	1,300	830–1,700
4	12	120	72–224	1,090	180–2,900	4,450	810–9,600
4	24	156	112–288	865	450–1,100	4,850	930–11,000
5	0[b]	160	80–256	550	120–760	0	0–0

[a] Counts were made 4 days after 21 rabbits received (subcutaneously) 5×10^7 CFU of *S. aureus* 2698 bacteria and either control suspension or 3×10^9 PFU of LS2a phage at the times shown. Where 0 is quoted as a count this means <10 CFU or PFU.
[b] Control suspension.

For three rabbits, we recovered more phages than were administered; this, to our knowledge, is the first direct evidence of administered phage multiplying in the tissues infected by gram-positive bacteria. Many previous models of *S. aureus* infection have studied animals that do not normally suffer from *S. aureus* infection (e.g., mice) by using either large infecting doses (14, 7) or foreign bodies (6). Since rabbits, like humans, readily suffer from *S. aureus* infections, they are more appropriate, and infections can be produced without foreign bodies and with only moderate bacterial doses. The use of strains derived from rabbits makes the model close to naturally occurring infection and the results applicable to farmed rabbits. With concerns about future untreatable strains, phage prophylaxis for human surgery might be appropriate.

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REFERENCES

1. **Adams, M. H.** 1959. Bacteriophages, p. 447–448. Interscience Publishers, Inc., New York, N.Y.
2. **Barrow, P., M. Lovell, and A. Berchieri, Jr.** 1998. Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. Clin. Diagn. Lab. Immunol. **5:**294–298.
3. **Biswas, B., S. Adhya, P. Washart, B. Paul, A. N. Trostel, B. Powell, R. Carlton, and C. R. Merril.** 2002. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Infect. Immun. **70:**204–210.
4. **Cerveny, K. E., A. De Paola, D. H. Duckworth, and P. Gulig.** 2002. Phage therapy of local and systemic disease caused by *Vibrio vulnificus* in iron-dextran-treated mice. Infect. Immun. **70:**6251–6262.
5. **Dubos, R. J., J. H. Straus, and C. Pierce.** 1943. The multiplication of bacteriophage in vivo and its protective effect against an experimental infection with Shigella dysenteriae. J. Exp. Med. **78:**161–168.
6. **Espersen, F., N. Frimodt-Møller, L. Corneliussen, U. Riber, V. Thamdrup Rosdahl, and P. Skinhøj.** 1994. Effect of treatment with methicillin and gentamicin in a new experimental mouse model of foreign body infection. Antimicrob. Agents Chemother. **38:**2047–2053.
7. **Matsuzaki, S., M. Yasuda, H. Nishikawa, M. Kuroda, T. Ujihara, T. Shuin, Y. Shen, Z. Jin, S. Fujimoto, M. D. Nasimuzzaman, H. Wakiguchi, S. Sugihara, T. Sugiura, S. Koda, A. Muraoka, and S. Imai.** 2003. Experimental protection of mice against lethal Staphylococcus aureus infection by novel bacteriophage phi MR11. J. Infect. Dis. **187:**613–624.
8. **Nakai, T., R. Sugimoto, K. H. Park, S. Matsuoka, K. Mori, T. Nishioka, and K. Maruyama.** 1999. Protective effects of bacteriophage on experimental *Lactococcus garviae* infection in yellowtail. Dis. Aquat. Org. **37:**33–41.
9. **Pages Manté, A., C. Artigas, and L. L. Costa.** 1992. Experimental model to better know the sensibility to Staphylococcus aureus in different commercial rabbit breeds. J. Appl. Rabbit Res. **15:**1440–1447.
10. **Smith, H. W., M. B. Huggins, and K. M. Shaw.** 1987. The control of experimental Escherichia coli diarrhoea in calves by means of bacteriophages. J. Gen. Microbiol. **133:**1111–1126.
11. **Smith, H. W., and M. B. Huggins.** 1983. Effectiveness of phages in treating experimental Escherichia Coli diarrhoea in calves, piglets and lambs. J. Gen. Microbiol. **129:**2659–2675.
12. **Smith, H. W., and M. B. Huggins.** 1982. Successful treatment of experimental Escherichia coli infections in mice using phage: its general superiority over antibiotics. J. Gen. Microbiol. **128:**307–318.
13. **Soothill, J. S.** 1994. Bacteriophage prevents destruction of skin grafts by Pseudomonas aeruginosa. Burns **20:**209–211.
14. **Soothill, J. S.** 1992. Treatment of experimental infections of mice with bacteriophages. J. Med. Microbiol. **37:**258–261.
15. **Weigel, L. M., and D. B. Clewell.** 2003. S. R. Gill et al. Genetic analysis of a high level Vancomycin-resistant isolate of Staphylococcus aureus. Science **302:**1569–1570.