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MAJOR ARTICLE

MgrA Governs Adherence, Host Cell Interaction, and Virulence in a Murine Model of Bacteremia Due to *Staphylococcus aureus*

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Background. MgrA is an important global virulence gene regulator in *Staphylococcus aureus*. In the present study, the role of *mgrA* in host-pathogen interactions related to virulence was explored in both methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) strains.

Methods. In vitro susceptibilities to human defense peptides (HDPs), adherence to fibronectin (Fn) and endothelial cells (ECs), EC damage, α-toxin production, expression of global regulator (eg, *agr RNAIII*) and its downstream effectors (eg, α-toxin [*hla*] and Fn binding protein A [*fnbA*]), MgrA binding to *fnbA* promoter, and the effect on HDP-induced *mprF* and *dltA* expression were analyzed. The impact of *mgrA* on virulence was evaluated using a mouse bacteremia model.

Results. *mgrA* mutants displayed significantly higher susceptibility to HDPs, which might be related to the decreased HDP-induced *mprF* and *dltA* expression but decreased Fn and EC adherence, EC damage, α-toxin production, *agr RNAIII*, *hla* and *fnbA* expression, and attenuated virulence in the bacteremia model as compared to their respective parental and *mgrA*-complemented strains. Importantly, direct binding of MgrA to the *fnbA* promoter was observed.

Conclusions. These results suggest that *mgrA* mediates host-pathogen interactions and virulence and may provide a novel therapeutic target for invasive *S. aureus* infections.

Keywords. *Staphylococcus aureus*; *mgrA*; host factors; virulence and bacteremia.

Staphylococcus aureus is an important human pathogen that causes a wide range of serious infections, including life-threatening bacteremia [1]. The emergence of methicillin-resistant *S. aureus* (MRSA) further emphasized this public health issue and has become a global problem [1]. Of concern, up to 30% of patients with *S. aureus* bacteremia do not respond to antibiotic treatment even when a gold-standard anti-MRSA antibiotic (eg, vancomycin or daptomycin) is used [2, 3]. With the critical shortage of new anti-MRSA antibiotics, there is an urgent need to understand the intersection of host and pathogen factors that contribute to the pathogenesis and to develop novel strategies to prevent and/or treat these severe infections.

The ability of *S. aureus* to cause infections is attributed to its complex expression of multiple gene products, including surface adhesins and exoproteins [4]. The adhesins allow bacteria to attach to host cells and extracellular matrix during the early colonization phase [5]. Of these adhesins, fibronectin (Fn) binding protein A (FnBPA; encoded by *fnbA*) has been demonstrated to be involved in binding and internalization into host cells (eg, endothelial cells [ECs]), which is important in *S. aureus* endovascular infections [6]. Exoproteins may facilitate tissue damage and spread into the bloodstream during later infection [7]. α-toxin (encoded by *hla*) is a major pore-forming exoprotein produced by the majority of *S. aureus* strains, and it targets a broad range of host cells (eg, ECs) that ultimately leads to tissue damage and dissemination [8, 9]. MgrA is one of the major master regulators and regulates the expression of many virulence factors in *S. aureus*, such as α-toxin, capsule, and protease, by binding to their respective promoters [10, 11]. In addition, *mgrA* is ubiquitous among clinical *S. aureus* isolates, including MRSA and methicillin-susceptible *S. aureus* (MSSA) [4]. However, the role of MgrA in host-pathogen interactions has not been well studied. In the current study, we showed that *mgrA* mediates host-pathogen interactions that might contribute to virulence in a mouse model of bacteremia due to MSSA and MRSA.

METHODS

Bacterial Strain and Plasmids

One representative MSSA strain (RN6390, a laboratory strain related to 8325-4 [11]) and 1 MRSA strain (MW2, a USA400

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strain [12]) were used as parental strains. A *mgrA* deletion in strain RN6390 (ALC2530; a *mgrA* deletion mutant of RN6390 [Δ*mgrA*::*ermC*]) was achieved by using a temperature-sensitive erythromycin^r shuttle plasmid, pCL52.2 [13]. *mgrA* was inactivated in MRSA strain MW2 by transducing the Δ*mgrA*::*ermC* mutation from ALC2530, using phage ϕ11 [13]. The *mgrA* mutant strains were complemented with *mgrA* by transforming it with plasmid pEPSA5::*mgrA* as described previously [14]. Unless otherwise stated, all *S. aureus* strains were grown at 37°C in tryptic soy broth (TSB; Difco) or TSB agar plates.

Susceptibility to Human Defense Peptides (HDPs)

Human neutrophil peptide 1 (hNP-1) was purchased from Peptides International (Louisville, KY). Thrombin-induced platelet microbicidal proteins (tPMPs) were prepared from thrombin-stimulated platelets isolated from fresh rabbit blood [15]. The bioactivity of the tPMPs was assessed by a *Bacillus subtilis* ATCC 6633 susceptibility assay [16, 17]. The HDP susceptibilities of our study strains were tested by exposing 10^5 colony-forming units (CFU)/mL or 10^3 CFU/mL of *S. aureus* cells from the exponential growth phase (incubation time, approximately 3 hours) to hNP-1 at 1.25 μg/mL or tPMPs at 6.25 μg/mL, respectively for 2 hours at 37°C [16–18]. These HDP concentrations were selected on the basis of extensive pilot studies, in which we identified peptide levels that did not rapidly result in *S. aureus* killing over a 2-hour exposure period (data not shown). The results are expressed as the percentage (±standard deviation [SD]) of the initial inoculum that survived exposure to HDPs.

Adherence to Fibronectin (Fn)

To assay the ability of *S. aureus* cells to adhere to Fn, 6-well tissue culture plates were coated with purified human Fn (50 μg/mL, Sigma Chemicals) for 18 hours at 4°C and then treated with 3% bovine serum albumin (Sigma Chemicals) for 2 hours to prevent nonspecific adhesion [19]. *S. aureus* cells grown to exponential phase (incubation time, approximately 3 hours), when MgrA expression is most optimal [20], were added to the plates (5×10^3 CFU/well) and incubated for 1 hour at 37°C [19]. After washing with phosphate-buffered saline, TSB agar was added to each well, and plates were incubated overnight at 37°C. Adherence was expressed as the percentage (±SD) of the initial inoculum bound to Fn [19].

Confocal Microscopy

To confirm adherence to Fn, a fluorescence-labeled Fn (FN-488; Cytoskeleton, Denver, CO) was used according to the manufacturer's protocol [21, 22]. The maximum excitation and emission spectra of FN-488 are 450 nm and 550 nm, respectively [21]. Briefly, *S. aureus* exponential-phase cells (10^8 CFU/mL) were incubated with FN-488 (4 μg/mL) for 30 minutes at 37°C, washed with phosphate-buffered saline, and imaged by confocal microscopy [22].

α-Toxin Production

A semiquantitative assay of α-hemolysin production was performed by dropping 3 μL of 10^8 CFU/mL of *S. aureus* exponential-phase cells onto 5% sheep blood agar plates, followed by incubation for 24 hours at 37°C [23]. Clear zones around the bacterial colonies on the blood agar plates indicate α-hemolytic activity.

EC Adherence

Human microvascular ECs (HMEC-1) were obtained from the Centers for Disease Control and Prevention and maintained as previously described [24, 25]. For the EC adherence assay, confluent HMEC-1 in 6-well plates were washed with Hank's balanced salt solution (HBSS). *S. aureus* exponential-phase cells were added to the plates at a final inoculum of 5×10^3 CFU/well and incubated for 1 hour at 37°C. Unbound *S. aureus* cells were removed by washing the wells with HBSS. The number of adherent bacteria was determined by lysing HMEC-1 with cold distilled water, followed by quantitative culture [26]. Adherence was expressed as the percentage (±SD) of the initial inoculum bound to HMEC-1 [26].

EC Damage

A well-established 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to test the EC damage [24, 27]. Briefly, *S. aureus* exponential-phase cells were added to confluent HMEC-1 in 24-well plates (5×10^5 ECs/well), corresponding to a multiplicity of infection (MOI) of 50 and 500 (as established by pilot studies) for the MW2 and RN6390 strain sets, respectively. After 3 hours of incubation at 37°C, the wells were washed with HBSS, medium containing lysostaphin (10 μg/mL; Sigma Chemicals) was added to lyse extracellular *S. aureus* cells, and plates were incubated at 37°C [24]. After 18 hours of incubation, 100 μL of MTT (5 mg/mL, Sigma Chemicals) was added, and plates were incubated for 2 hours. The medium was replaced with 150 μL of 0.04 M HCl to stop the reaction. HMEC-1 without *S. aureus* exposure were considered a 0% damage control. HMEC-1 damage is presented as a percentage (±SD), calculated as 1 − [OD_{560} of samples/OD_{560} of control] [24].

RNA Isolation and Real-Time Quantitative Reverse Transcription–Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from exponential-phase *S. aureus* cells, as the level of *mgrA* expression reaches its peak during exponential phase [19], using an RNeasy Kit (Qiagen, Valencia, CA). Then DNase-treated RNA was transcribed into complementary DNA. Real-time qRT-PCR was performed using an ABI Prism 7000 instrument (Applied Biosystems) and the SYBR green PCR master kit (Applied Biosystems). The amplification of *agr RNAIII*, *hla*, and *fnbA* was conducted using primers as described previously [28–30]. *gyrB* was used to normalize the transcript quantification, and relative

expression of the study genes was calculated by the $\Delta\Delta C_T$ method [28].

In addition, to assess the impact of *mgrA* on the expression of genes involved in HDP susceptibility (eg, *mprF* and *dltA*), as well as induction of *mprF* and *dltA* by HDPs, RNA samples were isolated from exponential-phase *S. aureus* cells exposed to hNP-1 (5 μg/mL) for 30 minutes as described previously [31]. The concentration of hNP-1 was selected on the basis of our pilot studies to identify the peptide level that did not rapidly kill *S. aureus* over a 30 minute-exposure period (≥90% survival). Amplification of *mprF* and *dltA* was conducted using primers as described previously [32, 33]. Then, the relative levels of *mprF* and *dltA* expression, with or without hNP-1 exposure, were calculated as described above.

Gel Shift Analysis

To determine whether MgrA interacts directly with *fnbA* promoter, purified MgrA and a gel-purified fragment containing the *fnbA* promoter region were used. Cloning and purification of the His6-tagged fusion MgrA were performed as described elsewhere [13]. A 287-bp fragment containing the *fnbA* promoter region was amplified by PCR, using previously described primers [34]. A specific competitor DNA representing the promoter fragment of *hla* [11] and a nonspecific competitor DNA from within the *fnbA* coding sequence (primers were the same as for the *fnbA* real-time qRT-PCR) were also used. Briefly, the *fnbA* promoter DNA (500 ng) with or without the specific or nonspecific competitor DNA (2500 ng) was incubated at room temperature for 20 minutes with various amount of purified MgrA in binding buffer (10 mM Tris-HCl, 150 mM KCl, 0.1 mM DTT, and 0.1 mM ethylenediaminetetraacetic acid) [35]. The reaction mixtures were analyzed in a precast 10% native Tris-glycine gel (Novex, San Diego, CA). The band shifts were stained with a well-studied fluorescence-based SYBR green electrophoretic mobility shift assay (EMSA) kit (Molecular Probes EMSA, Panomics, Fremont, CA) and detected by exposure to UV_{302} according to the manufacturer's instructions [36, 37].

Mouse Bacteremia Model

To better define the role of *mgrA* in vivo in regard to staphylococcal virulence, a murine bacteremia model was used [38]. The Institutional Animal Care and Use Committee of the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center approved all animal study protocols. Female CD1 Swiss mice (weight, 18–23 g) were infected intravenously with 5×10^7 CFU/animal (a 95% effective dose established by pilot studies) of each study *S. aureus* strain (approximately 8 mice/group). Ten days after infection, mice were euthanized, and their kidneys, spleen, livers, and lungs were removed and quantitatively cultured. *S. aureus* counts in the target tissues are specified as mean $\log_{10}$ CFU/g of tissue (±SD). In addition, the body weight of animals was monitored during the experimental period.

Statistical Analysis

The 2-tailed Student *t* test was used to analyze the in vitro data. Differences in *S. aureus* density in the target tissues were compared by analysis of variance. *P* values of <.05 were considered statistically significant.

RESULTS

HDPs Susceptibilities

Susceptibility to killing by HDPs from polymorphonuclear leukocytes (eg, hNP-1) and platelets (eg, tPMPs) is a critical factor in the pathogenesis of *S. aureus* endovascular infection [26]. We demonstrated that both *mgrA* mutant strains in the MSSA and MRSA backgrounds exhibited significantly reduced survival due to hNP-1 and tPMP killing, compared with their respective parental and *mgrA*-complemented strains ($P < .0001$; Figure 1). For instance, the survival percentages among MW2 parental strains in the presence of hNP-1 and tPMPs were 26.6% and 44.0%, respectively, and the percentages among *mgrA*-complemented strains were 28.5% and 39.3%, respectively; these values were significantly higher than those for *mgrA* mutant strains (17.1% and 17.4%, respectively; Figure 1).

Effect of *mgrA* on Induction of *mprF* and *dltA* Expression

It is known that *mprF* and *dltA*, which regulate the cell membrane surface charge, are involved in the susceptibility to HDPs [31]. To assess the underlying mechanism of MgrA on susceptibility to HDPs, MRSA and MSSA strain sets were exposed to a sublethal concentration of hNP-1, and transcription levels of *mprF* and *dltA* were measured. We demonstrated that *mgrA* mutants exhibited lower levels of *mprF* and *dltA* expression than their isogenic parental and *mgrA*-complemented strains in the absence of hNP-1, indicating that *mgrA* positively regulates *mprF* and *dltA* expression (Figure 1C and 1D). Importantly, levels of *mprF* and *dltA* expression were significantly induced by hNP-1 in the MRSA and MSSA parental and *mgrA*-complemented strains but not in the *mgrA* mutant strains (Figure 1C and 1D).

Adherence to Fn

Through bridging the staphylococcal cell wall–anchored FnBPs to the mammalian cell-surface integrin α5β1 (FnBP-Fn-α5β1), Fn-mediated adherence of *S. aureus* has been implicated in the pathogenesis of *S. aureus* infections [39]. We found that adhesion to Fn in *mgrA* mutant strains was significantly reduced (by 2.6–7.8-fold) as compared to their respective parental and *mgrA*-complemented strains ($P < .001$; Figure 2A). To confirm the role of *mgrA* in the ability of *S. aureus* to adhere to Fn, a well-studied fluorescence-labeled Fn was used [21, 22]. Confocal microscopy of the 2 *mgrA* strain sets revealed that a substantially lower percentage of MSSA and MRSA mutants had bound to Fn as compared to their respective parental and *mgrA*-complemented strains (Figure 2B).

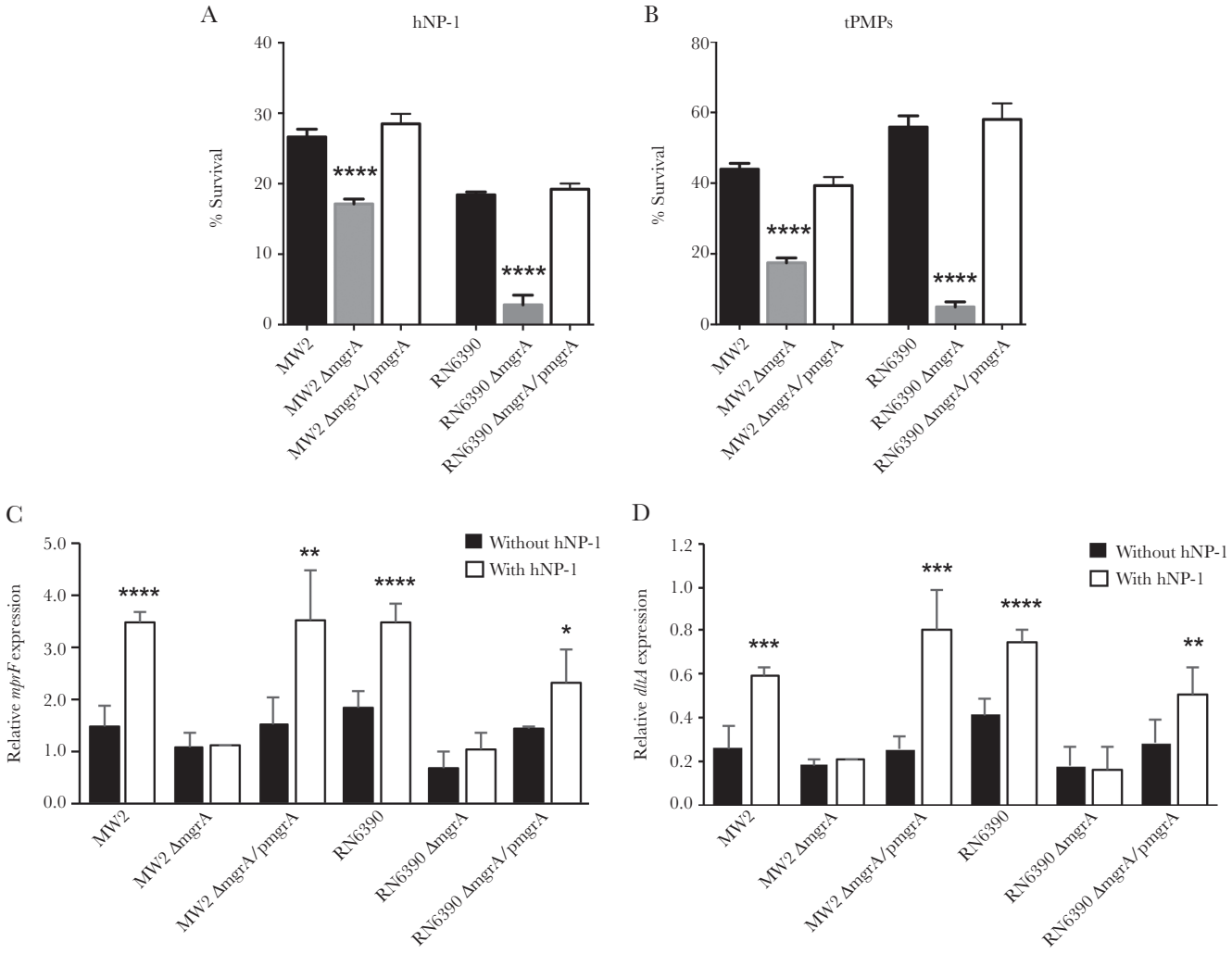

Figure 1. In vitro susceptibilities to human neutrophil peptide 1 (hNP-1; *A*) and thrombin-induced platelet microbicidal proteins (tPMPs; *B*) and relative expression of *mprF* (*C*) and *dltA* (*D*) in the absence and presence of sublethal concentration of hNP-1 in MW2 and RN6390 parental *Staphylococcus aureus* strains and their respective isogenic *mgrA* mutants and *mgrA*-complemented strain sets. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001 vs their respective parental and mgrA-complemented strains or condition without hNP-1 exposure.

EC Adherence and Damage

In line with the Fn adherence results described above, we found that the *mgrA* mutant strains exhibited a significantly reduced capacity for EC adherence, compared with their respective wild-type strains (Figure 3A). For example, *mgrA* mutants in the MSSA and MRSA backgrounds had an approximately 2.4-fold decrease in EC adherence, compared with their respective parental and *mgrA*-complemented strains ($P < .001$; Figure 3A). It has been reported that EC damage is involved in staphylococcal pathogenesis by providing a route for dissemination of *S. aureus* to distant sites [40]. In the present study, we found that both *mgrA* mutant strains in the MSSA and MRSA backgrounds caused significantly less EC damage than their respective parental and *mgrA*-complemented strains ($P < .0001$; Figure 3B). For instance, the mutant MRSA strain damaged 6% of ECs, which is significantly less damage than that caused by its isogenic parental strain (approximately 48%) and *mgrA*-complemented strain (approximately 36%; $P < .0001$; Figure 3B).

α-Toxin Production

Previous studies indicated that α-toxin production is positively associated with EC damage [41]. Consistent with previous findings, we found that *mgrA* mutants showed substantially weaker α-toxin activity than their respective parental and *mgrA*-complemented strains (Figure 3C), which is consistent with the EC damage data presented above.

Role of *mgrA* in *agr RNAIII*, *hla*, and *fnbA* Expression

As reported from prior studies, *mgrA* positively regulates *agr RNAIII* to promote *hla* expression and binds to the *hla* promoter to augment gene expression [11]. Consistently, our

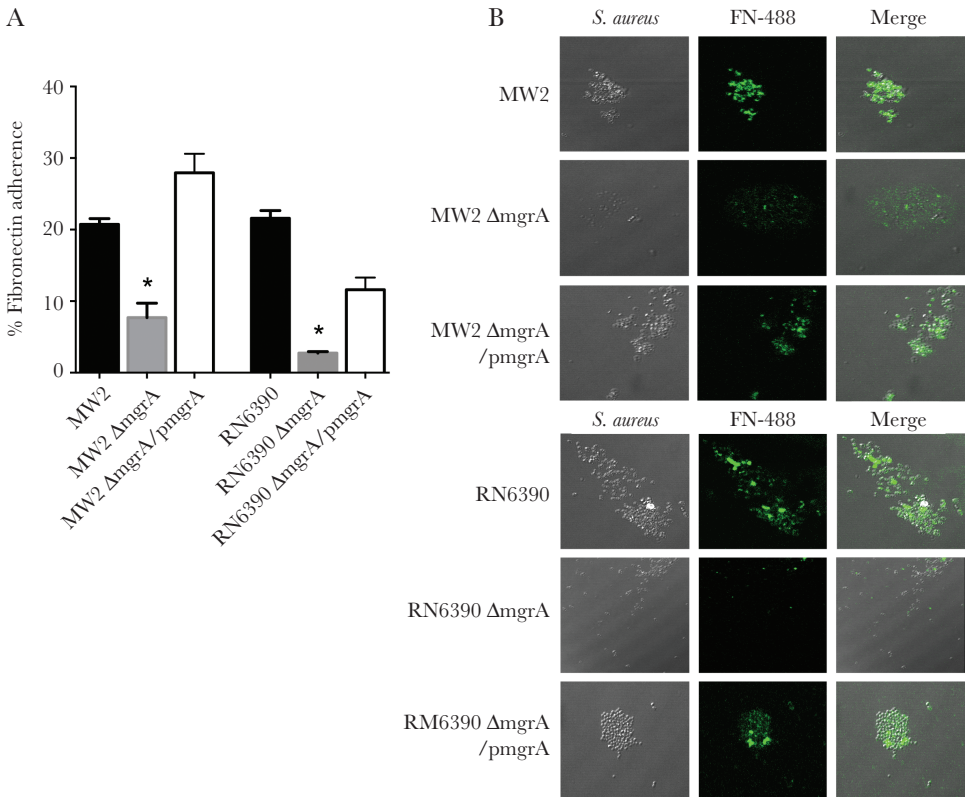

Figure 2. *A,* In vitro fibronectin (Fn) adherence by the study strain sets (A). *B,* Confocal microscopy of fluorescence-labeled Fn (FN-488) bound to the *Staphylococcus aureus* strain sets. *$P < .001$ vs their respective parental and *mgrA*-complemented strains.

study showed that both *mgrA* mutant strains in the MSSA and MRSA backgrounds had reduced *agr RNAIII* and *hla* expression, compared with their respective parental and *mgrA*-complemented strains ($P < .001$; Figure 4A). It has been reported that *agr* downregulates both *fnbA* expression and Fn binding [19]. However, decreased expression of *agr RNAIII* and *fnbA* in the *mgrA* mutants was observed, compared with findings for their respective wild-type strains in the current study, which is in contrast to findings from previous studies [19]. Gel shift analysis showed that MgrA bound to the *fnbA* promoter region, which might have contributed to the unexpected enhanced *fnbA* expression in the *mgrA* mutants (Figure 4B). For instance, a retarded MgrA-P_{fnbA} complex could be detected with 0.5 µg of MgrA. Notably, as the concentrations of MgrA increased, the retarded MgrA-P_{fnbA} complex showed a more predominant band, with complete conversion at 4.0 µg of MgrA. This binding could be outcompeted by adding the specific competitor DNA (Figure 4B). In addition, adding the nonspecific competitor DNA did not significantly affect MgrA-P_{fnbA} binding (Figure 4B). These results indicate that MgrA-P_{fnbA} binding is specific. Importantly, decreased *fnbA* expression profiles in the *mgrA* mutants positively correlated with Fn and EC adherence phenotypes.

Mouse Model of Bacteremia

All mice infected with the *mgrA* mutant strains lost significantly less body weight than mice infected with their respective isogenic parental and *mgrA*-complemented strains, especially late during the observation period (Figure 5A and 5B). For instance, 5 days after infection, the changes in body weight between mice infected with parental strains and those infected with *mgrA* mutants became significantly different in the MW2 strain set ($P < .05$; Figure 5B). Notably, both *mgrA* mutant strains in the MSSA and MRSA backgrounds exhibited significantly attenuated virulence, with at least 2-log reductions in the *S. aureus* density in all target tissues (ie, kidney, spleen, liver, and lung), compared with their respective parental strains ($P < .05$; Figure 5C and 5D). However, for most target tissues, *S. aureus* densities had partially returned to those of the parental strains when *mgrA* was complemented (Figure 5C and 5D).

DISCUSSION

MgrA, a member of the SarA protein family, is a key global regulator that regulates many virulence genes (eg, *hla*, *spa*, and *cap5*) in *S. aureus* [10, 11]. It has been reported that MgrA affects biofilm formation, autolysis, and virulence in animal models [4, 10, 11, 42, 43]. The current investigation builds on previous work

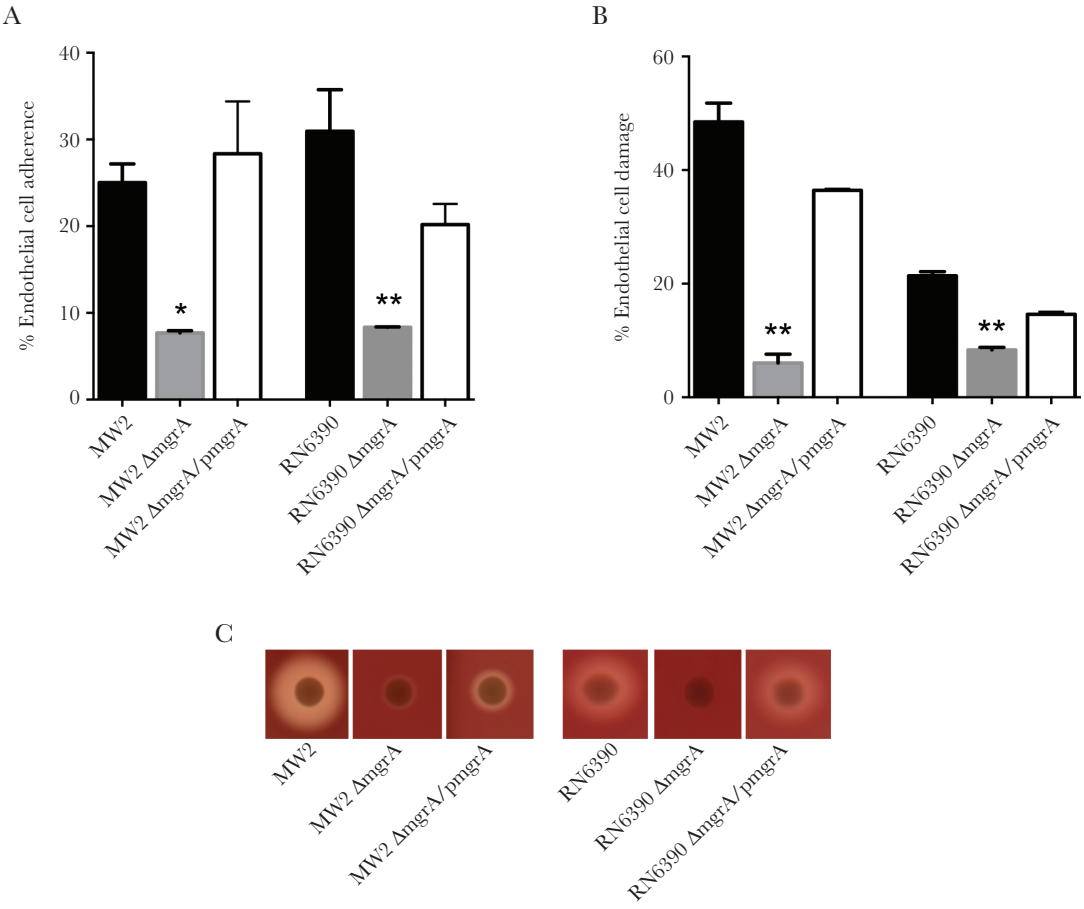

Figure 3. Endothelial cell (EC) adherence (*A*) and damage (*B*) and α-toxin activity (zones of clearance on the sheep blood plate; *C*) among MW2 and RN6390 parental *Staphylococcus aureus* strains and their respective mgrA mutant and *mgrA*-complemented strain sets. $^{*}P < .001$ and $^{**}P < .0001$ vs their respective parental and *mgrA*-complemented strains.

by exploring the mechanisms of virulence mediated by MgrA through host factors. Several key findings, which shed new light on the role of MgrA in the host-pathogen relationship, emerged from these studies.

The ability of *S. aureus* to cause disease is at least in part due to evasion of the host's innate defense response, which includes resistance to HDPs [44]. HDPs, including those of hematogenous origin (eg, neutrophils [for hNP-1] and platelets [for tPMPs]), kill many important blood-borne pathogens, especially *S. aureus* [45]. Reduced susceptibility to HNP-1 and tPMPs in vitro has been shown to correlate with enhanced in vivo virulence in *S. aureus* endovascular infections [26]. In the current study, we showed that *mgrA* mutant strains in both the MSSA and MRSA backgrounds were significantly more susceptible to HDPs than the respective wild-type and *mgrA*-complemented *S. aureus* strains. Therefore, MgrA appears to play an important role in HDP susceptibility, which contributes to *S. aureus* pathogenesis.

Since most HDPs target the bacterial cell membrane to initiate their bactericidal mechanism(s), we hypothesized that MgrA may directly and/or indirectly interact with *S. aureus* cell membrane factors (eg, *mprF* and *dlt*). Peschel et al demonstrated that *mprF*, a lysyl-phosphatidylglycerol synthase, adds positively charged lysine to the negatively charged PG molecule within the staphylococcal cell membrane. This process results in an increased net positive surface charge, thus reducing the binding of HDPs by *S. aureus* [46]. In addition, it has been shown that the *dltABCD* operon also contributes to the net positive surface charge by covalently incorporating D-alanine into cell wall teichoic acids and then promotes resistance to distinct HDPs in *S. aureus* [47]. In the current study, we tested the effect of *mgrA* on *mprF* and *dltA* expression with or without hNP-1 exposure and demonstrated that *mgrA* positively regulated *mprF* and *dltA* transcription in both the MSSA and MRSA strain backgrounds. Importantly, hNP-1 exposure significantly induced *mprF* and *dltA* expression in the parental and *mgrA*-complemented strains but not in the *mgrA* mutant strains. These data indicate that *mgrA* is involved in HDP susceptibility at least in part through its regulation of *mprF* and *dltA* expression.

In addition, *S. aureus* pathogenicity is closely related to its capacity to bind to extracellular matrix (eg, Fn) and host

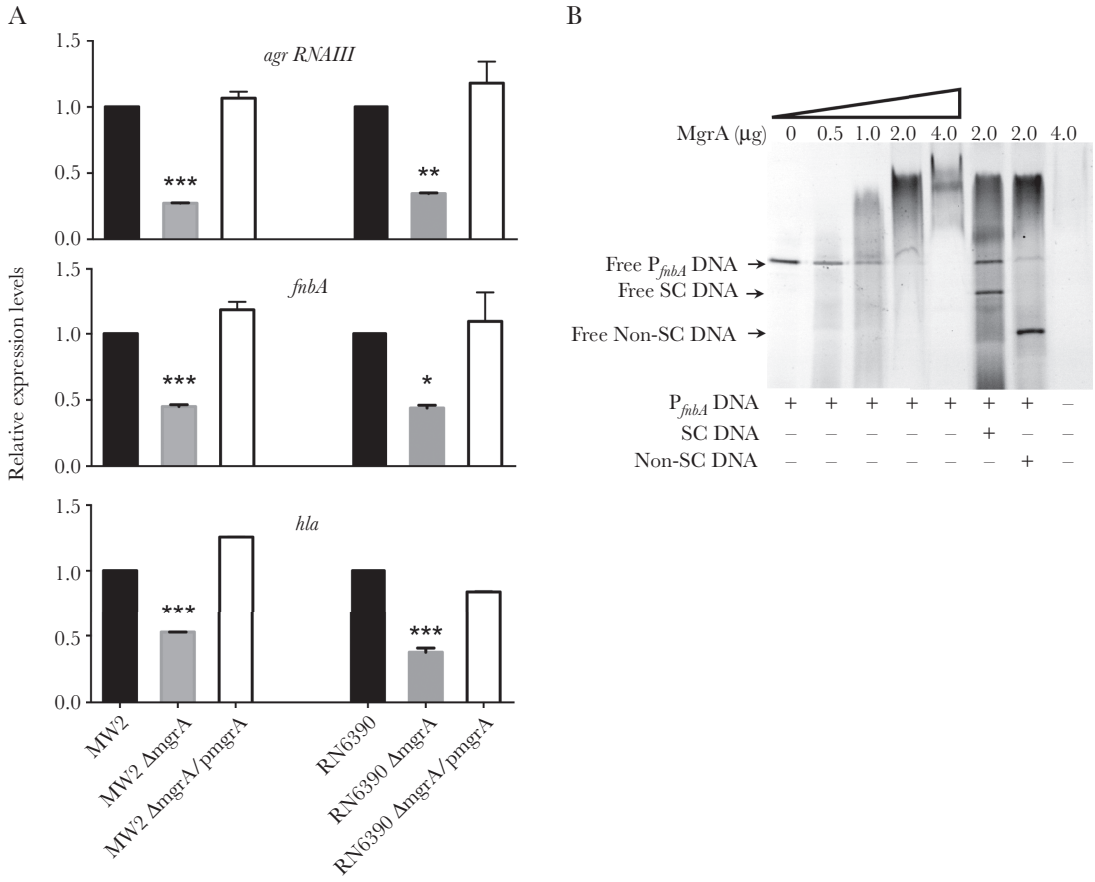

Figure 4. *A*, Relative expression of *agr RNAIII*, *fnbA*, and *hla* in MW2 and RN6390 parental *Staphylococcus aureus* strains and their respective *mgrA* mutant and *mgrA*-complemented strain sets. Expression levels are relative to that of the parental strain, which is arbitrarily set at 1. **P* < .05, ***P* < .001, and ****P* < .0001 vs their respective parental and *mgrA*-complemented strains. *B*, Binding of purified MgrA with the 287-bp *fnbA* promoter DNA (P_{fnbA}), as determined by gel shift assay. The first lane represents the *fnbA* promoter DNA alone. Increasing amounts of purified MgrA (0.5, 1.0, 2.0, and 4.0 μg) were applied. The last lane represents purified MgrA alone (4.0 μg). In competition assays, MgrA was incubated with a presence of 5-fold excess of specific competitor (SC; 235-bp *hla* promoter DNA) or nonspecific competitor (non-SC; 162 bp from within the *fnbA* coding sequence).

cells (eg, ECs) [6]. In the present study, we observed that significantly fewer *mgrA* mutant organisms adhered to Fn than their respective isogenic wild-type and *mgrA*-complemented strains regardless of MRSA or MSSA backgrounds. It is known that host cell adhesion mainly involves Fn forming a bridge between α5β1 integrin on the cellular side and FnBPs on the bacteria [6, 48]. We therefore assume that *mgrA* may also influence EC adherence. Indeed, significantly decreased EC adherence profiles were found in *mgrA* mutants, which would theoretically provide a disadvantage for *S. aureus* pathogenesis. As a component of the FnBP-Fn-α5β1 pathway, FnBPs, especially FnBPA, are essential factors for Fn binding and Fn-mediated EC adherence [6]. Thus, we tested the expression of *fnbA* (encoding FnBPA). Consistent with the Fn and EC binding results, our results revealed that expression of *fnbA* in *mgrA* mutant strains was significantly less than that in parental and *mgrA*-complemented strains, indicating reduced Fn binding ability, and that subsequently weaker EC adherence might be due to the decreased *fnbA* expression. However, the observations that *mgrA* upregulates *agr RNAIII* [11] and *agr RNAIII* downregulates *fnbA* [19] are in contrast to our observations showing that expression of *fnbA* was decreased in *mgrA* mutant strains. Thus, we hypothesize that, as a member of the SarA global regulatory protein family, MgrA may bind to target promoter DNA in a similar fashion [49], The regulation of *fnbA* expression by direct minding of MgrA to the *fnbA* promoter can thus occur independent of *agr* [34]. We performed a gel shift assay and found that MgrA bound to the *fnbA* promoter fragment in a dose-dependent fashion. These data indicate that MgrA could activate *fnbA* transcription by directly binding to its promoter. To our knowledge, this is the first study to show that *mgrA* upregulates *fnbA* expression by directly binding to the *fnbA* promoter.

In support of the role of MgrA in *S. aureus* virulence that was observed in other studies [10, 42], deletion of *mgrA* led to decreased virulence in our experimental murine bacteremia model, with significantly less animal weight loss and

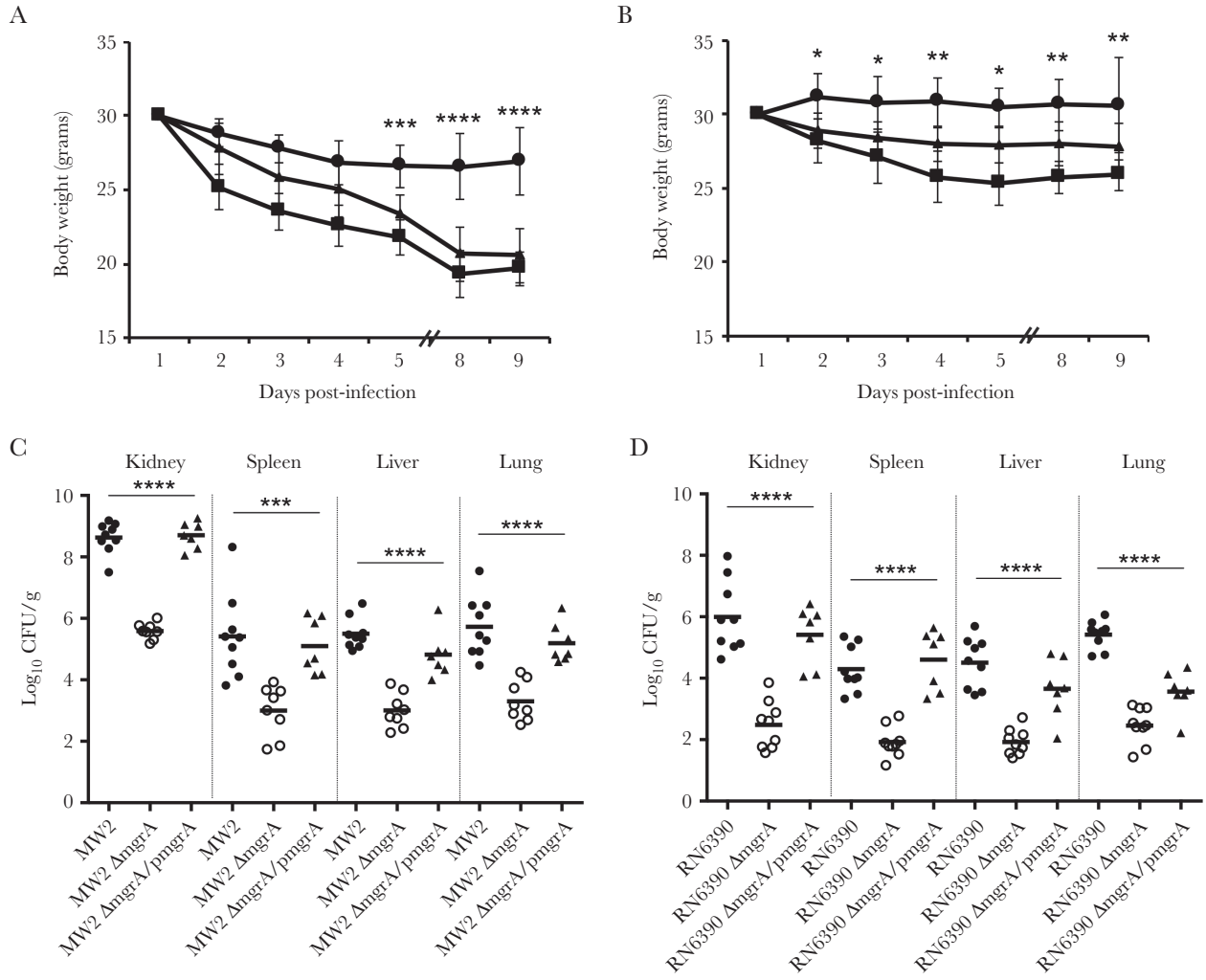

Figure 5. *A* and *B*, Body weight changes among mice after infection of parental (squares), *mgrA* mutant (solid circles), and *mgrA*-complemented (triangles) *Staphylococcus aureus* strains (*A*, MW2 strain set; *B*, RN6390 strain set). *C* and *D*, *S. aureus* densities in target tissues in the mouse bacteremia model (*C*, MW2 strain set; *D*, RN6390 strain set). Each dot represents 1 animal. Horizontal black bars indicate mean S. *aureus* densities. **P* < .05, ***P* < .01, ****P* < .001, and **** *P* < .0001 vs their respective parental and *mgrA*-complemented strains.

decreased *S. aureus* densities in target tissues, compared with respective parental and *mgrA*-complemented strains, regardless of MSSA or MRSA background. In agreement with our results, other investigations have shown that mutation in *mgrA* attenuated *S. aureus* virulence in endocarditis and bacteremia models caused by other *S. aureus* strain backgrounds [10, 42]. The MgrA-mediated reduced virulence in *S. aureus* might be related to *S. aureus*–host interactions, including (1) increased susceptibilities to polymorphonuclear leukocytes and platelet-specific HDPs through regulations of genes (eg, *mprF* and *dltA*) involved *S. aureus* membrane surface charges; (2) lower *fnbA* expression, which subsequently leads to less adherence to Fn and ECs; and (3) decreased expression of global regulators (eg, *agr RNAIII*) and virulence factors (eg, *hla*). These combinatorial pathogen-host interactions could potentially influence virulence in *S. aureus*.

In conclusion, our studies provide valuable insights into MgrA-mediated pathogen-host interactions, including their effect on susceptibility to HDPs, Fn and EC binding, α-toxin, and EC damage, that are necessarily involved in the virulence in *S. aureus*. MgrA might be a potential target to optimize anti–*S. aureus* therapeutic strategies.

Notes

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