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Effect of Highly Purified Coagulase and Culture Filtrate on Virulence and Immunity of a Coagulase-Negative Mutant of *Staphylococcus aureus* BB

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The virulence of the coagulase-deficient mutant BB-Cgl$^-$ 1301 (50% lethal dose [LD_{50}] for mice by the intraveneous route) was compared with that of its parental strain, *Staphylococcus aureus* BB. The BB strain produced free coagulase of serotype I, whereas the mutant 1301 did not. Mice were infected with strain 1301, alone or in combination with a highly purified coagulase type I or type II solution, or with concentrated culture filtrates of parent strain BB or mutant strain 1301. The ratios of the LD_{50}s of 1301 and its combinations to that of BB ranged from 34.9 to 461. Combining strain 1301 with a concentrated culture filtrate of BB (BB-CF2.5) was the most effective for enhancement of its virulence. When mice were infected with a combination of strain 1301 and BB-CF2.5, the LD_{50} of strain 1301 (1.72 mg of cells [wet weight]) was decreased to 0.13 mg (1.3×10^8 CFU). This LD_{50} yielded the smallest ratio, 34.9, as compared with the LD_{50} of BB (0.00373 mg). In contrast, when the mice subcutaneously immunized with strain 1301 and BB-CF50 were intravenously challenged by strain BB, the LD_{50} for the immunized mice was 17.4 times the LD_{50} for the unimmunized control mice (0.0429 mg as compared with 0.00246 mg), indicating that combination was the most effective for enhancement of mouse immunization with strain 1301. However, combining strain 1301 with the highly purified sample of coagulase increased neither the virulence nor the immunizing power of mutant strain 1301.

Staphylococcus aureus has been known to produce many kinds of extracellular toxins and enzymes, such as α-toxin, β- and δ-hemolysin, DNase, staphylokinase, leucocidin, lipase, gelatinase, etc. Many studies have discussed the role of these extracellular products in staphylococcal infection and immunity. Elston and Fitch (5) reported that 99.4% of the clinical strains of *S. aureus* tested released coagulase and 98.8% produced DNase. Forsgren (7) found that 100% of the coagulase-positive strains tested produced DNase and 98.9% carried protein A in their cell walls. Hale and Smith (8) found that coagulase could prevent staphylococcal cells from phagocytosis and promote their invasiveness. Hasegawa (9) reported that in mice, a marked decrease in the virulence of *S. aureus* 248 was found in coagulase-deficient mutants, but a less marked decrease occurred in DNase-negative mutants. Many researchers have been interested in coagulase and have tried to obtain pure coagulase preparations. Duthie and Haughton (3) employed precipitation of culture supernatant with cadmium sulfate, followed by dialysis and several cycles of ammonium sulfate fractionation, in the purification of coagulase. Zolli and San Clemente (20) used three cycles of dialysis in ethanol-water mixtures, followed by column chromatography through Sephadex G-200. Siwecka and Jeljaszewicz (17) followed the work of Duthie and Haughton (3) and Zolli and San Clemente (20) and showed that highly active preparations are electrophoretically homogeneous. Recently, Igarashi et al. (11) isolated staphylocoagulase with affinity chromatography with a bovine prothrombin-Sepharose 4B column and gel filtration on Sephadex G-25.

In the present study, the virulence and immunizing power of coagulase-negative mutant 1301 of *S. aureus* BB were investigated, and highly purified samples of coagulase of serotypes I and II, as well as concentrated broth culture filtrates

of the parental and mutant strains, were examined for their effects on the virulence and immunizing power of the mutant Cgl$^-$ 1301.

MATERIALS AND METHODS

Bacterial strains. In this study, we used *S. aureus* BB and its coagulase-negative mutant, *S. aureus* BB-Cgl$^-$ 1301 (10). They were designated the parent BB and the mutant 1301. There were no significant differences between the parent strain BB and the mutant strain 1301 except for the ability to produce free coagulase. Their major biological characteristics are shown in Table 1. The parent strain BB produced free coagulase type I, which was classed according to the coagulase-typing procedure of Zen-yoji et al. (19). When the bouillon cultures of parent strain BB were tested with rabbit plasma, the mixtures were coagulated completely after incubation at 37°C for 1 h, but their filtrate and rabbit plasma were not coagulated even after incubation at 37°C for 6 h. The bouillon cultures of mutant strain 1301 and rabbit plasma were not coagulated after incubation at 37°C for 6 h and standing at room temperature overnight (10). *S. aureus* 104 and *S. aureus* st-213 were used as standard coagulase-producing strains of free coagulase types I and II, respectively (11).

Culture media. Nutrient broth prepared with NaCl (Kanto Kagaku Co.), Lender meat extract (Mikuni Kagaku Co.), Arei peptone (Kyoei Seiyaku and Co. Ltd.), and nutrient agar (Eiken Co.) were used for culturing and colony counts. Brain heart infusion (Difco Laboratories) supplemented with agar (Wako Pure Chemical Industries, Ltd.) was also used for culturing.

Coagulase test and activity. Coagulase rabbit plasma (Eiken) was used. The broth cultures, culture filtrates, or purified coagulase solutions (0.5 ml) were each serially diluted twofold with saline, mixed with rabbit plasma (0.5 ml) previously diluted 1:5 with saline, and incubated at 37°C for 1 to 6 h. Activity was expressed as the reciprocal of the highest dilution titer.

Coagulase purification. A new, simplified method was developed for the large-scale purification of coagulase from culture filtrates of *S. aureus* 104 and *S. aureus* st-213, using Sepharose 4B covalently linked with bovine prothrombin (11). After the coagulase was absorbed strongly by the affinity column, it was eluted with 1.0 M NaSCN. The yield of coagulase activity ranged from 75 to 85%. The purified coagulase showed a single symmetrical peak by ultracentrifugal analysis ($S_{20,w}$ = 6.47) and gave a single precipitin line against anti-purified coagulase serum, as revealed by the immunodiffusion test. However, the preparation was shown to contain three active components by the isoelectric focusing method, suggesting some microheterogeneity. These highly purified coagulase type I and type II solutions were designated type I and type II, respectively.

Preparation of a concentrated culture filtrate. Brain heart infusion (600 ml) in 1,000-ml Erlenmeyer flasks was inoculated with 0.1 to 0.2 ml of overnight broth cultures of parent strain BB or mutant strain 1301 and incubated at 37°C for 18 h. Cultures were then centrifuged for 40 min at 8,000 to 10,000 × *g*. Supernatants were passed through a membrane filter having a pore diameter of 450 nm (Millipore Corp.) and concentrated 2.5- to 50-fold via a Diaflo PM-10 membrane (Amicon Corp.). Preparations of parent strain BB and mutant strain 1301 culture filtrates which were concentrated 2.5- to 50-fold were designated BB-CF2.5, BB-CF50, 1301-CF2.5, and 1301-CF50.

Mice. ICR mice were bought from Clea Co. All were females about 3 to 4 weeks of age and weighed 13 to 20 g each. Mice were maintained on commercial food and tap water and housed at 24°C and 60% humidity.

Preparation of a bacterial suspension for challenge and immunization. Thick brain heart infusion agar plates (about 50 ml of brain heart infusion agar per petri dish, 9 cm in diameter) were inoculated with 0.3 ml of overnight broth cultures and incubated at 37°C overnight. Bacterial growth was harvested with an inoculating loop, weighed, and suspended in broth-saline (1:40), concentrated culture filtrates, or highly purified coagulase dissolved in 0.1 M NH_4HCO_3 (pH 8.3). One milligram of bacteria (wet weight) contained about 10^9 cells, and this count was determined and confirmed for each new suspension.

Mouse challenge. The cells for challenge were prepared in 0.1 ml for intravenous inoculation.

Immunization methods. Bacterial suspensions for immunization were adjusted to 2 × 10^8 CFU/ml. A series of four injections (10^7 CFU/0.05 ml, each) was made subcutaneously at weekly intervals into alternate hips.

LD_{50} determination. Six to eight groups of five mice each were inoculated intravenously with decreasing twofold bacterial doses. After 15 days of observation, 50% lethal dose (LD_{50}) determinations were made by the Behrens-Kärber method (15). These values were determined by using the wet weight of the cells.

RESULTS

Virulence (LD_{50}) of *S. aureus* BB and BB-Cgl$^-$ 1301 alone and in combination with highly purified coagulase solutions and with concentrated broth culture filtrates. Mice weighing 13 to 16 g each were all infected intravenously in the same manner to determine the LD_{50}s of the coagulase-positive parent strain BB and the coagulase-negative mutant strain 1301 alone and in various combinations (Table 2). The activities of the highly purified coagulase type I and type II solutions were 1,024 and 2,048 at 37°C for 1 h. For the concentrated broth culture filtrates BB-CF2.5 and BB-CF50, the coagulase activities were 4 and 128, respectively at 37°C for 1 h. The LD_{50} of parent strain BB was determined separately during each trial. In three subseries, the LD_{50}s of mutant strain 1301 and its combinations were compared with that of parent strain BB. The ratio of the LD_{50}s of mutant strain 1301 alone and in combination to the LD_{50} of parent strain BB were calculated on the basis of wet weight (Table 2). The ratio of the LD_{50} of mutant strain 1301 alone to the LD_{50} of parent strain BB was 461 (1.72/0.00373 mg). The ratios of the LD_{50}s of mutant strain 1301 combined with type I and mutant strain 1301 combined with type II

TABLE 1. Biological characteristics of *S. aureus* BB and its coagulase-negative mutant *S. aureus* BB-Cgl$^-$ 1301

S. aureus strain	Biological characteristic[a]															
	NaCl (7.5%) tolerance	Mannitol fermentation	Coagulase	Clumping factor	DNase	α-Toxin	β-Hemolysin	δ-Hemolysin	Glucose fermentation	Gelatinase	Fibrinolysin	Protease	Acetylmethylcarbinol	Phosphatase	Egg yolk reaction	Colony type
BB[b]	+	+	+++	++	++++	+	++++	+′	+++	+++	−	+	+++	+++	+	Compact
BB-Cgl$^-$ 1301[c]	+	+	−	++	+++	+	++	+′	+++	+++	−	+	+++	+++	+	Compact

[a] For each biological test, the least discernible reaction was assigned a value of one +. These biological characteristics were determined as follows. The NaCl resistance, mannitol fermentation, and production of coagulase, clumping factor, and DNase were tested respectively with *Staphylococcus* medium 110, mannitol salt agar, the tube method with rabbit plasma, the slide glass method with rabbit plasma, and DNase test agar. The production of α-toxin, β-hemolysin, and δ-hemolysin were tested on rabbit erythrocyte-nutrient plates (2.5%, vol/vol), sheep erythrocyte-nutrient agar plates (2.5%, vol/vol), and human erythrocyte-nutrient plates (2.5%, vol/vol). The production of fibrinolysin, protease, and egg yolk reaction were tested respectively on heated human plasma-heart infusion agar plates (20%, vol/vol), skin milk-heart infusion agar plates (4%, wt/vol), and egg yolk-heart infusion agar plates (10%, vol/vol). The production of gelatinase was tested on *Staphylococcus* medium 110. Glucose fermentation, production of acetylmethylcarbinol and phosphatase, and the colony types of serum-soft agar were determined by the method of Ushioda et al. (18), Barritt (2), Barber and Kuper (1), and Finkelstein and Sukin (6), respectively.

[b] *S. aureus* BB, the parental strain.

[c] *S. aureus* BB-Cgl$^-$ 1301, a coagulase-negative mutant strain induced with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine from *S. aureus* BB. When the mutant was characterized for growth in nutrient broth; for colony size, form, and color on the nutrient agar plates; and for biological characteristics, there were no significant differences between the mutant and its parental strain except for the ability to produce coagulase.

TABLE 2. Ratios of LD_{50}s of the coagulase-negative mutant *S. aureus* BB-Cgl$^-$ 1301, alone and in combination with highly purified coagulase solutions and with concentrated culture filtrates, to that of parental strain *S. aureus* BB

Infecting strain and material	LD_{50} (mg of cells [wet wt])[a]	LD_{50} ratio by intravenous infection[b]
Parent BB[c]	0.00373	
Mutant 1301[d]		
Alone	1.72	461
With Type II[e]	0.858	230
With BB-CF2.5[f]	0.130	34.9
Parent BB	0.00985	
Mutant 1301 with type I[g]	1.49	151
Parent BB	0.00210	
Mutant 1301 with 1301-CF2.5[h]	0.493	235

[a] Bacterial concentration; 1 mg of wet cells = ca. 10^9 viable units.

[b] Mutant strain 1301 alone or in combination LD_{50}/parent BB LD_{50}.

[c] Parental strain *S. aureus* BB.

[d] Coagulase-negative mutant strain *S. aureus* BB-Cgl$^-$ 1301.

[e] Highly purified coagulase type II solution from culture filtrate of *S. aureus* st-213.

[f] Concentrated (2.5 times) culture filtrate of *S. aureus* BB.

[g] Highly purified coagulase type I solution from culture filtrate of *S. aureus* 104.

[h] Concentrated (2.5 times) culture filtrate of *S. aureus* BB-Cgl$^-$ 1301.

to the LD_{50} of parent strain BB were 151 (1.49/0.00985 mg) and 230 (0.858/0.00373 mg), respectively. The ratios of the LD_{50}s of mutant strain 1301 combined with BB-CF2.5 and mutant strain 1301 combined with 1301-CF2.5 to the LD_{50} of parent strain BB were 34.9 (0.130/0.00373 mg) and 235 (0.493/0.00210 mg), respectively. The virulence of mutant strain 1301 in combination with BB-CF2.5 was 6.7-fold (235/34.9) stronger than that of mutant strain 1301 in combination with 1301-CF2.5, and 13.2-fold (461/34.9) stronger than that of mutant strain 1301 alone. These ratios indicate that the coagulase type I and type II solutions and the culture filtrate 1301-CF2.5 do not effectively increase the virulence of mutant strain 1301 in mice. However, the culture filtrate BB-CF2.5 increases the virulence of mutant strain 1301 by more than 13-fold when given intravenously to mice.

During the 15-day observation period for the LD_{50} determination, the dead mice from the first subseries (Table 2) were autopsied. When the mice were infected with parent strain BB, mutant strain 1301, mutant strain 1301 combined with type II, or mutant strain 1301 combined with BB-CF2.5, 33.3% (6 of 18), 91.7% (11 of 12), 58.8% (10 of 17), and 42.9% (6 of 14), respectively, of all mice in each group died within the first three days of the period. Of a total of 18 dead mice, abscesses were found in the kidneys of 13 (72.2%); in other organs (joint, liver, etc.) and in bone and muscle, of 12 (66.7%); and in the kidneys and other organs of 10 (55.6%) after infection with parent strain BB. However, when mice were infected with mutant strain 1301 alone and in combination with BB-CF2.5, only kidney abscesses were found in 8.5% (1 of 12) and 7.1% (1 of 14), respectively, of the dead mice. No abscesses were found in dead mice infected with mutant strain 1301 combined with type II.

After the 15-day observation period for the LD_{50} determination, the surviving mice from the first subseries (Table 2) were sacrificed for determination of spleen weight. Of the total 22 surviving mice infected with parent strain BB, 28 infected with mutant strain 1301, 23 infected with mutant strain 1301 combined with type II, and 26 infected with mutant strain 1301 combined with BB-CF2.5, the spleen-to-body weight percentages were 2.09 (357.1 mg/17.6 g), 0.73 (144.9 mg/19.5 g), 0.78 (156.0 mg/20.3 g), and 0.73 (155.0 mg/21.2 g), respectively. These percentages indicate that there are significant differences in the timing of death, the degree of abscess formation, and spleen weight between the mice infected with parent strain BB and those infected with mutant strain 1301. Infecting mice with mutant strain 1301 combined with type II and mutant strain 1301 combined with BB-CF2.5 did not result in an increase of abscesses formation or of spleen weight, but did result in a decrease in the number of dead mice in the first three days.

Effect of subcutaneous immunization with *S. aureus* BB-Cg$^-$ 1301, highly purified coagulase solutions, and concentrated culture filtrates. Each of three subseries included an untreated control group. Mice weighing 16 to 20 g each were immunized subcutaneously with 10^7 living cells four times at weekly intervals. Mice were injected with living cells of mutant strain 1301 alone, in combination with types I and II, and in combination with BB-CF50 and 1301-CF50 (Table 3). Moreover, mice were injected with types I and II and with BB-CF50 alone under the same conditions (Table 3). During this immunizing period, most of the mice injected with mutant strain 1301 or with type I or II did not form subcutaneous abscesses on their hips. However, some mice of the other five groups produced abscesses and patches of baldness. On the day

TABLE 3. Comparison of the immune effect (ratio of LD_{50}) produced by *S. aureus* BB-Cgl$^-$ 1301, highly purified coagulase solutions, and concentrated culture filtrates in mice challenged intravenously with *S. aureus* BB

Immunizing strain or material	LD_{50} (mg of cells [wet wt])[a]	LD_{50} ratio by intravenous infection[b]
Untreated control	0.00246	
Mutant 1301[c]		
With type II[d]	0.00985	4.00
With 1301-CF50[e]	0.00747	3.04
With BB-CF50[f]	0.0429	17.4
Type II	0.00429	1.74
Untreated control	0.00493	
Mutant 1301		
Alone	0.00650	1.32
With type I[g]	0.00566	1.15
Type I	0.00650	1.32
Untreated control	0.00488	
BB-CF50	0.0149	3.05

[a] Bacterial concentration; 1 mg of wet cells = ca. 10^9 viable units.

[b] Immunized mouse LD_{50}/untreated control mouse LD_{50}.

[c] Coagulase-negative mutant *S. aureus* BB-Cgl$^-$ 1301.

[d] Highly purified coagulase type II solution from culture filtrate of *S. aureus* st-213.

[e] Concentrated (50 times) culture filtrate of *S. aureus* BB-Cgl$^-$ 1301.

[f] Concentrated (50 times) culture filtrate of *S. aureus* BB.

[g] Highly purified coagulase type I solution from culture filtrate of *S. aureus* 104.

before challenge with the parent BB, mice were weighed and examined physically for lesions, and some of the mice were sacrificed to confirm that the immunizing cells of the mutant 1301 were not found in the kidneys, liver, and spleen. The mean weight ranged from 26.2 to 30.0 g in eight immunized and three untreated control mice groups. Lesions were formed in 5% (2 of 40), 45% (18 of 40), and 83% (33 of 40) of the mice immunized with mutant strain 1301 combined with type II, with 1301-CF50, and with BB-CF50, respectively. Patches of baldness were formed in 38 (15/40)% of the mice immunized with BB-CF50.

Seven days after the fourth injection, the mice were challenged intravenously with parent strain BB to determine the LD_{50}s. The LD_{50}s of these immunized mice were compared with those of the untreated controls. The ratios of the former to the latter are shown in Table 3. The maximal ratio, 17.4 (0.0429/0.00246 mg), was obtained when the mice were immunized with mutant strain 1301 combined with BB-CF50, but when the mice were immunized with mutant strain 1301 or with BB-CF50 alone, the ratios were 1.32 (0.00650/0.00493 mg) and 3.05 (0.0149/0.00488 mg), respectively (Table 3). When the mice were immunized with mutant strain 1301 combined with type I or II, mutant strain 1301 combined with 1301-CF50, and with types I and II alone, the ratios were 1.15 (0.00566/0.00493 mg), 4.00 (0.00985/0.00246 mg), 3.04 (0.00747/0.00246 mg), and 1.32 (0.00650/0.00493 mg), and 1.74 (0.00429/0.00246 mg), respectively. These ratios indicate that immunization with mutant strain 1301 combined with BB-CF50 is effective in protecting mice from challenge with parent strain BB, whereas immunization with mutant strain 1301 combined with type I or with type I alone is not.

During the 15-day observation period for the LD_{50} determination, time of death and abscess formation in the dead mice were observed in the immunized mice as well as in the untreated control mice. After the LD_{50} determination, abscesses were found in the surviving mice of the immunized groups and in untreated control mice. Of the untreated control mice in the first subseries (Table 3), abscesses were found in the kidneys of 20 (95.2%), in the other organs of 7 (33.3%), and in both the kidneys and the other organs of 7 (33.3%) of the 21 dead mice and in the kidneys of 8 (42.1%), in the other organs of 12 (63.2%), and in both the kidneys and other organs of 5 (26.3%) of the 19 survivors. These results indicate that immunization with mutant strain 1301 combined with BB-CF50 or with type I, and immunization with type I alone, is not effective to suppress abscesses formation in mice.

DISCUSSION

The overnight broth cultures of parent strain BB showed a coagulase activity of 64 when incubated with rabbit plasma at 37°C for 1 h, whereas the solutions of type I or II coagulase showed coagulase activities of 1,024 and 2,048, respectively, when incubated at 37°C for 1 h. However, our experiments showed that the virulence of mutant strain 1301 was not increased by combining it with type I coagulase. Moreover, subcutaneous immunization with mutant strain 1301 combined with type I coagulase was not effective in protecting mice from challenge with parent strain BB. These unexpected results might be caused by the method of combining mutant strain 1301 with coagulase or by the route of infection. There might be some difference in quality and function between coagulase type I produced in vivo and that produced in vitro. When the staphylococci produced coagulase in vivo, some material effective in increas-

ing the virulence of the organisms might also be produced. In contrast, mutant strain 1301 combined with BB-CF2.5 or with BB-CF50 was effective in increasing the virulence of mutant strain 1301 in mice and was useful in protecting mice from challenge with parent strain BB. The mixtures of BB-CF2.5 and BB-CF50 with rabbit plasma showed coagulase activities in vitro of only 4 and 128, respectively. These results suggest that there is something in BB-CF other than free coagulase which is effective in enhancing the virulence and immunity of mutant strain 1301.

After the determination of the LD_{50}, the mice that survived the challenge were sacrificed and examined for abscess formation (Table 2). When the mice were infected wtih parent strain BB, abscesses were found in the kidneys of 13 (59.1%), in the other organs of 20 (90.1%), and in both the kidneys and the other organs of 13 (59.1%) of the 22 survivors. However, of the mice infected with mutant strain 1301, mutant strain 1301 combined with type II coagulase, and mutant strain 1301 combined with BB-CF2.5, only kidney abscesses were found in 67.9% (19 of 28), 73.9% (17 of 23), and 73.1% (19 of 26), respectively, of the survivors. In consideration of the abscess formation in the dead mice and in the survivors, abscess formations in organs other than the kidneys are related more directly to time of death and spleen weight after infection with parent strain BB than are kidney abscesses. The living bacterial cells in the kidney abscesses of mice infected with mutant strain 1301 were determined to be coagulase negative. The kidney abscesses found in mice infected with mutant strain 1301 were white, and those in mice infected with parent strain BB were yellow. The immunizing cells of mutant strain 1301 were not found in kidneys, livers, or spleens of the immunized mice (Table 3). When mice were immunized with mutant strain 1301 combined with BB-CF50, the maximal value, 17.4, was obtained (ratio of LD_{50} of immunized mice to that of untreated mice) (Table 3). Immunization with this combination was useful in protecting mice from challenge by parent strain BB but did not decrease abscesses formation in dead or surviving mice. In a previous study (10), mice were immunized with parent strain BB and challenged with parent strain BB under the same conditions, and the ratio (immunized-mouse LD_{50}/untreated control mouse LD_{50}) was 60.6.

Meanwhile, many papers have been published on the virulence and coagulase of *S. aureus*. Ekstedt and Yotis (4) have reported on the effect of coagulase on the virulence of coagulase-negative strains. When coagulase-negative, hemolytic *S. aureus* strains are suspended in solutions of partially purified coagulase and injected intracerebrally into mice, significant mortality results. Such virulence enhancement could not be demonstrated when the coagulase was given intravenously and followed by intracerebral challenge. Kapral and Li (12) have found that two mutants, one lacking the soluble type of coagulase (18Z-C) and the other lacking the bound type of coagulase (18Z-B), were still virulent in rabbits. The third mutant, which produced both kinds of coagulase (18Z-D), lost its virulence in rabbits. These three mutants were isolated from a single strain of *S. aureus* 18Z which was known to be virulent in rabbits.

Li and Kapral (14) studied the survival of certain coagulase-negative mutants in the organs of intravenously infected rabbits. Coagulase-negative *S. aureus* mutants behave in the same manner as the parent strain from which they were derived. The populations within the lungs, liver, and spleen decreased gradually over a period of 11 days. However, within the kidneys of the host, these strains continued to multiply. Another mutant of the parent strain was incapable of multiplication in the kidneys. This avirulent mutant possessed no coagulase but did possess all other tested factors elaborated by the parent strain. Rogers and Tompsett (16) have reported on the survival of staphylococci within human leukocytes and found that a significant number of microorganisms of pathogenic strains (coagulase positive) were able to survive within human leukocytes. Such intracellular survival was found to be associated with evidence of destruction of the leukocytes. In contrast, nonpathogenic (coagulase negative) staphylococcal strains failed to survive within human polymorphonuclear leukocytes after ingestion. Karas and Kapral (13) have also reported on the behavior of staphylococcal strains in the organs of intravenously infected mice. In quantitative studies in mice infected with a virulent parent strain of *S. aureus* and its derived coagulase-negative mutant at two intravenous dose levels, these authors showed that, as in rabbits, these strains multiply only in the kidneys but are eventually eliminated from the spleen, liver, and lungs. Hasegawa and San Clemente (10) isolated the coagulase-deficient and the DNase-negative mutants from *S. aureus* BB and examined the parent and mutant strains for virulence and immunizing power in mice.

In the present study, the virulence and immunizing power of the coagulase-negative mutant 1301 of *S. aureus* BB were investigated and the highly purified samples of coagulase of serotypes I and II, as well as the concentrated broth culture filtrates of the parental and mutant strains, were examined for their effects on the virulence and immunizing power of mutant Cgl^- 1301.

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