

Salicylic Acid Diminishes *Staphylococcus aureus* Capsular Polysaccharide Type 5 Expression[∇]

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Capsular polysaccharides (CP) of serotypes 5 (CP5) and 8 (CP8) are major *Staphylococcus aureus* virulence factors. Previous studies have shown that salicylic acid (SAL), the main aspirin metabolite, affects the expression of certain bacterial virulence factors. In the present study, we found that *S. aureus* strain Reynolds (CP5) cultured with SAL was internalized by MAC-T cells in larger numbers than strain Reynolds organisms not exposed to SAL. Furthermore, the internalization of the isogenic nonencapsulated Reynolds strain into MAC-T cells was not significantly affected by preexposure to SAL. Pretreatment of *S. aureus* strain Newman with SAL also enhanced internalization into MAC-T cells compared with that of untreated control strains. Using strain Newman organisms, we evaluated the activity of the major *cap5* promoter, which was significantly decreased upon preexposure to SAL. Diminished transcription of *mgrA* and upregulation of the *saeRS* transcript, both global regulators of CP expression, were found in *S. aureus* cultured in the presence of SAL, as ascertained by real-time PCR analysis. In addition, CP5 production by *S. aureus* Newman was also decreased by treatment with SAL. Collectively, our data demonstrate that exposure of encapsulated *S. aureus* strains to low concentrations of SAL reduced CP production, thus unmasking surface adhesins and leading to an increased capacity of staphylococci to invade epithelial cells. The high capacity of internalization of the encapsulated *S. aureus* strains induced by SAL pretreatment may contribute to the persistence of bacteria in certain hosts.

Staphylococcus aureus is an opportunistic pathogen that causes both community-acquired and life-threatening nosocomial infections (35). Although *S. aureus* can colonize mucosal surfaces of healthy humans, it is also a major cause of skin and soft tissue infections and has the invasive potential to cause severe infections, including osteomyelitis, endocarditis, and bacteremia with metastatic complications (35). The pathogenicity of *S. aureus* depends upon successful adaptation of the microorganism to the host and the coordinated expression of virulence factors. *S. aureus* is usually surrounded by a thin capsule, and capsular polysaccharides (CP) of serotypes 5 (CP5) and 8 (CP8) are the most prevalent ones in clinical isolates from humans (40). *S. aureus* CP5 and CP8 are antiphagocytic and positively contribute to the virulence of this pathogen (27, 40). Production of CP5 (or CP8) may be deeply modified by different global regulators (*agr*, *arlRS*, *saeRS*, and *sarA*) and transcriptional factors (*mgrA*, σ^B) (30, 31, 34, 55, 59). A recent report has shown that the *sbcDC* locus mediates repression of CP5 production as a part of the SOS response in *S. aureus* (5). Furthermore, the expression of CP5 (7) is highly sensitive to diverse environmental signals, such as iron concentration, specific nutrients, CO₂ concentration, or subinhibitory concentrations of ciprofloxacin and mitomycin C (5, 17, 26, 40).

Aspirin is a nonsteroidal anti-inflammatory agent that is

regularly taken by hundreds of millions of individuals worldwide due to its known analgesic and cardiovascular protective activities. The main aspirin biometabolite, salicylic acid (SAL), affects the expression of bacterial virulence factors (45). A study performed using a rabbit model of endocarditis demonstrated that SAL causes a reduction in *S. aureus* virulence (23, 24). Biofilm formation and attachment of *S. aureus* strain 8325 to the *Arabidopsis thaliana* root surface were disrupted by a low concentration of SAL (48). It has also been shown that the effects of SAL on *S. aureus* include activation of the *sigB* operon via both *rsbU*-dependent and -independent mechanisms (24, 42). Moreover, growth of *S. aureus* in the presence of SAL reduced bacterial susceptibility to multiple antimicrobials (16, 44, 46, 47).

S. aureus can adhere to and invade nonprofessional phagocytic cells (9, 12, 54). The relevance of this finding is that intracellular *S. aureus* may be a source of persistent or recurrent infection (60). Results from our laboratory demonstrated that the production of CP5 (or CP8) reduced the internalization of *S. aureus* into epithelial cells (4, 57). Growth of *Klebsiella pneumoniae*, a capsule-containing microorganism, in the presence of SAL also resulted in reduced synthesis of CP (6, 7). In this study, we evaluated whether SAL negatively affects CP5 expression in *S. aureus*, thus enhancing the capacity of *S. aureus* to invade epithelial cells.

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MATERIALS AND METHODS

Bacterial isolates and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. Bacteria were stored in Trypticase soy broth (TSB) (Difco, Detroit, MI) with 20% glycerol at –20°C until use. *S. aureus* was routinely cultured at 37°C and 200 rpm for 18 h in Casamino Acids-yeast extract-

TABLE 1. *Staphylococcus aureus* strains and plasmids used in this study

Strain or plasmid	Description	Reference
<i>S. aureus</i> strains		
Newman	Clinical isolate (ATCC 25904); CP5 producer	8
RN6390	Laboratory strain related to 8325-4	22
Reynolds (CP5)	Capsular polysaccharide serotype 5 strain Reynolds	62
Reynolds (CP-)	Reynolds (CP5) isogenic mutant that does not express CP5	62
ALC2547	<i>mgrA</i> mutant of Newman ($\Delta mgrA::ermC$)	20
ALC4483	Newman <i>sae</i> single-deletion mutant	28
ALC1842	Strain Newman with pALC1766	59
ALC5163	Strain Newman with pALC4991	28
ALC6141	Strain Newman with pALC2566	19
ALC3257	Strain Newman with pALC1484	33
Plasmids		
pALC1484	Derivative of pSK236 containing the promoterless <i>gfp_{uvr}</i> gene preceded by an <i>S. aureus</i> ribosome binding site	33
pALC1766	Derivative of pALC1484 containing the main <i>cap5</i> promoter driving the expression of the <i>gfp_{uvr}</i> gene	59
pALC4991	Derivative of pALC1484 containing the <i>sae</i> P3 promoter driving the expression of the <i>gfp_{uvr}</i> gene	28
pALC2566	Derivative of pALC1484 containing the <i>mgrA</i> promoter driving the expression of the <i>gfp_{uvr}</i> gene	19

glycerophosphate (CYGP) broth without glucose (CYGP_w) (38). When necessary, SAL was added to the culture medium at 50 μ g/ml (0.36 mM). For cell invasion experiments, bacterial cells were collected by centrifugation, washed with sterile saline solution, and suspended in invasion medium (see below) to a density of ca. 10^7 CFU/ml.

Antigenic extracts. Bacterial extracts from wild-type Newman and *mgrA* and *saeRS* isogenic mutant strains (Table 1) were prepared as previously described (56). Briefly, *S. aureus* was cultured for 24 h at 37°C on Columbia agar (Difco) supplemented with 2% NaCl, to enhance CP production, plus 0.36 or 2 mM SAL. Controls were cultured in the same medium with no SAL added. The colonies from one plate were harvested in 1 ml of 10 mM phosphate-buffered saline (PBS) (0.15 M NaCl, pH 7.2), and the cell suspensions were autoclaved for 1 h at 121°C. Bacteria were pelleted by centrifugation at $10,000 \times g$, and the supernatants containing the cell extracts were passed through 0.45- μ m filters and stored at -20°C. Bacterial extracts from isogenic Reynolds (CP5) and Reynolds (CP-) (nonencapsulated) reference strains of *S. aureus* were used as positive and negative controls, respectively.

CP expression by immunoprecipitation. CP5 expression in *S. aureus* Newman was determined by immunoprecipitation in the presence of 0.36 or 2 mM SAL as previously described (21). Briefly, a 1% agarose gel was prepared on a glass slide, and wells were punched in a circular fashion around a central hole. Absorbed type 5 antiserum (25) was added to the central well, and serial dilutions of bacterial extracts [including known serotype 5 Reynolds (CP5) and nonencapsulated Reynolds (CP-) extracts as controls] were applied to the outer wells. Immunodiffusion was conducted in a moist chamber at room temperature. After 24 h, the precipitin lines were examined by staining with Coomassie brilliant blue.

Cell culture. The established bovine mammary epithelial cell line MAC-T (18) was generously provided by Nexia Biotechnologies (Quebec, Canada). MAC-T cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL), insulin (5 μ g/ml), hydrocortisone (5 μ g/ml), penicillin (100 U/ml), and streptomycin sulfate (100 μ g/ml) (Sigma Chemical Co., St. Louis, MO). Prior to each experiment, MAC-T cells were seeded at 1.5×10^5 cells/well in 24-well tissue culture plates and grown for 24 h at 37°C with 5% CO₂.

Internalization assays. Internalization assays were performed as described previously (4). Briefly, confluent MAC-T cell monolayers (approximately 2.5×10^5 cells/well) were inoculated with *S. aureus* suspended in fresh growth medium without antibiotics (invasion medium) to a multiplicity of infection (MOI) of 40. Plates were subjected to centrifugation at $1,000 \times g$ for 20 min to deposit bacteria on the monolayer surface and to synchronize the bacterial internalization into cells. After incubation for 1 h at 37°C under 5% CO₂, the wells were washed with PBS, and then 1 ml of invasion medium supplemented with 25 μ g of lysostaphin (Sigma) was added to each well to kill extracellular bacteria. Incubation of cocultures with lysostaphin proceeded for an additional 2 h at 37°C with 5% CO₂. Supernatants were then collected and plated on Trypticase soy agar (TSA) to verify 100% bacterial killing by lysostaphin. The monolayer was washed with sterile PBS, treated for 5 min at 37°C with 100 μ l of 0.25% trypsin-0.1% EDTA (Gibco BRL), and lysed by the addition of 900 μ l of 0.025% Triton X-100 (USB,

Cleveland, OH) in sterile distilled water to release intracellular staphylococci. The CFU number was determined by quantitative plating on TSA. MAC-T cell viability was evaluated by trypan blue exclusion.

Transcriptional fusion studies. After overnight culture, *S. aureus* strains harboring different recombinant plasmids (Table 1) were diluted 1:100 and grown with or without 50 μ g/ml SAL at 37°C with shaking in CYGP_w broth. Aliquots were transferred hourly to microtiter plates and assessed for cell density (optical density at 650 nm [OD₆₅₀]) and fluorescence for 9 h in an FL600 fluorescence spectrophotometer (BioTek Instruments, Winooski, VT). Promoter activities were plotted as mean fluorescence/OD₆₅₀ ratios to minimize variations due to changes in cell density between experiments, using the average values for triplicate readings.

Real-time PCR. Bacterial RNA was extracted using Trizol (Gibco BRL) and 0.1-mm silica beads in a reciprocating shaker (Biospec, Bartlesville, OK) according to the manufacturer's instructions. RNA was subjected to DNase treatment, using a Turbo DNAfree kit (Ambion, Austin, TX) according to the manufacturer's protocol. cDNA synthesis was performed with a Transcriptor first-strand cDNA synthesis kit (Roche, Basel, Switzerland), using random hexamer primers. Quantitative real-time PCR was performed using LightCycler FastStart DNA Master SYBR green I (Roche) equipment and kits. cDNA was subjected to real-time PCR using the following primers: *cap5K*-f, 5'-CCA GTG AAT TGT TTG CAA CG-3'; *cap5K*-r, 5'-CAT TTT CCC AAT AAA TGT TGA AAG-3'; *mgrA*-f, 5'-GGG ATG AAT CTC CTG TAA AC-3'; *mgrA*-r, 5'-GCT GAA GCG ACT TTG TCA GA-3'; *saeRS*-f, 5'-ATG CTA ATA CCG TGA ATG TCC A-3'; *saeRS*-r, 5'-TGG CCG TTA AAC CAC ATT AAA-3' (3.1-, 2.4-, and 2.0-kb *sae* transcripts were amplified using these primers); *gyrB*-f, 5'-GGT GCT GGG CAA ATA CAA GT-3'; and *gyrB*-r, 5'-TGG GAT ACC ACG TCC GTT AT-3'. The *gyrB* gene was used as a calibrator and as an internal control to normalize data. Cycling conditions were 95°C for 10 min followed by 45 cycles of 95°C for 10 s, 55°C for 10 s, and 72°C for 15 s and 1 cycle of 40°C for 30 s. The number of copies of each sample transcript was determined with the aid of LightCycler software. The $-\Delta\Delta C_T$ value represents the difference in threshold cycle (C_T) between the target and control (*gyrB*) genes treated with SAL minus the difference in C_T between the untreated target and control genes (29).

Statistical analysis. Nonparametric data were analyzed with the Mann-Whitney test, using GraphPad software (version 4.0; GraphPad Prism). *P* values of <0.05 were considered significant.

RESULTS

Effect of SAL on internalization of encapsulated *S. aureus*. Previous studies have reported a reduction in staphylococcal adherence when bacteria are cultured in the presence of SAL (10, 36, 43). In the present study, we investigated whether *S. aureus* strains Newman and RN6390 treated with SAL exhibited a reduced ability to invade epithelial cells. *S. aureus* New-

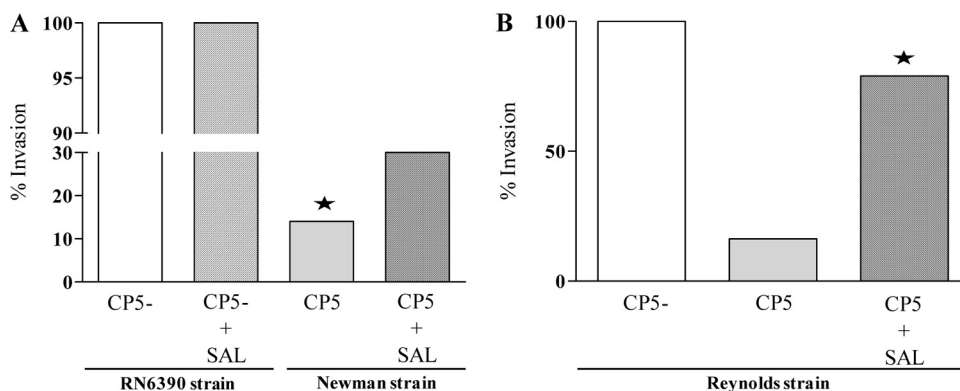


FIG. 1. Effect of SAL on internalization of *S. aureus* into MAC-T epithelial cells. Confluent MAC-T cells were tested for staphylococcal invasion. Each bar represents the “percentage of invasion,” defined as the median of intracellular CFU/ml ($n = 9$ to 12) from *S. aureus* strains related to the median of intracellular CFU/ml from *S. aureus* RN6390 or Reynolds (CP-) without SAL treatment (100% invasion). The asterisks represent significant differences between strain Newman treated and not treated with SAL ($P = 0.0001$) (A) and between Reynolds (CP5) organisms treated and not treated with SAL ($P = 0.0089$; Mann-Whitney test) (B). Comparison of Reynolds (CP5) treated with SAL and Reynolds (CP-) showed no significant differences.

man organisms express CP5, whereas *S. aureus* RN6390 cells do not express CP5 due to a mutation in the essential *capE5* gene (61). The SAL concentration (0.36 mM) used in the experiments did not affect the growth of the *S. aureus* strains used in this study (data not shown) and represents the levels achievable in human serum after ingestion of aspirin in pharmacological (antithrombotic) doses (11, 37). Two hours after addition of lysostaphin to the infected MAC-T cells, the number of intracellular CFU of *S. aureus* RN6390 cells pretreated with SAL did not differ significantly compared with that of cells without SAL pretreatment (Fig. 1A). Preexposure of *S. aureus* Newman to SAL significantly enhanced the internalization of the bacteria into MAC-T cells compared with that of untreated bacteria (Fig. 1A). This finding was consistent with previous results from our laboratory demonstrating that *S. aureus* strains with no capsule or reduced capsule formation displayed an increased ability to invade MAC-T cells compared with those that express either CP5 or CP8, possibly due to unmasking of surface adhesins beneath the capsule (4, 57). The differences observed in the present study were likely attributed to the influence of SAL on CP expression. To test this hypothesis, we performed a similar study with *S. aureus* Reynolds (CP5) and the isogenic nonencapsulated Reynolds (CP-) derivative under the conditions described above. As shown in Fig. 1B, Reynolds (CP5) organisms pretreated with SAL were internalized by the MAC-T cells in significantly higher numbers than those of Reynolds (CP5) organisms not exposed to SAL. Moreover, the invasiveness of the nonencapsulated derivative of the Reynolds strain was not significantly affected by preexposure to SAL (data not shown). Taken together, our results demonstrated that encapsulated strains of *S. aureus* pretreated with SAL exhibited an increased capacity for invasion of MAC-T epithelial cells, probably due to decreased CP expression.

cap expression is diminished by SAL treatment. The effect of SAL on *cap5* promoter activity was ascertained by expression of the *gfp*_{uvr} reporter gene driven by the major *cap5* promoter. As shown in Fig. 2A, SAL induced a significant decrease in the activity of the strain Newman *cap5* promoter. The promoter activities of *saeRS* and *mgrA*, two known regu-

lators involved in down- and upmodulation of CP expression, respectively, were also measured after SAL exposure. These assays established that treatment with SAL reduced the *mgrA* promoter activity (Fig. 2B). However, the main *sae* promoter (28) was not affected by SAL treatment at the time point studied (Fig. 2B). Furthermore, real-time PCR experiments were performed to estimate the quantities of *cap5K*, *saeRS*, and *mgrA* transcripts formed under the effects of SAL. Our

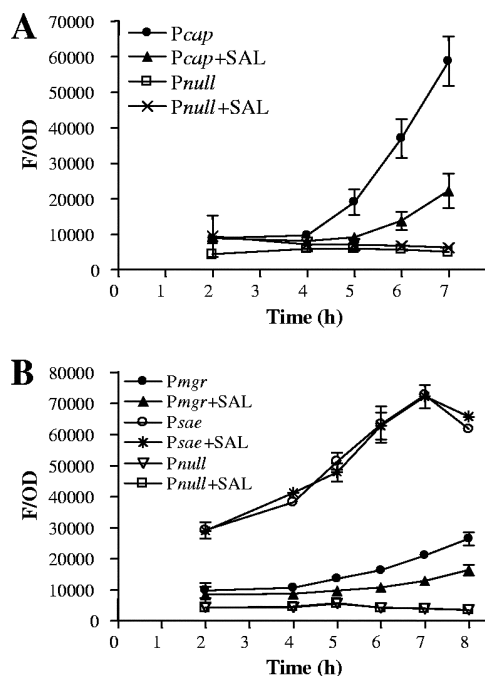


FIG. 2. Effect of SAL on *cap5* (A), *mgrA* (B), and *sae* P3 (B) promoter activities. Expression of *gfp* driven by the target promoters was measured during the growth cycle, and fluorescence values were expressed as green fluorescent protein fluorescence related to the OD₆₅₀ (F/OD) in order to minimize variations in fluorescence due to varying cell density. The data represent the arithmetic means \pm standard deviations for triplicate measurements from three or four independent experiments.

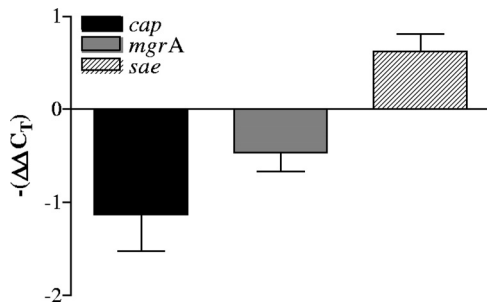


FIG. 3. Real-time PCR analysis of *cap*, *mgrA*, and *sae* transcription. Changes in gene expression are shown as fold changes $[-(\Delta\Delta C_T)]$ following growth with 50 $\mu\text{g/ml}$ of SAL to an OD_{650} of 1.4. The data represent the means \pm standard deviations for duplicate measurements from three independent experiments.

results showed that the levels of expression of *cap5K* and *mgrA* transcripts decreased in *S. aureus* organisms treated with SAL. In contrast, SAL treatment increased the level of *saeRS* transcript expression (Fig. 3). These results demonstrate that pretreatment of *S. aureus* Newman organisms with SAL decreased transcription of *cap5* and *mgrA*, an upregulator of CP expression. Decreased transcription of *cap5* and *mgrA* was accompanied by enhanced expression of *saeRS*, a downregulator of CP expression, thus contributing to an overall decrease in CP expression upon SAL exposure.

SAL reduces phenotypic CP expression. The effect of SAL on CP production was also evaluated by immunoprecipitation, using monospecific antiserum to CP5. This method was suitable for semiquantitative estimation of the amount of CP produced using twofold serial dilutions of CP extracts (32). A high concentration of SAL (2 mM) was used to investigate the effect on CP expression. The results revealed a dose-dependent decrease of CP5 expression, caused by SAL, in *S. aureus* Newman organisms (Fig. 4). The highest CP5 expression level was seen in the control (bacteria not treated with SAL). These results showed that CP5 production by the Newman strain organisms was decreased by treatment with SAL.

DISCUSSION

Previous results from our laboratory demonstrated that non-encapsulated *S. aureus* strains were internalized more efficiently into MAC-T cells than were their encapsulated (CP5⁺ or CP8⁺) counterparts (4, 57). In the present study, we demonstrated that pretreatment of encapsulated (CP5⁺) *S. aureus* strain Newman organisms with SAL increased the ability of the bacteria to invade MAC-T cells. This observation correlated with a diminished production of CP5. Our experiments were conducted in medium without glucose to avoid the repression of CP production (52). Besides, the concentration of SAL (0.36 mM) used matched the antiplatelet concentration achievable in human plasma (11, 37). It was recently reported that *S. aureus* 8325-4 (nonencapsulated) (61) cultured in the presence of SAL exhibited reduced adhesion to human umbilical vein endothelial cells (43). In our studies performed with strain RN6390 (an 8325 strain derivative), we were unable to confirm these experimental results. This discrepancy, however, can be explained by the different cell line used and, perhaps more

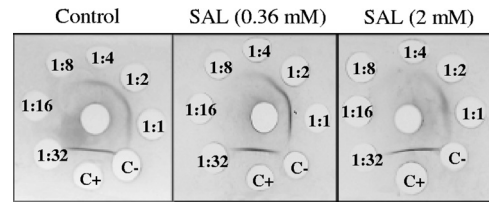


FIG. 4. Immunodiffusion analysis of CP5 extracts. Thirty microliters of monospecific antiserum to serotype 5 was added to the center well. Thirty-microliter samples of undiluted and twofold serially diluted (indicated inside the well) CP5 extracts of Newman strain organisms, cultured or not with different concentrations of SAL, were added to the outer wells. Precipitin lines were visualized after staining with Coomassie brilliant blue. C+, positive control [undiluted extract of *S. aureus* Reynolds (CP5) strain]; C-, negative control [undiluted extract of *S. aureus* Reynolds (CP-) strain]. Precipitin lines were visible up to a 1:4 dilution of capsular extracts in the control without SAL, up to a 1:2 dilution at a 0.36 mM SAL concentration, and at only a 1:1 dilution at the highest SAL concentration (2 mM).

importantly, the major differences in the experimental design and bacterial growth conditions utilized.

Internalization of *S. aureus* into mammalian cells depends upon fibronectin bridging between fibronectin binding proteins (FnBPs) and the integrin $\alpha_5\beta_1$ (53). Kupferwasser et al. (24) described that pretreatment of several *S. aureus* strains with SAL (0.36 mM) resulted in low levels of adhesion to cell matrix proteins, such as fibronectin and fibrinogen. In that study, the reduction in matrix binding protein expression on the surface of *S. aureus* represented composite pooled data derived from *S. aureus* laboratory strains RN6390, COL, Newman, and ISP479C as well as from several clinical (methicillin-resistant *S. aureus* [MRSA]) *S. aureus* isolates. Grundmeier et al. (15) have shown that both *fnbA* and *fnbB* of *S. aureus* Newman contain a centrally located point mutation resulting in a stop codon. This causes a truncation of both FnBPs at the end of the C domain. Consequently, this led to deficient adherence of strain Newman compared with that of *S. aureus* strains with wild-type FnBPs. Our results also confirmed that strain Newman (7×10^4 CFU/ml) organisms were internalized into MAC-T cells at lower rates than those of strain RN6390 (4.9×10^5 CFU/ml) organisms. However, when both strains were cultured in the presence of SAL, only the internalization capability of strain Newman was affected. Moreover, the increased invasion of MAC-T cells after SAL treatment of Newman organisms may suggest that another factor(s) besides CP may be affected by SAL, thus improving cellular internalization (2). We are now conducting experiments to evaluate this hypothesis. The preliminary results of SDS-PAGE analysis of Newman, AH12 (Newman *eap* mutant), and complemented strain AH12pCXEap surface proteins revealed increased Eap expression after SAL treatment in strain Newman (L. P. Alvarez et al., unpublished data). Thus, the increased invasion observed after SAL treatment may be due not only to the diminished expression of CP but also to an increased expression of certain adhesins, such as Eap.

The low CP5 production by *S. aureus* induced by SAL treatment was also seen at the transcriptional level. Previous studies showed that expression of the *cap* operon is mainly driven by the major promoter located at the beginning of the operon (41, 51). The expression of the major *cap5* promoter was thus

monitored with a *cap5* promoter-*gfp*⁺ reporter gene fusion by measuring fluorescence emission. Indeed, the activity of the *cap5* promoter was diminished by exposure to SAL. Down-regulation of CP5 by SAL was also confirmed by analyzing the *cap5K* (CP5-specific region) mRNA, using real-time PCR. Blickwede et al. (3) observed that mRNA expression of *cap5A* was decreased in *S. aureus* strain Newman after treatment with subinhibitory concentrations of florfenicol (an antibiotic used in veterinary medicine), indicating that *cap* expression can be affected by different pharmacological agents. In the present study, when the expression of CP5 was evaluated by immunoprecipitation using monospecific antiserum to CP5, we observed a dose-dependent reduction of CP5 production by *S. aureus* Newman after SAL treatment. In a study on Gram-negative bacteria, Domenico et al. (6) observed a decrease of *K. pneumoniae* CP production by SAL, in a concentration-dependent manner. In particular, at 0.21 mM SAL, CP was reduced nearly 60%. In both cases, SAL exerted its effect on CP production at serum concentrations normally attained after ingestion of aspirin at doses within the therapeutic range.

Expression of the *cap* operon and CP expression in *S. aureus* are known to be under multiple levels of control and affected by several environmental stimuli (49). Previous studies have shown that the global regulators *agr*, *mgrA*, *arlRS*, σ^B , *sarA*, and *saeRS* control CP production at the transcriptional level (30, 31, 34, 55, 59). It should be noted that the results from several previous reports are contradictory. In this regard, the *sae* locus has been shown to repress the *cap5* genes (55). In contrast, Rogasch et al. (50) did not find any influence of *SaeRS* on the transcription of the *cap* operon in *S. aureus* strain Newman or COL. On the other hand, the *sbcD* and *sbcC* genes were involved in the repression of CP5 production through an *arl-mgr*-dependent pathway at subinhibitory concentrations of ciprofloxacin or mitomycin C (5). As expected, we observed that after SAL treatment of *S. aureus* strain Newman, the activity of the *mgrA* promoter was diminished. In agreement with this finding, the level of *mgrA* transcript, as determined by real-time PCR, was also decreased after SAL exposure. Similarly, Riordan et al. (49) demonstrated that salicylate induction downregulated *mgrA* transcripts. In addition, the *mgrA* mutant of the Newman strain did not express CP under any of the conditions studied here (data not shown). Several studies identified four overlapping transcripts (3.1-, 2.4-, 2.0-, and 0.7-kb mRNAs) of the *sae* locus (1, 39, 55). The main *sae* promoter (28), located upstream of *saeP*, is strongly autoregulated and also repressed by SigB in strains 8325-4, ISP479R, UAMS-1, and COL (13). This *sae* promoter appears to be activated by H₂O₂ and subinhibitory concentrations of α -defensins (13). Conversely, the main *sae* promoter did not respond to high salt concentrations (1). We did not observe any effect on the main *sae* promoter's activity by SAL treatment of *S. aureus* Newman strain organisms at the time points studied. However, the level of *sae* transcripts (3.1-, 2.4-, and 2.0-kb transcripts included) increased after SAL exposure. Previous studies showed that the *sae* transcripts T3 (3.1 kb), T1 (2.4 kb), and T4 (0.7 kb) were highly expressed in strain Newman compared with those in strain 8325-4 (14, 55).

Since the *sae* system of the Newman strain is not activated by *agr* or repressed by *sigB* (13), we suggest that the diminished CP5 production by *S. aureus* observed upon SAL exposure may

be caused mainly by high expression of *saeRS* and reduced expression of *mgrA*. The relevance of our findings is underscored by the fact that aspirin, a main source of salicylic acid in the human host, is being taken by millions of human beings worldwide without medical prescription and is widely prescribed for defined purposes, including prevention of cardiac infarction (58). Therefore, modulation of the invasive capacity of bacteria may play a role in pathogenesis, prophylaxis, and treatment of staphylococcal infections in certain at-risk populations colonized with *S. aureus*. It should not be forgotten, however, that nonencapsulated *S. aureus* strains have a greater ability to invade epithelial cells and may persist for long periods inside the host. Furthermore, persistent or recurrent infection may be favored by SAL administration.

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