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The Surface Protein Pls of Methicillin-Resistant *Staphylococcus aureus* Is a Virulence Factor in Septic Arthritis

Elisabet Josefsson,[1]* Katri Juuti,[2] Maria Bokarewa,[1] and Pentti Kuusela[3,4]

Department of Rheumatology, University of Göteborg, Göteborg, Sweden[1]; Department of Biological and Environmental Sciences, Division of General Microbiology, University of Helsinki, Finland[2]; Department of Bacteriology and Immunology, The Haartman Institute, University of Helsinki, Finland[3]; and Division of Clinical Microbiology, Helsinki University Central Hospital, Helsinki, Finland[4]

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Pls, a surface protein of certain methicillin-resistant *Staphylococcus aureus* strains, is associated with poor bacterial adherence to solid-phase fibronectin and immunoglobulin G, as well as with reduced invasion of cultured epithelial cells. Here the importance of Pls for the development of septic arthritis and sepsis was investigated by using a mouse model. Mice inoculated with a *pls* knockout mutant developed a much milder arthritis and showed less grave weight reduction than mice infected with the wild-type Pls$^+$ clinical isolate. Also, the *pls* mutant induced a significantly lower frequency of mortality than the wild-type strain. The bacterial load of the kidneys was larger in mice infected with the Pls$^+$ strain than in animals challenged with the *pls* mutant. However, there was no evident inflammatory effect due to the Pls molecule alone, as indicated by knee injection of purified Pls. In conclusion, the results show that Pls is a virulence factor for septic arthritis and sepsis.

The binding of bacterial surface proteins to host extracellular matrix components is postulated to be crucial for bacterial colonization and survival in the host. Several interactions between host matrix components and *Staphylococcus aureus* surface proteins have been described (for a review, see reference 4). Surface proteins of *S. aureus* have been shown to be virulence factors in various infection models, although the virulence mechanisms have not yet been revealed. The staphylococcal surface proteins protein A and clumping factor A, which promote binding to immunoglobulin G and fibrinogen, respectively, have been shown to be virulence factors in models of septic arthritis and endocarditis (13, 9, 12).

One *S. aureus* surface protein with interesting properties is Pls (plasmin-sensitive protein). Pls has been shown to attenuate the bacterial binding to immobilized fibronectin and immunoglobulin G (18), as well as invasion of epithelial cells (10). On the other hand, Pls appears to mediate adhesion to cellular lipids and glycolipids and to promote bacterial cell-cell interactions (7). The molecule is large, 1,637 amino acids, and consists of a nonrepeat region preceded by short repeats close to the N terminus and followed by 4.5 longer repeats, a serine-aspartate (SD) dipeptide repeat region, and the wall- and membrane-spanning region containing an LPDTG motif (18). According to the terminology now generally adopted for the *S. aureus* LPXTG proteins, the N-terminal repeats and the nonrepeat region should be called an A domain and the longer repeats should be called a B domain (14). The *Staphylococcus epidermidis* accumulation-associated protein, Aap, is very similar to Pls in both domain structure and amino acid sequence. Also, SasG and SasA, two recently described LPXTG proteins of *S. aureus*, show homology with the Pls sequence (14). There is evidence that both Pls and SasG are involved in staphylococcal attachment to human desquamated nasal epithelial cells (15). The *pls* gene is part of the staphylococcal cassette chromosome *mec*, which carries the *mecA* gene encoding methicillin resistance, and so far has been found only in type I staphylococcal cassette chromosome *mec* (8). *pls* carriage was originally linked to a negative phenotype in first-generation latex agglutination assays (6, 11).

Thus, it seems that Pls may have both adhesive and antiadhesive functions. These opposite functions raise a question concerning the effect of Pls on staphylococcal virulence. Bacterial arthritis is a rapidly progressive and highly destructive joint disease that in 5 to 15% of the cases leads to death (5). *S. aureus* is the major cause of human infectious arthritis (5). In this study a well-established mouse model of septic arthritis was used to explore the importance of Pls to the bacterial virulence (3, 16). Pls turned out to be an important virulence factor for sepsis, for induction of arthritis, and for sepsis-induced death.

MATERIALS AND METHODS

Mice. Female NMRI mice were obtained from B&K Universal AB (Sollentuna, Sweden) and were maintained in the animal facility of the Department of Rheumatology, University of Göteborg, Sweden. All mice were maintained according to the local ethic board animal husbandry standards. They were housed at densities of up to 10 animals per cage with a 12-h light-dark cycle, and they were fed standard laboratory chow and water ad libitum. The animals were 8 to 9 weeks old at the start of the experiments.

Bacterial strains. *S. aureus* strains 1061 (6, 11), 1061 *pls*::Tc^r (18), and 1061 *pls*::Tc^r(pPLS4) (18) were used in infection studies. Strain 1061 is a Pls-expressing clinical methicillin-resistant *S. aureus* (MRSA) isolate. Strain 1061 *pls*::Tc^r is an allele replacement mutant and does not express Pls on the surface. Strain 1061 *pls*::Tc^r(pPLS4) is the 1061 *pls*::Tc^r strain complemented with the *pls* gene in the pCU1 plasmid and expresses Pls on the surface.

Bacteria were grown on tryptic soy agar plates (containing 5 μg of chloramphenicol/ml for cultivation of the complemented strain) for 48 h, harvested, and

* Corresponding author. Mailing address: Department of Rheumatology, University of Göteborg, Guldhedsgatan 10, S-413 46 Göteborg, Sweden. Phone: 46-31-3426475. Fax: 46-31-823925. E-mail: elisabet.josefsson@rheuma.gu.se.

kept frozen at −20°C in phosphate-buffered saline (PBS) containing 5% bovine serum albumin (wt/vol) and 10% (vol/vol) dimethyl sulfoxide.

Before injection into animals, the bacterial suspensions were thawed, washed in PBS, and adjusted to appropriate cell concentrations. The number of viable bacteria was measured in conjunction with each challenge by cultivating organisms on blood agar plates and by counting colonies after 24 h of incubation at 37°C.

Purification of Pls protein. Pls was purified from a lysostaphin digest of strain 1061 using affinity chromatography on immobilized wheat germ agglutinin lectin as described previously (6).

Experimental protocols for infection studies. In all experiments 15 mice per group were injected intravenously in the tail vein with a certain number of bacteria in 0.2 ml of PBS. The tails were warmed under an infrared bulb before intravenous injection. The mice were regularly weighed and examined for arthritis and general appearance. In cases of severe systemic infection (as judged by reduced alertness and ruffled coat), mice were killed by cervical dislocation.

In the first experiment mice were infected with 4.7×10^7 CFU/mouse of strain 1061 or 7.8×10^7 CFU/mouse of the *pls* mutant 1061 *pls*::Tc^r. The experiment was terminated 14 days postchallenge. Kidneys were examined for bacterial infection, and the paws were investigated histopathologically.

In the second experiment 15 mice per group received 3.4×10^7 CFU/mouse of strain 1061 or 3.8×10^7 CFU/mouse of strain 1061 *pls*::Tc^r. Serum samples were obtained at day 7, and the interleukin-6 (IL-6) levels in the samples were measured. The experiment was terminated on day 11. One pair of limbs (fore and hind) and the kidneys were examined for bacterial infection.

In the third experiment mice were infected with 3.4×10^7 CFU/mouse of strain 1061 or 2.8×10^7 CFU/mouse of strain 1061 *pls*::Tc^r. The mice were sacrificed at day 8. Tumor necrosis factor alpha (TNF-α) levels were measured in sera, and the kidneys were examined for bacterial infection.

In the fourth experiment mice were infected with 2.5×10^7 CFU/mouse of strain 1061, 3.8×10^7 CFU/mouse of strain 1061 *pls*::Tc^r, or 4.0×10^7 CFU/mouse of strain 1061 *pls*::Tc^r(pPLS4). Bacterial growth was determined in the blood 24 h after infection. The experiment was terminated at day 10. IL-6 and TNF-α levels in sera were determined. One pair of limbs and the kidneys were examined for bacterial infection.

In the last experiment, in order to study mortality, the mice received 1.8×10^8 or 1.7×10^8 CFU/mouse of the wild-type strain or strain 1061 *pls*::Tc^r. The general state of health was monitored each day until day 22, when the experiment was terminated. During the experiment, the mice judged too ill to survive another 24 h were sacrificed and considered dead due to sepsis.

Clinical evaluation of arthritis. Clinical arthritis was evaluated blindly by a neutral observer. All mice were examined individually, and the limbs were inspected visually. The inspection yielded a score of 0 to 4 for each limb (0, normal appearance; 1, very mild arthritis; 2, mild arthritis; 3, moderate arthritis; 4, marked arthritis). Arthritis was defined as visible erythema and/or swelling of at least one joint. The arthritic index was constructed by adding the scores from all four limbs for each animal (1).

Histopathological examination. Histological examination of joints was performed using a modification (9) of a previously described method (17).

Bacteriological examination of infected animals. In order to detect bacterial growth in joints, one pair of joints (ankle and wrist) was topically disinfected with ethanol, dissected, swabbed, and streaked on *Staphylococcus* medium 110 agar plates (BBL, Becton Dickinson, Sparks, MD). The joint area was considered positive for *S. aureus* when 10 or more colonies were found on the plate after 24 h of incubation at 37°C.

To examine bacterial growth in blood, blood samples were obtained from a tail vein. To detect bacteria in the kidneys, the kidneys were removed, homogenized, and serially diluted in PBS. Blood or kidney suspensions (100 μl) were spread on blood agar plates, and bacterial colonies were counted after 24 h of incubation at 37°C. The results were expressed as the number of CFU per ml of blood or per kidney pair.

Colonies recovered from joints and kidneys were tested for catalase and coagulase activities and scored for antibiotic resistance to ensure stability of markers and to exclude contamination.

Cytokine measurements. Serum IL-6 levels were determined by a method described previously (2). The levels of TNF-α were determined using a sandwich enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Intra-articular injection of Pls. Seven mice per group were injected in one knee joint either with 2 μg of Pls protein in 20 μl of PBS or with the vehicle alone. The animals were sacrificed 4 days after challenge, and the knees were collected for histopathological examination.

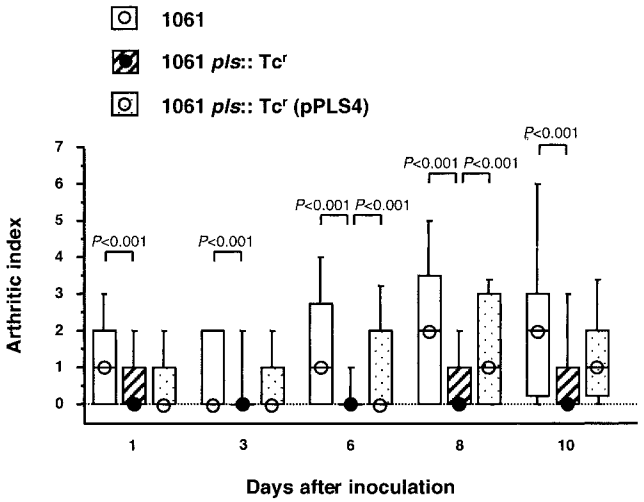

FIG. 1. Development of arthritis in mice during 10 days after inoculation with 2.5×10^7 to 3.4×10^7 CFU of *S. aureus* wild-type strain 1061, 2.8×10^7 to 3.8×10^7 CFU of strain 1061 *pls*::Tc^r, or 4.0×10^7 CFU of strain 1061 *pls*::Tc^r(pPLS4) at day zero (pooled results from experiments 2, 3, and 4). The circles indicate medians; the boxes indicate interquartile ranges; and the whiskers indicate 80% central ranges. For days 1 to 8, $n = 40$ to 45 for the wild type, $n = 45$ for the *pls* mutant, and $n = 13$ to 15 for the complemented *pls* mutant. For day 10, $n = 27$ for the wild type, $n = 30$ for the *pls* mutant, and $n = 11$ for the complemented *pls* mutant. Data were analyzed by the Kruskal-Wallis test. For each test, significance was set at $P = 0.005$ by Bonferroni's adjustment, with simultaneous significance set at $P = 0.05$.

Statistical analysis. Statistical evaluation was done by using the Mann-Whitney U test when two groups were compared, the Kruskal-Wallis test with a subsequent post hoc analysis when there were three groups to compare, or the Kaplan-Meier test for survival analysis. The Bonferroni method was used to adjust the significance level when multiple comparisons were performed. A P value of <0.05 was considered significant. Scores are reported below as medians, interquartile ranges (IQR), and 80% central ranges, and numerical continuous data are reported as means ± standard errors of means.

RESULTS

Induction of arthritis. In the first experiment mice received 4.7×10^7 CFU of the wild-type strain or 7.8×10^7 CFU of the *pls* mutant. During the following 14 days the wild-type-infected mice developed more severe arthritis than mice infected with the *pls* mutant, although the difference was not statistically significant (data not shown). Also, the wild-type strain induced more severe synovitis and bone and cartilage destruction of the knee joints than the *pls* mutant, as judged by histological examination (data not shown). However, the mortality in both groups was high (53% for the wild-type-infected mice and 20% for mice infected with the mutant at day 14), suggesting that the bacterial inoculum was too big to optimally study arthritis and too small to study mortality.

In the next three experiments, to study arthritis optimally, mice were inoculated with 2.5×10^7 to 3.4×10^7 and 2.8×10^7 to 3.8×10^7 CFU of the wild-type strain and the *pls* mutant, respectively. The *pls* mutant-inoculated groups developed clearly less severe arthritis than the wild-type-infected groups developed. The differences were statistically significant at all times ($P < 0.001$) (Fig. 1). The frequency of arthritic mice was at least double in the wild-type-inoculated groups compared to

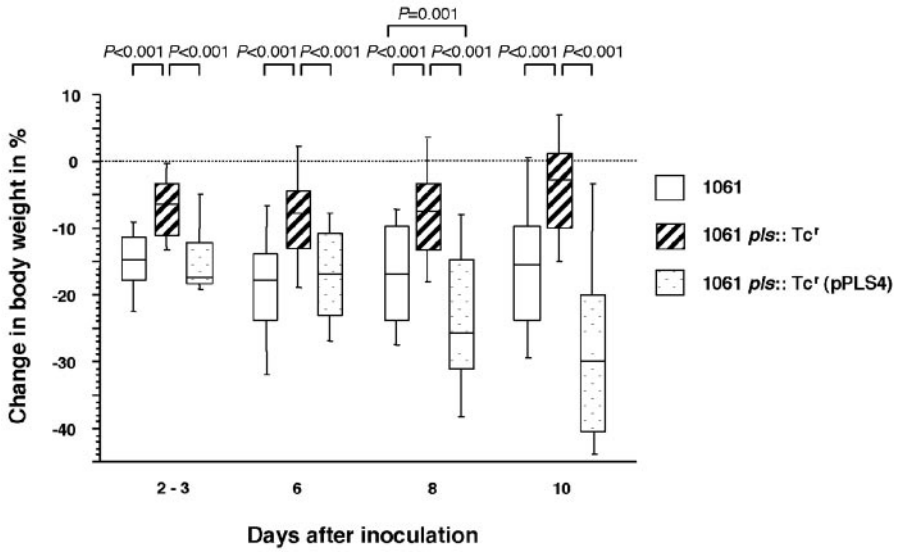

FIG. 2. Changes in body weight in mice after inoculation with 2.5×10^7 to 3.4×10^7 CFU of *S. aureus* wild-type strain 1061, 2.8×10^7 to 3.8×10^7 CFU of 1061 *pls*::Tc^r, or 4.0×10^7 CFU of 1061 *pls*::Tc^r(pPLS4), expressed as percentages of decrease compared with the weight at day zero (pooled results from experiments 2, 3, and 4). The center lines indicate medians; the boxes indicate interquartile ranges; and the whiskers indicate 80% central ranges. For days 2 to 3 to day 8, $n = 40$ to 45 for the wild type, $n = 45$ for the *pls* mutant, and $n = 13$ to 15 for the complemented *pls* mutant. For day 10, $n = 27$ for the wild-type, $n = 30$ for the *pls* mutant, and $n = 11$ for the complemented *pls* mutant. Data were analyzed by the Kruskal-Wallis test. For each test, significance was set at $P = 0.006$ by Bonferroni's adjustment, with simultaneous significance set at $P = 0.05$.

the mice infected with the *pls* mutant strain during the whole experimental period (data not shown).

In order to study whether the impaired arthritogenic capacity of the *pls* mutant could be restored, another group of mice were infected with 4.0×10^7 CFU of the *pls* complemented strain 1061 *pls*::Tc^r(pPLS4) (Pls$^+$) in the fourth experiment. The *pls* complementation significantly increased the arthritogenic response of infected mice compared to the *pls* mutant ($P < 0.001$) (day 6 to 8) (Fig. 1). Also, the frequency of arthritic mice was about double in the group infected with the *pls* complemented strain compared to the *pls* mutant-infected mice (data not shown). However, the arthritis indices for animals infected with the complemented strain did not quite reach the levels in mice infected with the wild type (Fig. 1).

Effect of soluble Pls. To test if the Pls protein is inflammatory by itself, knees were injected with the protein or with the vehicle. The inflammatory reaction was examined histologically 4 days later. There was no evident difference in the inflammatory responses between the mice injected with Pls and the mice injected with the vehicle; five of seven Pls-injected mice developed mild inflammation in the knee, while three of seven control mice exhibited mild inflammation.

Changes in body weight. The mice that were infected with the wild-type strain showed significantly greater body weight loss than the mice that were infected with the *pls* mutant (experiments 2 to 4; $P < 0.001$) (Fig. 2). While the weight reduction caused by the *pls* mutant strain reached a maximum of 7.6% of the original body weight at day 6, the reduction in the wild-type-infected group was more than twice as large (17.8%). After day 6 these animals gradually started to gain weight again (Fig. 2). Complementation of the *pls* mutation caused reversion to the wild-type phenotype, in that the complemented bacteria induced a weight loss similar to that induced by the wild type until day 6 (Fig. 2). In contrast to the other groups, after day 6 the animals infected with the complemented strain continued to lose weight until they had lost almost 30% at day 10 (Fig. 2). Also, in experiments 1 and 5 with the high doses of bacteria the mice infected with the wild-type strain lost more weight than the mice infected with the *pls* mutant. In these experiments the differences in weight reduction were not statistically significant, however (data not shown).

Bacterial numbers in various organs. (i) Kidneys. For experiments 2, 3, and 4, in which the mice were infected with a lower bacterial inoculum, the bacterial load in the kidneys of the animals infected with the wild-type strain was much higher than that in the kidneys of animals infected with the *pls* mutant (Fig. 3A). The median bacterial numbers per kidney pair in these animal groups were 2.7×10^7 CFU and 4.5×10^5 CFU, respectively (Fig. 3A). The median bacterial number in kidneys from mice infected with the complemented *pls* mutant strain was 8.3×10^7 CFU (Fig. 3A). In all groups there was great variation in kidney bacterial numbers between individual animals (see IQR values in Fig. 3A). However, there was a statistically significant difference between the bacterial numbers obtained from animals challenged with the Pls$^+$ and Pls$^-$ strains (for the Pls$^-$ strain versus the wild-type or complemented *pls* mutant strain, $P < 0.01$). For animals which had received a larger inoculum (experiment 1) there was no statistically significant difference in the bacterial loads of kidneys from wild-type- and *pls* mutant-infected mice; the median for the wild-type group was 3.9×10^5 CFU (IQR, 0.9×10^5 to 257×10^5 CFU) ($n = 7$), and the median for the *pls* mutant group was 17×10^5 CFU (IQR, 0 to 800×10^5 CFU) ($n = 12$).

(ii) Paws. The growth of bacteria in paws showed the same pattern as the growth in kidneys, although the difference be-

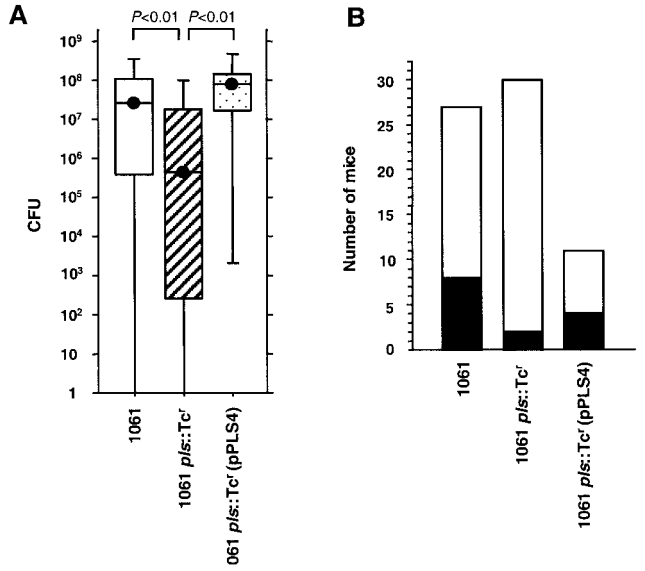

FIG. 3. (A) Bacterial growth in kidneys 8 to 11 days after inoculation with 2.5×10^7 to 4.0×10^7 CFU/mouse of strain 1061, strain 1061 *pls*::Tc^r, or strain 1061 *pls*::Tc^r(pPLS4). The circles indicate medians; the boxes indicate interquartile ranges; and the whiskers indicate 80% central ranges (data pooled from experiments 2, 3, and 4; $n = 39$ for the wild type, $n = 45$ for the *pls* mutant, and $n = 11$ for the complemented *pls* mutant). Data were analyzed by the Kruskal-Wallis test. For each test, significance was set at $P = 0.025$ by Bonferroni's adjustment, with simultaneous significance set at $P = 0.05$. (B) Bacterial growth in paws 10 to 11 days after bacterial inoculation. The solid portions of bars indicate the number of mice with bacterial growth in paws; the open and solid portions of bars together indicate the total number of mice examined (pooled data from experiments 2 and 4).

tween animal groups challenged with the Pls$^+$ and Pls$^-$ strains was not statistically significant (Fig. 3B). Bacterial growth in the paws was found in 8 of 27 mice (30%) and in 4 of 11 mice (36%) infected with the wild-type strain and with the complemented *pls* mutant, respectively, while paw growth was monitored in only 2 of 30 (7%) of the *pls* mutant-infected mice (Fig. 3B).

(iii) Blood. Bacteremia was measured 24 h after inoculation (experiment 4). There were very few bacteria detected in the blood at this stage, and no obvious difference between the groups could be seen. The median bacterial numbers for mice inoculated with the wild-type, the *pls* mutant, and the complemented *pls* mutant were 80 CFU/ml of blood (IQR, 40 to 180 CFU/ml of blood), 60 CFU/ml of blood (IQR, 10 to 173 CFU/ml of blood), and 80 CFU/ml of blood (IQR, 5 to 250 CFU/ml of blood), respectively.

Cytokine levels. The mean IL-6 levels in sera were 3.2 and 2.7 ng/ml when they were measured 7 days after challenge with 3.4×10^7 to 3.8×10^7 CFU of the wild-type and *pls* mutant strains, respectively (experiment 2) (Table 1). The corresponding values after 10 days were 2.5 and 0.8 ng/ml when animals were infected with 2.5×10^7 to 3.8×10^7 CFU of the same bacteria (experiment 4) (Table 1). Challenge of mice with 4.0×10^7 CFU of the complemented *pls* mutant resulted in reversion of IL-6 production to the level found in wild-type-infected animals (experiment 4) (Table 1). The IL-6 levels were lower in mice infected with Pls$^-$ bacteria than in mice infected with the Pls$^+$ strains, although the difference was not statistically significant (Kruskal-Wallis test).

TABLE 1. Serum levels of IL-6 and TNF-α in mice infected with wild-type strain 1061, the *pls* mutant, or the *pls* complemented mutant

Expt	Day	Strain	No. of mice	IL-6 concn (pg/ml)[a]	TNF-α concn (pg/ml)[a]
2	7	Wild type	14	3,231 ± 566	ND[b]
		pls mutant	15	2,669 ± 985	ND
3	8	Wild type	12	ND	74 ± 7
		pls mutant	15	ND	114 ± 37
4	10	Wild type	13	2,483 ± 775	95 ± 12
		pls mutant	15	788 ± 223	79 ± 8
		pls complemented mutant	11	2,168 ± 800	95 ± 14

[a] The cytokine responses are expressed as means ± standard errors of the means.
[b] ND, not determined.

The serum TNF-α levels were hardly over the detection limit in many of the mice, and there were no differences in the TNF-α levels between mice which were infected with the Pls$^+$ and Pls$^-$ strains (Table 1). The mean values were 74 and 95 pg/ml when the levels were measured at days 8 and 10 in animals challenged with 3.4×10^7 and 2.5×10^7 CFU of the wild-type strain (experiments 3 and 4) (Table 1). Infection with 2.8×10^7 and 3.8×10^7 CFU of the *pls* mutant led to TNF-α values of 114 and 79 pg/ml at the same times, respectively (experiments 3 and 4) (Table 1). At day 10 the mean level of TNF-α in mice infected with 4.0×10^7 CFU of the complemented *pls* mutant was 95 pg/ml (experiment 4) (Table 1).

Effect on mortality. In one experiment higher doses of bacteria were used to study the effect of Pls on sepsis-induced death. Challenge of mice with the wild-type strain (1.8×10^8 CFU) gave rise to a significantly higher mortality rate than challenge with the mutant strain (1.7×10^8 CFU) ($P = 0{,}014$) (Fig. 4). After 5 days 40% of the wild-type-infected mice had died, whereas all the mice infected with the mutant were still alive. During the follow-up period (22 days) only 33% of the wild-type-infected animals survived, whereas the survival percentage of the mice infected with the *pls* mutant was 73% (Fig.

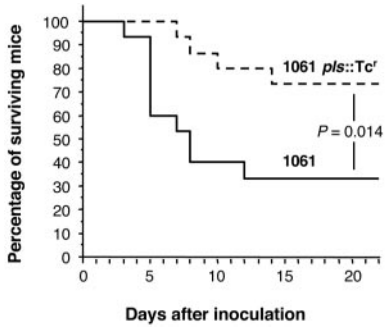

FIG. 4. Percentage of surviving mice after inoculation of 1.7×10^8 to 1.8×10^8 CFU of *S. aureus* wild-type 1061 or 1061 *pls*::Tc^r. Statistical evaluation was done by Kaplan-Meier analysis. $P = 0.014$ as determined by the log rank test ($n = 15$ at the start of the experiment).

4). The maximum mortality in these groups occurred in 12 and 14 days, respectively.

DISCUSSION

The present results clearly show that Pls on the staphylococcal surface modifies the virulence characteristics of the bacterium. First, intravenous challenge of mice with *S. aureus* expressing Pls caused a joint infection more frequently and led to more severe arthritis than a comparable challenge with the isogenic *pls* mutant. Second, the animals infected with the Pls-expressing strain developed more serious signs of sepsis, as detected by loss of body weight, greater bacterial growth in kidneys and paws, and elevated serum IL-6 levels, than the mice infected with the *pls* mutant. Third, when higher doses of bacteria were used, infection by the wild-type strain also resulted in higher mortality than infection by the *pls* mutant.

In this model the outcome of infection is different depending on the bacterial dose used. Lower doses are optimal for induction of arthritis, whereas higher doses lead to death. Consequently, by varying the dose, a wide range of information can be achieved.

We have shown previously that although the complemented *pls* mutant possesses Pls in its cell wall, from which it can be solubilized with lysostaphin digestion, all the adhesive properties of the complemented mutant do not completely revert to the attenuated wild-type levels (18). In this study those findings might be parallel to the fact that the levels of the arthritic properties of the complemented strain did not reach the levels of the wild type. However, the complemented bacteria induced body weight loss to the same degree as the wild-type bacteria during the follow-up for 6 days, but thereafter the mice continued to lose weight, while the animals infected with the wild-type staphylococci started to regain weight. This pattern observed for the complemented strain (i.e., making the wild-type characteristics worse) was also shown by the bacterial growth data for kidneys and paws but not by the cytokine data. These data may have been due to the slightly higher number of complemented bacteria or to possible overexpression of Pls on the surface of complemented bacteria (18), which might have changed the disease manifestation from localized arthritis to systemic responses.

The effect of Pls on virulence has also been studied using another infection model. Huesca et al. (7) showed that in a mouse abscess model Pls expression in itself seemed to neither increase nor decrease the virulence. An epidemic Pls^+ MRSA strain was not markedly changed in virulence compared to another epidemic, nonisogenic, but closely related Pls^- strain.

It is difficult to estimate the mechanism by which Pls works as a virulence factor in our experimental septic arthritis model. However, the failure of the purified Pls molecule alone to induce arthritis favors a mechanism in which Pls must be associated with the bacterial surface in order to be able to induce the observed consequences. In order to cause disease, bacteria have to survive in blood, spread from the bloodstream to the joint, colonize the joint, and survive there, at least for a while. These individual events likely require different properties of the bacteria. Regarding adhesion, at some stages it might be beneficial to the bacteria not to adhere, to enhance the speed or ability of spreading. At other stages, such as joint colonization, adhesion might be a prerequisite. Pls seems to be a versatile molecule; it both mediates and reduces *S. aureus* adhesion to different host structures. The reported ability of Pls to bind to glycolipids and cellular lipids (7) could promote arthritis development and induction of the systemic infection. On the contrary, Pls-mediated attenuation of bacterial binding to fibronectin (18) could lead to more efficient spread in the body. Finally, at later growth phases surface Pls is processed into a smaller form (18). This indicates that the binding characteristics of Pls could vary during the life cycle of a bacterium, given that the cleaved parts of the molecule would be needed for its binding and binding prevention activities. All this suggests that Pls may play an important role in regulation of adhesion at various stages during infection and that the adhesion properties which Pls provides to the bacterium benefit virulence.

Further experiments are needed to find out if the changed adhesion properties of Pls^+ strains observed in vitro play a role in this experimental animal infection model. It would also be interesting to find out whether infections caused by Pls^+ MRSA strains in humans lead to arthritis more often than infections caused by Pls^- strains.

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REFERENCES

1. **Abdelnour, A., S. Arvidson, T. Bremell, C. Rydén, and A. Tarkowski.** 1993. The accessory gene regulator (*agr*) controls *Staphylococcus aureus* virulence in a murine arthritis model. Infect. Immun. **61:**3879–3885.
2. **Bremell, T., A. Abdelnour, and A. Tarkowski.** 1992. Histopathological and serological progression of experimental *Staphylococcus aureus* arthritis. Infect. Immun. **60:**2976–2985.
3. **Bremell, T., S. Lange, A. Yacoub, C. Ryden, and A. Tarkowski.** 1991. Experimental *Staphylococcus aureus* arthritis in mice. Infect. Immun. **59:**2615–2623.
4. **Foster, T. J., and M. Höök.** 1998. Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol. **6:**484–488.
5. **Goldenberg, D. L.** 1998. Septic arthritis. Lancet **351:**197–202.
6. **Hildén, P., K. Savolainen, J. Tyynelä, M. Vuento, and P. Kuusela.** 1996. Purification and characterisation of a plasmin-sensitive surface protein of *Staphylococcus aureus*. Eur. J. Biochem. **236:**904–910.
7. **Huesca, M., R. Peralta, D. N. Sauder, A. E. Simor, and M. J. McGavin.** 2002. Adhesion and virulence properties of epidemic Canadian methicillin-resistant *Staphylococcus aureus* strain 1: identification of novel adhesion functions associated with plasmin-sensitive surface protein. J. Infect. Dis. **185:**1285–1296.
8. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **45:**1323–1336.
9. **Josefsson, E., O. Hartford, L. O'Brien, J. M. Patti, and T. Foster.** 2001. Protection against experimental *Staphylococcus aureus* arthritis by vaccination with clumping factor A, a novel virulence determinant. J. Infect. Dis. **184:**1572–1580.
10. **Juuti, K. M., B. Sinha, C. Werbick, G. Peters, and P. I. Kuusela.** 2004. Reduced adherence and host cell invasion by methicillin-resistant *Staphylococcus aureus* expressing the surface protein Pls. J. Infect. Dis. **189:**1574–1584.
11. **Kuusela, P., P. Hildén, K. Savolainen, M. Vuento, O. Lyytikäinen, and J. Vuopio-Varkila.** 1994. Rapid detection of methicillin-resistant *Staphylococcus aureus* strains not identified by slide agglutination tests. J. Clin. Microbiol. **32:**143–147.

12. **Moreillon, P., J. M. Entenza, P. Francioli, D. McDevitt, T. J. Foster, P. François, and P. Vaudaux.** 1995. Role of *Staphylococcus aureus* coagulase and clumping factor in the pathogenesis of experimental endocarditis. Infect. Immun. **63:**4738–4743.
13. **Palmqvist, N., T. Foster, A. Tarkowski, and E. Josefsson.** 2002. Protein A is a virulence factor in *Staphylococcus aureus* arthritis and septic death. Microb. Pathog. **33:**239–249.
14. **Roche, F. M., R. Massey, S. J. Peacock, N. P. J. Day, L. Visai, P. Speziale, A. Lam, M. Pallen, and T. J. Foster.** 2003. Characterization of novel LPXTG-containing proteins of *Staphylococcus aureus* identified from genome sequences. Microbiology **149:**643–654.
15. **Roche, F. M., M. Meehan, and T. J. Foster.** 2003. The *Staphylococcus aureus* surface protein SasG and its homologues promote bacterial adherence to human desquamated nasal epithelial cells. Microbiology **149:**2759–2767.
16. **Sakiniene, E., T. Bremell, and A. Tarkowski.** 1996. Addition of corticosteroids to antibiotic treatment ameliorates the course of experimental *Staphylococcus aureus* arthritis. Arthritis Rheumatism **39:**1596–1605.
17. **Sakiniene, E., T. Bremell, and A. Tarkowski.** 1999. Complement depletion aggravates *Staphylococcus aureus* septicaemia and septic arthritis. Clin. Exp. Immunol. **115:**95–102.
18. **Savolainen, K., L. Paulin, B. Westerlund-Wikström, T. J. Foster, T. K. Korhonen, and P. Kuusela.** 2001. Expression of *pls*, a gene closely associated with the *mecA* gene of methicillin-resistant *Staphylococcus aureus*, prevents bacterial adhesion in vitro. Infect. Immun. **69:**3013–3020.

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