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The *Staphylococcus aureus* Collagen Adhesin Is a Virulence Determinant in Experimental Septic Arthritis

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The importance of a collagen-binding adhesin in the pathogenesis of septic arthritis has been examined by comparing the virulence of two sets of *Staphylococcus aureus* mutants in an animal model. Collagen adhesin-negative mutant PH100 was constructed by replacing the chromosomal collagen adhesin gene (*cna*) in a clinical strain, Phillips, with an inactivated copy of the gene. Collagen adhesin-positive mutant *S. aureus* CYL574 was generated by introducing the *cna* gene into CYL316, a strain that normally lacks the *cna* gene. Biochemical, immunological, and functional analyses of the generated mutants and their respective parent strains showed that binding of ^{125}I-labeled collagen, expression of an immunoreactive collagen adhesin, and bacterial adherence to cartilage were directly correlated with the presence of a functional *cna* gene. Greater than 70% of the mice injected with the Cna$^+$ strains developed clinical signs of arthritis, whereas less than 27% of the animals injected with Cna$^-$ strains showed symptoms of disease. Furthermore, mice injected with the Cna$^+$ strain Phillips had remarkably elevated levels of immunoglobulin G1 and interleukin-6 compared with mice injected with the Cna$^-$ mutant PH100. Taken together, these results demonstrate that collagen adhesin plays an important role in the pathogenesis of septic arthritis induced by *S. aureus*.

Hematogenously acquired bacterial arthritis remains a serious medical problem. This rapidly progressive and highly destructive joint disease is difficult to eradicate, with less than 50% of the infected patients recovering without serious joint damage. *Staphylococcus aureus* is the most common etiological agent of both bacterial arthritis and acute osteomyelitis, causing up to 80% of the cases (7, 27).

An analysis of *S. aureus* strains isolated from patients diagnosed with osteomyelitis and septic arthritis revealed that almost all of the isolates contained a collagen-binding adhesin (24). In contrast, only one-third of the *S. aureus* strains isolated from patients with soft tissue infections expressed the collagen adhesin (24).

We have previously isolated a collagen-binding protein from the cell surface of *S. aureus* (22) and shown that this protein can act as an adhesin, mediating the attachment of *S. aureus* cells to cartilage (24). Furthermore, cloning and sequencing of the collagen adhesin gene, *cna*, from *S. aureus* FDA 574 has revealed a protein that can occur in at least two forms, with molecular masses of approximately 135 and 110 kDa, in different strains. A recombinant form of the adhesin inhibited the binding of *S. aureus* to soluble type II collagen (22) and the attachment of *S. aureus* cells to cartilage (24).

To determine the importance of the collagen adhesin as a virulence factor in staphylococcus-induced septic arthritis, we have constructed two classes of mutants. In the first class of mutants, the isolated collagen adhesin gene, *cna*, was inactivated in an *S. aureus* clinical isolate obtained from a patient with osteomyelitis. In the second type of mutant, the intact *cna* gene was introduced into an *S. aureus* strain that lacked the gene.

We have compared the virulence of the two classes of *S. aureus* mutants with that of their respective parent strains using a newly developed and characterized murine model of septic arthritis (4, 5). In this model, mice display histopathological signs of arthritis peaking approximately 2 to 3 weeks postinjection, with regard to both intensity and extension of arthritis, and leveling off thereafter. Clinically estimated signs of arthritis correlate closely to the histopathological evaluation (4). Tail lesions with inflammatory cells invading and destroying the disk and the bone tissue occur within 4 weeks after inoculation in approximately 50% of the mice. Furthermore, the arthritic mice often display a tremendous interleukin-6 (IL-6)-driven polyclonal B-cell activation (1, 4).

The results presented show that mice injected intravenously (i.v.) with *S. aureus* strains expressing the collagen adhesin were much more likely to develop arthritis compared with mice injected with the *S. aureus* mutant strains. Moreover, levels of immunoglobulin G1 (IgG1) and IL-6 in serum were dramatically elevated in mice injected with the Cna$^+$ clinical isolate compared with levels in serum in mice injected with the Cna$^-$ mutant or saline. Taken together these data demonstrate that the collagen adhesin is an important virulence factor in septic arthritis induced by staphylococci.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used in this paper: BHI, brain heart infusion; BSA, bovine serum albumin; EY, egg yolk; GM, gentamicin; PBS, phosphate-buffered saline; PBST, PBS containing Tween 20; and SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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TABLE 1. Description of strains and plasmids used in this study

Strain or plasmid	Description or construction	Reference
E. coli		
JBL44	F⁻ *hsd-20* (r_B^- m_B^-) Ara⁻ 14 *lacYI galK2 rpsL20* (Sm^r) *xyl-5 mtl-1 supE44* lambda⁻ *recA13 proA2* (pPQ132)	15
LE392	F⁻ *supE44 supF58 hsdR514* (r_K^- m_K^+) *galK2 galT22 metB1 trpR55 lacYI* (pCL84)	3
JM101	*supE thiΔ(lac-proAB)* (F′ *traD36 proAB⁺ lacI^q* ZΔM15), host for subcloning	Stratagene
S. aureus		
FDA 574	C E *ent⁺*	22
RN4220	r⁻, host for shuttle plasmids and subcloning	11
RN574	r⁻, Gm^r (pBLGM574$_{ts}$)	This study
JBL22	8325-4 r⁻(pPQ126)	15
Phillips	Clinical osteomyelitis isolate	This study
PH100	Collagen adhesin-negative mutant	This study
CYL316	8325-4 r⁻(pYL112Δ19)	13
CYL574	Collagen adhesin-positive mutant	This study
Plasmids		
Bluescript SK(+)	Amp^r, subcloning and propagation	Stratagene
pBLHIND	Bluescript SK(+) with destroyed *Hin*dIII site	This study
pBL574	pBLHIND derivative containing the cloned *cna* gene on an *Eco*RI-*Pst*I fragment	This study
pBLGM574	pBL574 derivative containing a gentamicin resistance determinant	This study
pBLGM574$_{ts}$	pBLGM574 derivative containing an *S. aureus* temperature-sensitive origin of replication	This study
pCL84	*S. aureus* integration vector, Sp^r Tc^r	13
pCL574	pCL84 derivative containing the cloned *cna* gene on an *Eco*RI-*Bam*HI fragment	This study

Bacterial strains. All bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strains were grown at 37°C in Luria Broth (GIBCO BRL, Grand Island, N.Y.) or spread onto Luria broth agar plates. *S. aureus* strains were grown either in BHI broth (Difco Laboratories, Detroit, Mich.) or on BHI agar plates. Bacteria were grown at 37°C unless otherwise noted, and antibiotics were added to media when appropriate. Baird-Parker agar base supplemented with EY tellurite enrichment solution (Difco Laboratories) was used to detect lipase production by *S. aureus* isolates.

Extracellular matrix molecules. Chicken type II collagen isolated from chicken sternum was a gift from Richard Mayne, University of Alabama, Birmingham. Human fibrinogen (Kabi, Stockholm, Sweden) was found to contain a small amount of fibronectin and was subsequently purified by gelatin-Sepharose chromatography (12). Human thrombospondin purified from activated human platelets as previously described (19) was a gift from Joanne Murphy-Ullrich, University of Alabama, Birmingham. The 29-kDa fragment of human fibronectin was generated by thermolysin digestion of intact fibronectin and isolated as described by McGavin and colleagues (18). Collagen, fibrinogen, and the 29-kDa fragment of fibronectin were labeled with ^{125}I by the chloramine-T method (10). Thrombospondin was labeled with ^{125}I by using Enzymobeads (Bio-Rad, Richmond, Calif.). Na^{125}I (specific activity, 15 mCi/μg) was obtained from Amersham Corp., Arlington Heights, Ill. The estimated specific activities of the radiolabeled ligands ranged from 2×10^6 to 5×10^7 cpm/μg of protein.

Isolation of the collagen adhesin gene. Genomic DNA was isolated from *S. aureus* cells as previously described (16). Different forms of the *cna* gene were generated by using PCR and genomic DNA from *S. aureus* FDA 574 as the template. Oligonucleotides (Oligo's Etc., Guilford, Conn.) CNA-I (5′ CCGAATTCGCACTTGTATTCGTTATAC 3′) and CNA-II (5′ ATTCTGCAGAGAACTAAGAATAGCCTT 3′) were used to amplify the entire *cna* gene as previously described (22, 24). These primers have *Eco*RI and *Pst*I restriction cleavage sites (underlined) incorporated into their sequences and flank the intact *cna* gene at the 5′ and 3′ ends, respectively. Primers CNA-III (5′ CCGGGATCCCAAGCTTGG TATCAAGAG 3′) and CNA-IV (5′ GCGGATTCTTACT TATCAAATGTATACGG 3′) were used to amplify a 333-bp fragment from within the *cna* gene which was used in the hybridization studies. After amplification, 15 μl of the PCR mixture was analyzed on a 1% agarose gel (SeaKem GTG; FMC BioProducts, Rockland, Maine). The amplified DNA was purified from agarose gels by using Elu-Quik (Schleicher & Schuell, Keene, N.H.) following the manufacturers' directions.

Construction of an *E. coli-S. aureus* allele replacement shuttle vector. The ampicillin-resistant plasmid Bluescript SK(+) was first digested with *Hin*dIII. Following complete digestion, the *Hin*dIII site was filled in by the addition of the four deoxynucleoside triphosphates and DNA polymerase I Klenow fragment, thereby destroying the only *Hin*dIII site within this vector. The resulting plasmid, designated pBLHIND, was linearized by digestion with *Eco*RI and *Pst*I restriction enzymes. Following PCR with primers CNA I and CNA II, as described above, the gel-purified 3.6-kb *cna* gene was digested with *Eco*RI and *Pst*I and ligated to pBLHIND to create plasmid pBL574, which was used to transform *E. coli* JM101. Positive transformants were screened by restriction digest analysis.

To inactivate the cloned *cna* gene, a 2.5-kb gentamicin resistance determinant was isolated from plasmid pPQ132 in *E. coli* JBL44 (15) by *Hin*dIII restriction digestion. Following digestion and gel purification, the 2.5-kb gentamicin resistance marker was ligated to a unique *Hin*dIII site located within the *cna* gene present on plasmid pBL574. The new plasmid, designated pBLGM574, was then transformed into *E. coli* JM101.

To create the complete shuttle vector pBLGM574$_{ts}$, the *S. aureus* temperature-sensitive origin of replication derived from plasmid pPQ126 was isolated from *S. aureus* JBL22, which had been grown at a permissive temperature (30°C). A 2.3-kb *Hpa*I restriction fragment containing the origin of replication was gel purified and then blunt end ligated to a *Sma*I site in plasmid pBLGM574. Plasmid pBLGM574$_{ts}$ was

then transformed into *E. coli* JM101, and positive transformants were identified by restriction enzyme mapping.

Isolation of collagen adhesin-negative mutants. Shuttle vector pBLGM574$_{ts}$, containing the inactivated *cna* gene, was introduced into *S. aureus* RN4220 (11) by electroporation (Electro Cell Manipulator 600; Biotechnologies & Experimental Research Inc., San Diego, Calif.) as described by Augustin and Götz (2). The transformation mixture was plated onto BHI agar plates containing GM (10 μg/ml). The plates were incubated at 30°C for 48 h. Minipreparation plasmid DNA isolated from Gm^r *S. aureus* RN574 grown at 30°C was used to electroporate the osteomyelitis *S. aureus* isolate strain Phillips. The bacterial mixture was plated onto BHI agar plates containing GM (10 μg/ml) and incubated at 30°C overnight. *S. aureus* Gm^r transformants were then inoculated into BHI broth (GM, 10 μg/ml) and grown overnight at 42°C. Overnight cultures were diluted to 10^{-4}, replated onto solid media (BHI with GM at 10 μg/ml), and grown overnight at 42°C. The passage of cells from liquid to solid media was repeated for three successive cycles. Potential collagen adhesin-negative mutants were tested for collagen-binding activity (see below). An isolated mutant, PH100, was further characterized.

Construction and isolation of collagen adhesin-positive mutants. Oligonucleotides CNA-I and CNA-II were used in PCR to amplify the *cna* gene from *S. aureus* FDA 574. The amplified *cna* gene was digested with *Eco*RI and *Pst*I, gel purified, ligated to *Eco*RI-*Pst*I-digested Bluescript SK(+), and transformed into *E. coli* JM101. DNA from the transformants was isolated, purified, and subjected to *Eco*RI and *Bam*HI restriction digestion. The entire 3.6-kb *cna* gene was subsequently recleaved from the plasmid and gel purified.

Plasmid pCL84 was isolated from *E. coli* LE392, digested with *Eco*RI and *Bam*HI, purified, and ligated to the previously isolated *cna* gene. The ligation mixture was used to transform *E. coli* JM101. DNA isolated from the transformants was screened by restriction digest analysis. One transformant harboring the proper plasmid construct, designated pCL574, was purified and used to electroporate *S. aureus* CYL316. After electroporation, transformants were selected by plating on BHI agar plates containing tetracycline (3 μg/ml). Tetracycline-resistant transformants were analyzed by restriction digestion. Lipase activity was detected by growth on Baird-Parker media containing EY tellurite supplement. One positive isogenic isolate, designated CYL574, was further characterized.

Southern blot analysis. Genomic DNA was isolated and subjected to restriction enzyme digestion, fractionated by agarose gel electrophoresis, and transferred to a nylon membrane (MSI, Westboro, Mass.). Gel-purified DNA probes were labeled by random-primer labeling (Promega) with 50 μCi of [α-^{32}P]dCTP (Amersham). Unincorporated α-^{32}P was removed by passage through a NAP-5 column (Pharmacia).

Extracellular matrix protein binding assay. An in vitro binding assay was used to test staphylococcal strains for their ability to bind soluble ^{125}I-labeled extracellular matrix proteins. The amount of ^{125}I-labeled protein bound to bacteria was quantified essentially as described previously (25). All assays were done in duplicate.

SDS-PAGE and Western blot analysis. Lysostaphin cell surface extracts were prepared from the *S. aureus* strains and subjected to SDS-PAGE and Western blot (immunoblot) analysis as described earlier (22, 24, 25).

Biotin labeling of *S. aureus* cells and cartilage attachment assays. *S. aureus* strains were cultured under constant rotation for 15 h at 37°C in BHI broth. Bacteria were harvested by centrifugation, suspended in a bicarbonate buffer (50 mM $NaHCO_3$, pH 8.5), and heated to 88°C for 20 min to kill bacteria and inactivate hydrolyases. Bacteria were collected by centrifugation and resuspended in a 100 mM borate buffer, pH 8.2, to a final density of 10^{10} cells per ml, determined by comparing the A_{600} of the sample with a previously prepared standard curve relating A_{600} to cells counted in a Petroff Hausser chamber. Bacterial cells were labeled with biotin by the addition of 100 μl of 1% *N*-hydroxysuccinimidobiotin (Pierce, Rockford, Ill.) in *N*,*N*-dimethylformamide per ml of bacterial suspension. The staphylococcal cells were incubated with the *N*-hydroxysuccinimido-biotin for 2 h at room temperature with constant rotation. The biotinylation reaction was stopped by removing the supernatant after centrifugation of the bacterial cells. The bacteria were then washed three times in PBS to remove unincorporated biotin. The bacterial cell suspensions were then adjusted to 10^{10} cells per ml, and the cells were stored at 4°C in PBS, pH 7.4.

A modification of the attachment assay as described previously (24) was used. Briefly, uniform disks of bovine nasal cartilage were produced by piercing a block of cartilage with a 4-mm-diameter cork borer, removing the cylindrical cartilage sample, and cutting it crosswise with a scalpel blade into 1-mm-thick disks. Four disks of cartilage were incubated with 10^8 biotin-labeled staphylococcal cells in a total volume of 1 ml of PBST containing 0.1% BSA, with constant rotation at room temperature for 4 h. Each cartilage disk was washed extensively with PBST to remove unbound bacteria. Streptavidin conjugated with alkaline phosphatase (Boehringer Manheim, Indianapolis, Ind.) diluted 1:5,000 in PBS was added to the cartilage disks and incubated at room temperature for 30 min. The disks were again washed in PBST, and individual cartilage disks were placed in separate wells of a 96-well plate and covered with 100 μl of development solution for 30 min. The development solution was then transferred to a new plate, and the A_{405} was monitored in a microplate reader (Molecular Devices Corp., Menlo Park, Calif.).

Experimental septic arthritis. Experimental parameters for the mouse model of staphylococcus-induced septic arthritis have been described previously (4, 5). Mice were injected i.v., in the tail vein, with 0.2 ml of bacterial solution corresponding to 2×10^7 CFU per mouse.

***S. aureus* Phillips and isogenic mutant PH100.** Two separate experiments were conducted to evaluate the biological role of the collagen adhesin in vivo. For both experiments, all mice were visually examined for clinical arthritis and blood samples were also obtained and stored at −20°C until analysis. All sera were tested individually.

Histopathological analysis. In the first experiment, 10 mice were inoculated i.v. with 2×10^7 cells of the wild-type *S. aureus* strain Phillips and 10 mice were inoculated i.v. with 2×10^7 cells of the isogenic mutant *S. aureus* PH100. In addition to visual examination, after the sacrifice, the forepaws, the hind paws, and part of the tail of the 20 mice were immersed in 4% formalin for histopathological examination.

Bacteriological analysis. In the second experiment, 12 mice were inoculated i.v. with 2×10^7 cells of the wild-type *S. aureus* strain Phillips and another 12 mice were inoculated with the corresponding dose of the isogenic mutant *S. aureus* PH100, while 7 mice were injected i.v. with physiological saline. Two of the mice injected with strain Phillips died during the course of the experiment. The mice were weighed on four occasions during the bacteriological experiment. At

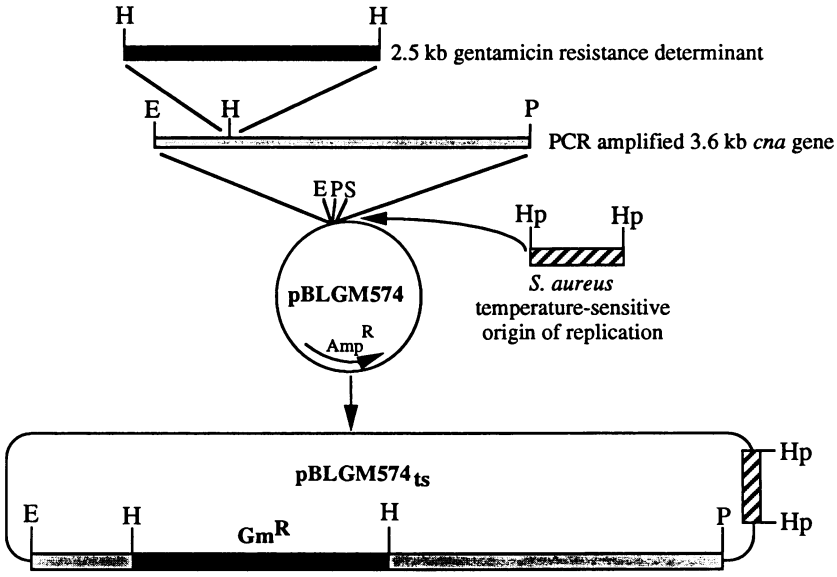

FIG. 1. Construction of the *E. coli-S. aureus* temperature-sensitive shuttle vector pBLGM574$_{ts}$ used for the allele relacement experiment. This plasmid contains the PCR-amplified *cna* gene from *S. aureus* FDA 574 insertionally inactivated by a gentamicin resistance determinant (Gm^R). The restriction cleavage sites *Hin*dIII (H), *Eco*RI (E), *Pst*I (P), *Sma*I (S), and *Hpa*I (Hp) are indicated. For details, see Materials and Methods.

sacrifice 21 days later, the talocrural joints, radiocarpal joints, the tail, the kidney, and the spleen were asceptically dissected. Bacteria were collected with charcoaled sticks, transferred to agar plates containing 5% human blood, and incubated for 48 h at 37°C. Bacterial colonies were tested for catalase and coagulase activities.

***S. aureus* CYL316 and collagen adhesin-positive mutant CYL574.** Fifteen mice were inoculated i.v. with 2×10^7 cells of the parent strain *S. aureus* CYL316, and 17 mice were inoculated i.v. with 2×10^7 cells of the mutant *S. aureus* CYL574. The mice were visually evaluated for clinical arthritis during a 21-day period and then sacrificed.

RESULTS

Construction and isolation of collagen adhesin mutants. An *S. aureus* collagen adhesin-negative mutant was constructed by an allele replacement strategy based on the findings of O'Reilly and coworkers (20). The *cna* gene from *S. aureus* FDA 574 encoding the cell surface collagen adhesin protein was amplified by PCR and inactivated by inserting a gentamicin resistance determinant within the coding sequence of the *cna* structural gene as described in Materials and Methods. Plasmid pBLGM574, containing the interrupted *cna* gene, was ligated to a DNA fragment containing an *S. aureus* temperature-sensitive origin of replication to create pBLGM574$_{ts}$, as schematically diagrammed in Fig. 1. Plasmid pBLGM574$_{ts}$ was subsequently introduced into *S. aureus* Phillips, a strain isolated from a patient with osteomyelitis. Initially, the transformed cells were grown at 30°C to expand the culture of plasmid-containing cells. The temperature was then shifted to 42°C to promote chromosomal incorporation of the recombinant plasmid and to allow allele replacement of the intact chromosomal copy of the *cna* gene with the *cna*::Gm mutation. One such mutant, PH100, first identified by its resistance to gentamicin, was isolated and further characterized.

A collagen adhesin-positive mutant was constructed through incorporation of a single copy of the *cna* gene into the chromosome of a *cna*-negative *S. aureus* strain. This was accomplished by cloning the PCR-amplified *cna* gene from *S. aureus* FDA 574 into the single-copy integration vector pCL84 to create plasmid pCL574, depicted in Fig. 2. This vector integrates into the *S. aureus* chromosome at the L54a *attB* site by site-specific recombination (13). The *attB* site is close to the 3′ end of the structural gene for a lipase (*geh*) (21), and integration into this site causes a loss of lipase production (14).

Parent strain CYL316 had detectable lipase activity, as evidenced by the halo of precipitated calcium salts of hydrolzed fatty acids surrounding the isolated colonies when plated and grown on egg yolk-containing medium (see Fig. 4B). Conversely, mutant strain CYL574 appears to have lost lipase activity, as is evident when these cells were plated on the same medium (see Fig. 4B). This strain was further characterized.

Southern hybridization analysis of the collagen adhesin mutants. Genomic DNA from parent strain Phillips and its isogenic derivative PH100 were digested with *Eco*RI and *Pst*I and probed with a random-primed PCR-amplified fragment of the *cna* gene. A single 5.5-kb band in Phillips DNA hybridized to the 333-bp *cna* probe (Fig. 3A, lane 1), whereas the probe hybridized to an approximately 6.1-kb band in the DNA from the putative collagen adhesin-negative mutant PH100 (Fig. 3A, lane 2). A 6.1-kb *Eco*RI-*Pst*I band is predicted on the basis of the restriction fragment pattern of the original plasmid pBLGM574$_{ts}$, which was used for allele replacement (Fig. 1). If a single crossover event had occurred, one would expect two hybridizing fragments to be detected in Fig. 3A, lane 2, one from the original copy of the *cna* gene and one from the interrupted version of the *cna* gene. During the isolation of the isogenic mutants, several clones that did undergo a single crossover event were isolated, but they were not studied further. In Fig. 3B, the genomic DNA, digested with either *Eco*RI-*Pst*I, *Hin*dIII, or *Bam*HI, was probed with the 2.5-kb *Hin*dIII gentamicin

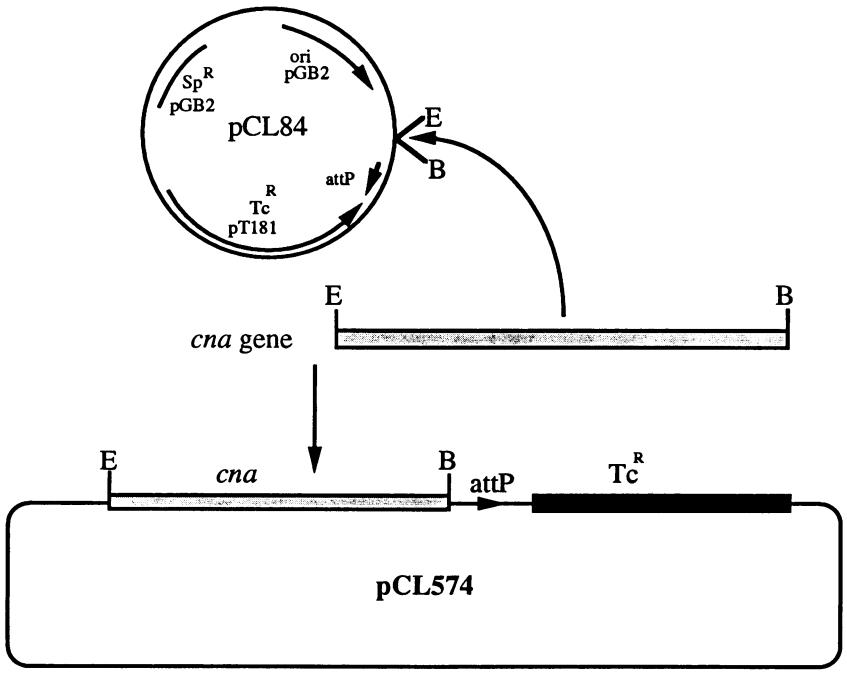

FIG. 2. Construction of integration vector pCL574. A cloned copy of the *cna* gene was digested with *Eco*RI and *Bam*HI and ligated to integration vector pCL84 (13) to create pCL574. Collagen adhesin-negative strain *S. aureus* CYL316 was then transformed with pCL574 to create *S. aureus* CYL574. The restriction cleavage sites *Eco*RI (E) and *Bam*HI (B) and antibiotic resistance markers for spectinomycin (Sp^R) and tetracycline (Tc^R) are indicated. For details, see Materials and Methods.

resistance marker used to inactivate the *cna* gene. The Gm^r determinant hybridized to a single band in all lanes containing DNA isolated from PH100 (Fig. 3B, lanes 2, 4, and 7), while no hybridization was detected with DNA isolated from parent strain Phillips (Fig. 3B, lanes 1, 3, and 6). These data agree with the Cna^- Gm^r phenotype exhibited by PH100 and also the Cna^+ Gm^s phenotype of parent strain Phillips.

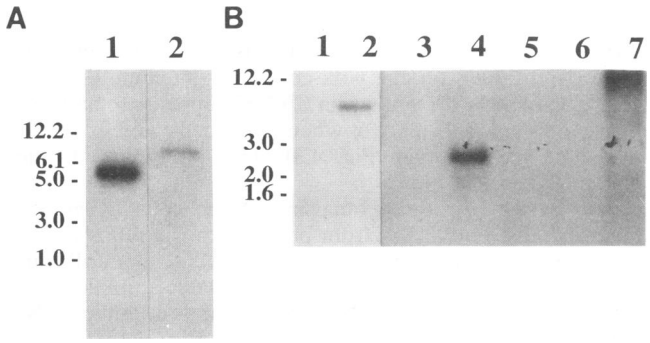

FIG. 3. Southern hybridization analysis of the allele replacement mutant PH100 and the parent strain Phillips. Chromosomal DNA isolated from parent *S. aureus* strain Phillips and isogenic mutant PH100 was digested with restriction enzymes and subjected to agarose gel electrophoresis. The DNAs were transferred to a nylon membrane and hybridized to probes random primer labeled with [α-^{32}P]dCTP. (A) A PCR-amplified 333-bp fragment from within the *cna* gene was used to probe the cleaved DNA. Lane 1, *Eco*RI- and *Pst*I-digested DNA from Phillips; lane 2, *Eco*RI- and *Pst*I-digested DNA from PH100. (B) The 2.5-kb gentamicin resistance determinant was used to probe DNA from *S. aureus* Phillips (lanes 1, 3, and 6) and allele replacement mutant PH100 (lanes 2, 4, and 7). The restriction endonucleases used to digest these DNAs were *Eco*RI and *Pst*I (lanes 1 and 2), *Hin*dIII (lanes 3 and 4), and *Bam*HI (lanes 6 and 7). Lane 5 contains no DNA. The numbers on the left indicate the sizes (in kilobase pairs) of nucleic acid standards.

To genetically analyze the putative *S. aureus* collagen adhesin-positive mutant, genomic DNA was prepared from parent strain CYL316 and the isolated mutant CYL574. The DNA was cleaved with *Eco*RI and *Bam*HI and probed with the same 333-bp *cna* fragment described above. A 3.6-kb band from CYL574 hybridized to the probe (Fig. 4A, lane 3), indicating that the *cna* gene had been incorporated into the chromosome, whereas no hybridization was detected with restriction fragments generated from parent strain DNA (Fig. 4A, lane 2). *Eco*RI- and *Bam*HI-digested plasmid pCL574, which was used to electroporate the parent strain, yielded a 3.6-kb fragment corresponding to the *cna* gene that also hybridized to the probe, as shown in Fig. 4A, lane 1.

Expression of the collagen adhesin protein. The presence of the collagen adhesin in lysostaphin extracts of staphylococci was analyzed by SDS-PAGE and immunoblotting. A Coomassie-stained protein corresponding to the expected molecular mass of the collagen adhesin (≈110 kDa) could be detected in the extract from *S. aureus* Phillips (Fig. 5, lane 1), whereas the same protein was absent in the extract from mutant PH100 (lane 2). When lysostaphin extracts from strain CYL316 (Fig. 5, lane 3) and its mutant derivative CYL574 (lane 4) were analyzed, no proteins with the correct predicted molecular weight could be detected. Presumably the expression level of the collagen adhesin in this strain was too low to be detected by Coomassie brilliant blue staining. The same samples were then subjected to Western blotting and probed with monospecific anti-collagen adhesin Fab fragments. A single immunoreactive band in the lysostaphin extracts from *S. aureus* Phillips (Fig. 5, lane 5) and the collagen adhesin-positive mutant CYL574 (lane 8) were

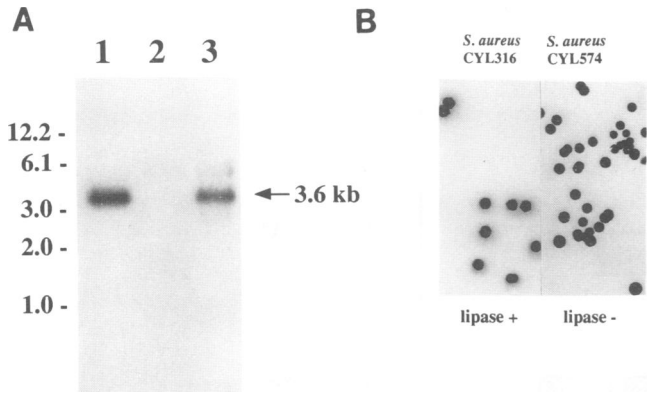

FIG. 4. Southern hybridization analysis of collagen adhesin-positive mutant CYL574 and the parent CYL316. (A) Plasmid pCL574 (lane 1) and chromosomal DNA isolated from parent strain CYL316 (lane 2) and mutant CYL574 (lane 3) were digested with *Eco*RI and *Bam*HI, fractionated on a 1% agarose gel, and transferred to a nylon membrane. The membrane was hybridized with the 333-bp fragment as described in the legend to Fig. 3. The numbers on the left indicate the sizes (in kilobase pairs) of nucleic acid standards. The intact *cna* gene is indicated by the arrow on the right. (B) Parent strain *S. aureus* CYL316 and mutant CYL574 were plated onto Baird-Parker medium containing EY tellurite supplement. Lipase activity was detected by the presence of a halo of precipitated salts surrounding CYL316 (lipase +). The collagen adhesin-positive mutant CYL574 did not possess lipase activity (lipase −).

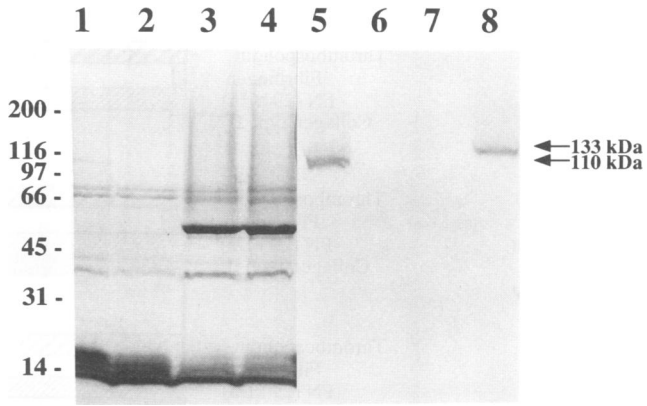

FIG. 5. Analysis of *cna* gene expression in *S. aureus*. Cell surface proteins solubilized by digestion of the *S. aureus* strains with lysostaphin were fractionated by SDS-PAGE on a 5 to 15% acrylamide gel and stained with Coomassie brilliant blue R250. Lane 1, strain Phillips; lane 2, mutant PH100; lane 3, strain CYL316; lane 4, mutant CYL574. Following SDS-PAGE, proteins were transferred to a nitrocellulose membrane and probed with Fab fragments prepared from a monospecific antibody against the collagen adhesin purified from *S. aureus* Cowan. Lane 5, strain Phillips; lane 6, mutant PH100; lane 7, strain CYL316; lane 8, mutant CYL574. The membranes were washed and incubated overnight at 4°C in Tris-buffered saline with rabbit anti-collagen adhesin Fab fragments. The membranes were again washed and incubated with alkaline phosphatase-conjugated goat anti-rabbit $F(ab')_2$ fragments (Tago, Inc., Burlingame, Calif.). The immunoreactive proteins were visualized by incubation of the membrane in an alkaline phosphatase color development solution containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate according to the manufacturer's directions (Promega, Madison, Wis.). Migration distances and molecular masses (in kilodaltons) of the standard proteins are indicated by the numbers on the left. The predicted size and migration distance of the full-length collagen adhesin from Phillips (110 kDa) and CYL574 (133 kDa), respectively, are indicated by the arrows on the right.

detected. However, immunoreactive bands were not detected in the extracts from the allele replacement mutant PH100 (Fig. 5, lane 6) or the collagen adhesin-negative parent strain CYL316 (lane 7). Furthermore, the molecular masses of the immunoreactive collagen adhesins, 110 and 133 kDa, from Phillips (3.0-kb *cna* gene) and CYL574 (3.6-kb *cna* gene from FDA 574), respectively, correlated with the sizes of the genes from which they were expressed (24).

Binding of extracellular matrix proteins to *S. aureus*. The binding of a variety of soluble ^{125}I-labeled extracellular matrix proteins to the *S. aureus* isolates was quantitated in an in vitro binding assay. Under the experimental conditions of this study, 10^8 cells of strain Phillips bound 62% of the type II collagen added (Fig. 6). Additionally, Phillips was shown to bind various amounts of ^{125}I-labeled fibronectin, fibrinogen, and thrombospondin (Fig. 6). Conversely, the allele replacement mutant PH100 bound only 0.3% of the added type II collagen, while the relative amount of binding to ^{125}I-labeled fibronectin, fibrinogen, and thrombospondin remained largely unchanged.

The same assay was conducted with *S. aureus* CYL574 and CYL316. In this case, one would expect to detect collagen-binding activity by the mutant CYL574 and not by parent strain CYL316. As shown in Fig. 6, the mutant bound approximately 10% of the ^{125}I-labeled type II collagen added, whereas the parent strain bound only 0.4%. The overall amount of collagen bound by CYL574 is somewhat smaller than that seen by Phillips (Fig. 6). The integration of the *cna* gene in the chromosome of CYL574 at a site different from its normal location may affect the level of transcription. Additionally, the regulatory factors may differ from one strain to another, and these may affect the expression of the *cna* gene. In fact, the amount of collagen bound by different Cna^+ strains may vary considerably (6, 9, 24). Thrombospondin binding, fibrinogen binding, and fibronectin binding to *S. aureus* CYL316 and CYL574 were at very similar levels and are comparable to those seen with *S. aureus* Phillips and PH100 (Fig. 6).

Taken together, these binding data indicate that the collagen adhesin on the cell surface of PH100 has been inactivated by the *cna*::Gm mutation and that a functional collagen adhesin is expressed on the surface of CYL574 cells. Furthermore, the mutation appears to be site specific in that the functional activities of other cell surface proteins expressed by the parent strain were essentially unaffected.

***S. aureus* attachment to cartilage.** Prior studies have indicated that expression of a collagen adhesin is required and sufficient for *S. aureus* cells to attach to cartilage (24). The ability of the two parent strains and the adhesin mutants to attach to cartilage was therefore examined. An in vitro attachment assay in which bacterial cells, surface labeled with biotin, were allowed to adhere to slices of bovine nasal cartilage was developed.

During a 4-h incubation, collagen adhesin-positive strains Phillips and CYL574 attached to the cartilage disks at levels 24- and 5-fold greater than that of collagen adhesin-negative strains PH100 and CYL316, respectively (Fig. 7).

Virulence of *S. aureus* Phillips and isogenic mutant PH100 in an animal model of septic arthritis. Two separate experiments were conducted to evaluate the capacity of *S. aureus* Phillips and its isogenic derivative PH100 to induce septic arthritis in mice.

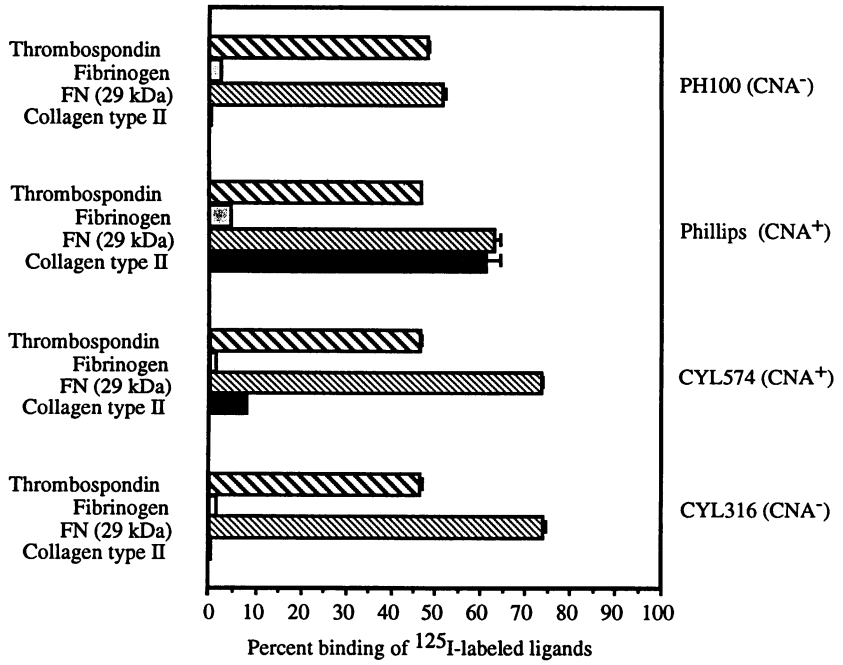

FIG. 6. Binding of various extracellular matrix proteins by different *S. aureus* strains. The different *S. aureus* strains indicated were incubated with ^{125}I-collagen type II (4×10^4 cpm), ^{125}I-thrombospondin (6×10^4 cpm), ^{125}I-fibrinogen (5×10^4 cpm), or the 29-kDa fragment of fibronectin (4×10^4 cpm) (FN) for 1 h at room temperature. Bacterial binding of the various radiolabeled ligands was quantitated as previously described (25).

(i) Histopathology. When the joints of the mice in the first experiment were analyzed for histopathological signs indicative of septic arthritis (Fig. 8A), all of the 10 mice inoculated with *S. aureus* Phillips displayed arthritis; of these, 8 were classified as having erosive arthritis, i.e., they exhibited destruction of joint cartilage and/or underlying bone (Table 2). In contrast, only 5 of the 10 mice injected with the isogenic mutant showed histopathological signs of arthritis; of these, 2 were classified as having erosive arthritis (Table 2). A representative micrograph of a grossly unaffected knee joint of a mouse that was inoculated with *S. aureus* PH100 is presented in Fig. 8B. Extra-articular manifestations of *S. aureus* infection such as osteitis and granulomas in the bone marrow adjacent to the joints occurred in eight of the mice inoculated with *S. aureus* Phillips, compared with one of the mice inoculated with *S. aureus* PH100 (data not shown). However, distant osteitic lesions occurred in three mice from each group, irrespective of the infecting organism (Table 2).

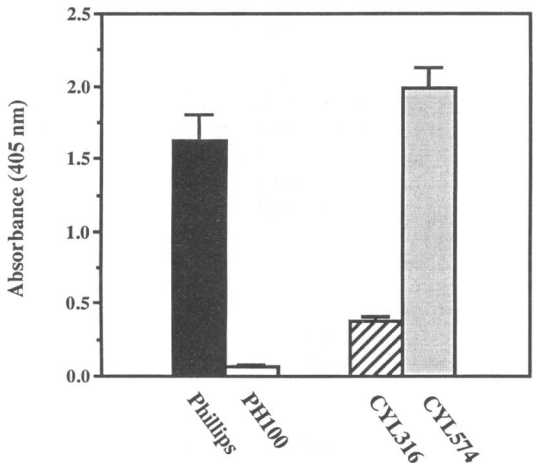

FIG. 7. Attachment of *S. aureus* cells to cartilage. Biotin-labeled cells of the different indicated *S. aureus* strains were allowed to attach to cartilage disks for 4 h. The unbound bacteria were removed by washing the cartilage disks in PBST. The remaining attached bacteria were detected with a streptavidin-alkaline phosphatase conjugate. The color A_{405} was monitored. For details, see Materials and Methods.

(ii) Bacteriology. In the second experiment, two groups of 12 mice each were injected with Phillips and PH100 and 7 mice were injected with saline. The body weights of the mice were monitored. Mice injected with *S. aureus* Phillips displayed a significantly smaller weight gain over the course of the experiment compared with the mice injected with the isogenic mutant PH100 (Table 3). Two of the mice injected with *S. aureus* Phillips died within the first week, but none of the mice inoculated with *S. aureus* PH100 died. In 8 of 10 mice challenged with *S. aureus* Phillips, significant numbers of viable *S. aureus* cells were isolated from the joints. One of these isolates was phage typed and confirmed to be identical to the injected bacterial strain. Two mice also displayed growth of *S. aureus* Phillips in the kidney. In the mice injected with the isogenic mutant PH100, we could not isolate any viable *S. aureus* cells from the joints, kidney, or spleen. *S. aureus* CYL574 was recovered from the mice and subsequently tested for its ability to bind collagen. There was no significant difference in collagen binding between the passaged isolate and the nonpassaged isolate.

Clinical arthritis. In the complete study, 14 of the mice injected with *S. aureus* Phillips showed clinical symptoms of arthritis. In contrast, injection of the isogenic mutant PH100

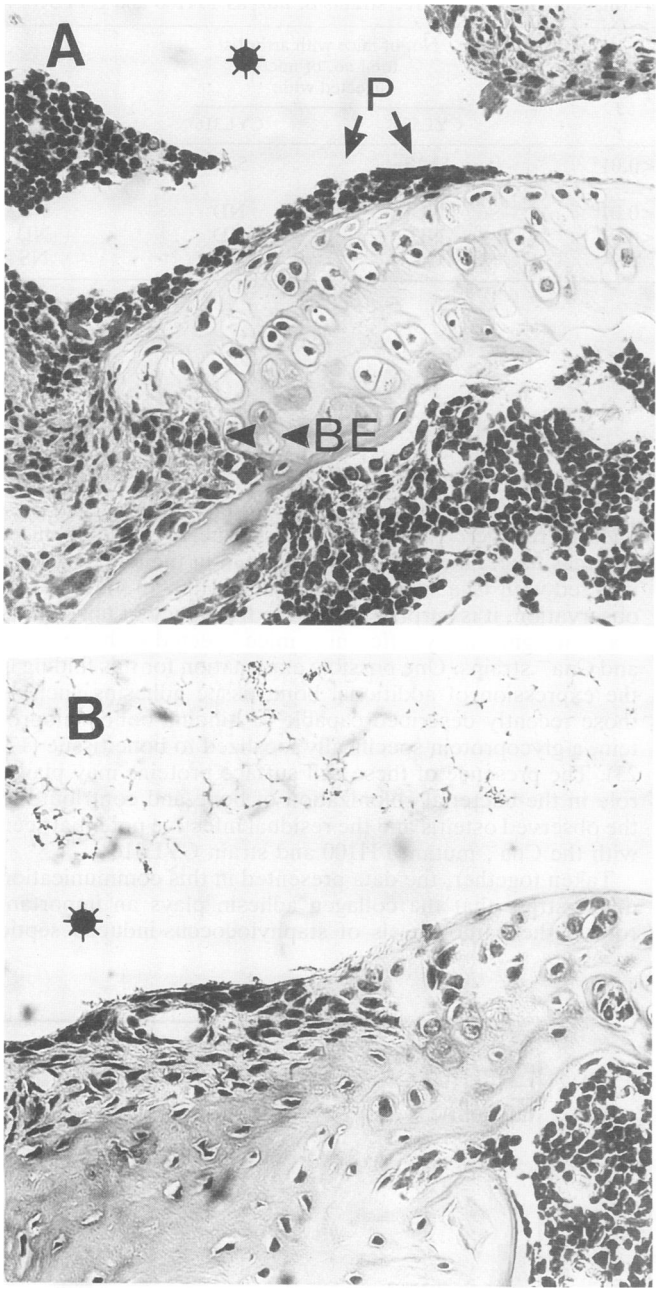

FIG. 8. Histopathological analysis. Histopathological micrographs showing the knee joints of two 8-week-old male Swiss mice 21 days after i.v. injection with *S. aureus*. (A) Strain Phillips. Synovial hypertrophy and pannus formation (P) are visible. At the cartilage-synovium junction there is bone erosion (BE) with macrophage-like cells at the front line of the erosion. Polymorphonuclear cells are seen in the joint cavity, which is marked with an asterisk. (B) Grossly unaffected knee joint after injection of isogenic mutant *S. aureus* PH100. Magnification, ×64.

induced arthritis in only six of the mice (Table 2). A significant difference ($P < 0.01$) in arthritogenicity between the wild-type *S. aureus* strain and the collagen adhesin-negative mutant was seen throughout both the histopathological and bacteriological experiments.

The murine model was also used to study the virulence of the second class of mutants, parent *S. aureus* CYL316 (Cna^-) and the derivative CYL574 (Cna^+). In this experiment, 17 mice were injected with CYL574 and 15 mice were injected with CYL316. Clinical arthritis was detected in 13 of the mice that were injected with CYL574, whereas only 5 mice injected with CYL316 displayed arthritic symptoms (Table 2). Osteitic lesions occurred only in two mice injected with the CYL316 (Table 2).

Taken together, our data demonstrate that the Cna^- strains PH100 and CYL316 have a reduced capacity to induce septic arthritis compared to the Cna^+ strains, Phillips and CYL574. However, Cna^- strains still express other putative virulence factors involved in the development of osteitic lesions. Hence the cell surface expression of the collagen adhesin plays an important role as a virulence factor in this murine model of *S. aureus*-induced septic arthritis.

Serological manifestations during *S. aureus* arthritis. During the progression of the bacterial infection a dramatic difference in immunoglobulin production between mice injected with parent *S. aureus* strain Phillips and its isogenic derivative PH100 was detected. Three weeks after injection with the parent strain, mouse serum IgG1 levels were sevenfold higher than levels in serum from mice injected with PH100. Levels of IgG3 and IgM in serum were also significantly higher in the Phillips-infected mice. Moreover, serum IL-6 levels were significantly elevated in the mice injected with *S. aureus* Phillips compared with those in mice injected with the isogenic mutant (Fig. 9). These data suggest that an IL-6-driven polyclonal B-cell activation occurs in response to infection with *S. aureus* strain Phillips (Cna^+) but to a much lesser extent as a consequence of exposure to its isogenic mutant PH100 (Cna^-).

DISCUSSION

In prior studies we have demonstrated that expression of the collagen adhesin is both necessary and sufficient to mediate the attachment of *S. aureus* to cartilage (24). Scanning electron microscopy studies have shown *S. aureus* cells to be intimately associated with cartilage and bone retrieved from the site of infection (26). Furthermore, essentially all strains isolated from patients with osteomyelitis and septic arthritis express a collagen adhesin, whereas only one-third of the strains obtained from patients diagnosed with soft tissue infections express the collagen adhesin (24). These observations have prompted us to formulate a hypothesis suggesting that the collagen adhesin on *S. aureus* cells plays an important role in the pathogenesis of septic arthritis and osteomyelitis by mediating bacterial colonization of cartilage and bone. To test this hypothesis we constructed two classes of *S. aureus* collagen adhesin mutants and compared the virulence of these strains with the parent strains in a septic arthritis animal model.

In a clinical isolate (Phillips), the *cna* gene was replaced through homologous recombination with an inactivated copy of the cloned *cna* gene, generating mutant PH100. Strain CYL316, which does not contain the *cna* gene, was the recipient of a intact copy of the cloned *cna* gene in a similar experiment, generating strain CYL574. The success of the genetic manipulations was confirmed by Southern hybridization studies. Furthermore, Cna^+ but not Cna^- strains expressed an immunoreactive collagen adhesin, bound ^{125}I-labeled type II collagen, and attached to cartilage. However, the mutants retained their ability to bind other extracellular matrix components such as fibronectin, fibrinogen, and thrombospondin. This indicates that the expression of other

TABLE 2. Decreased frequency of arthritis in mice inoculated with collagen adhesin-negative strains *S. aureus* PH100 and CYL316

Arthritis type	No. of mice with arthritis/ total no. of mice injected with:		*P*	No. of mice with arthritis/ total no. of mice injected with:		*P*
	Phillips	PH100		CYL574	CYL316	
Clinical	14/20	6/22	<0.01[a]	13/17	5/15	<0.05[a]
Histopathological						
Overall	10/10	5/10	<0.01[b]	ND[c]	ND	ND
Erosive	8/10	2/10	<0.01[b]	ND	ND	ND
Tail osteitis	3/10	3/10	NS[d]	0/17	2/15	NS

[a] Estimated by using the chi-square test with the Yates correction.
[b] Estimated by using the Fisher exact test.
[c] ND, not determined.
[d] NS, not significant.

putative *S. aureus* adhesins was not affected by the collagen adhesin mutation.

Human bacterial arthritis usually spreads in a hematogenous manner (8). Using an animal model that closely mimics human septic arthritis, we have demonstrated that dramatically fewer mice injected with the collagen adhesin-negative isogenic mutant PH100 or strain CYL316 exhibited clinical features and histopathological signs of arthritis compared with mice injected with the Cna$^+$ strain Phillips or mutant strain CYL574, respectively. Also, the mice injected with strain Phillips became more ill; displaying a higher intensity of arthritis and a delayed weight gain compared with the mice injected with strain PH100. Furthermore, levels of IgG1 and IL-6 in serum were dramatically elevated in mice injected with *S. aureus* Phillips compared with those in mice injected with Cna$^-$ mutant PH100 or with saline. The molecular basis for the apparent IL-6-driven polyclonal B-cell activation in mice injected with strain Phillips is presently unclear. In preliminary studies, we have not detected similar differences in serum IgG1 and IL-6 levels among mice injected with the Cna$^-$ strain CYL316 and the Cna$^+$ strain CYL574, respectively (data not shown). It is possible that the level of collagen adhesin expression among the Cna$^+$ strains differs and/or that these strains differ in additional components necessary for the immune response. Alternatively, collagen adhesin-mediated colonization of *S. aureus* to cartilage or bone tissue in the joint and subsequent bacterial proliferation result in the emergence of high concentrations of a bacterial component, e.g., a toxin that may trigger secretion of IL-6 by synoviocytes. Bacterial strains unable to colonize the joint or to produce the putative toxin at sufficient levels would not induce an inflammatory response.

The wild-type Cna$^+$ strain *S. aureus* Phillips was frequently recovered from the joint tissue of injected mice, whereas bacteria could not be detected in the joints of mice injected with Cna$^-$ isogenic mutant PH100. In view of this observation, it is surprising that the frequency of tail osteitis was not significantly different in mice injected with the Cna$^+$ and Cna$^-$ strains. One possible explanation for this finding is the expression of additional bone tissue adhesins such as those recently described capable of binding bone sialoprotein, a glycoprotein specifically localized to bone tissue (17, 23). The presence of these cell surface proteins may play a role in the bacterial colonization of bone and contribute to the observed osteitis and the residual infective potential seen with the Cna$^-$ mutant PH100 and strain CYL316.

Taken together, the data presented in this communication demonstrate that the collagen adhesin plays an important role in the pathogenesis of staphylococcus-induced septic

TABLE 3. Systemic effects of inoculation with wild-type parental strain *S. aureus* Phillips and the isogenic collagen adhesin-negative mutant PH100

Day	Result for mice injected with[a]:				*P*[b]
	Phillips		PH100		
	Mean body wt ± SD (g)	*n*	Mean body wt ± SD (g)	*n*	
0	17.4 ± 1.5	12	16.6 ± 2.2	12	NS
7	17.3 ± 2.2	10	24.7 ± 3.3	12	<0.001
14	23.3 ± 3.4	10	31.0 ± 3.1	12	<0.001
21	25.7 ± 4.6	10	34.5 ± 2.6	12	<0.001

[a] Swiss mice were injected i.v. at day 0 with 2×10^7 cells of *S. aureus* per mouse.
[b] Calculated by using Student's *t* test. NS, not significant.

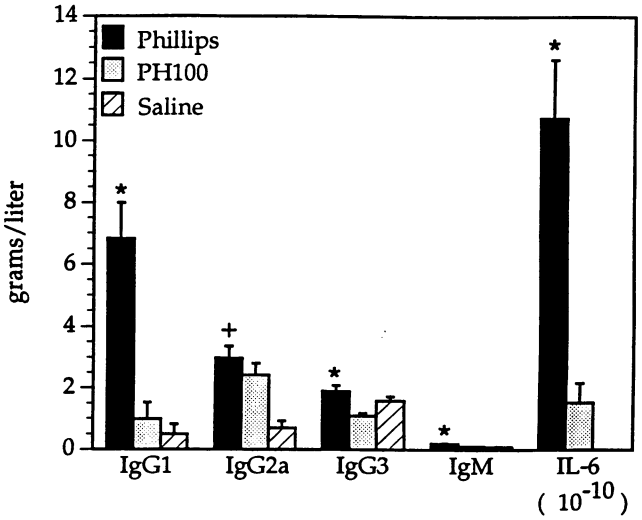

FIG. 9. Immunoglobulin and IL-6 concentrations in mouse serum samples. Twenty-one days after i.v. injection of *S. aureus* Phillips (Cna$^+$) or PH100 (Cna$^-$), the animals were sacrificed and the concentrations of the indicated immunoglobulins and IL-6 in serum were determined as described previously (4, 5). *P* values with regard to comparison between *S. aureus* Phillips and isogenic mutant PH100 were calculated by using Student's *t* test (*, $P < 0.001$; +, not significant).

arthritis by mediating bacterial colonization of the cartilage surface. Experiments are presently under way to determine whether a recombinant version of the adhesin can be used as a vaccine to protect animals against bone and joint infections.

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REFERENCES

1. **Abdelnour, A., and A. Tarkowski.** 1993. Polyclonal B-cell activation by an arthritogenic *Staphylococcus aureus* strain: contribution of T-cells and monokines. Cell. Immun. **147:**279–293.
2. **Augustin, J., and F. Götz.** 1990. Transformation of *Staphylococcus epidermidis* and other staphylococcus species with plasmid DNA by electroporation. FEMS Microbiol. Lett. **66:**203–208.
3. **Borck, K., and J. D. Beggs, W. J. Brammar, A. S. Hopkins, and N. E. Murray.** 1976. The construction *in vitro* of transducing derivatives of phage lambda. Mol. Gen. Genet. **146:**199–210.
4. **Bremell, T., A. Abdelnour, and A. Tarkowski.** 1992. Histopathological and serological progression of *Staphylococcus aureus*. Infect. Immun. **60:**2976–2985.
5. **Bremell, T., S. Lange, A. Yacoub, C. Rydén, and A. Tarkowski.** 1991. Experimental *Staphylococcus aureus* arthritis. Infect. Immun. **59:**2615–2623.
6. **Buxton, T. B., J. P. Rissing, J. A. Horner, K. M. Plowman, D. F. Scott, T. J. Sprinkle, and G. K. Best.** 1990. Binding of a *Staphylococcus aureus* bone pathogen to type I collagen. Microb. Pathog. **8:**441–448.
7. **Cierny, G., III.** 1990. Classification and treatment of adult osteomyelitis, p. 4337–4380. *In* C. M. Evarts (ed.), Surgery of the musculoskeletal system, 2nd ed., vol. 5. Churchill Livingstone, London.
8. **Goldenberg, D. L., and J. I. Reed.** 1985. Bacterial arthritis. N. Engl. J. Med. **312:**764–771.
9. **Holderbaum, D., T. Spech, L. A. Ehrhart, T. Keys, and G. S. Hall.** 1987. Collagen binding in clinical isolates of *Staphylococcus aureus*. J. Clin. Microbiol. **25:**2258–2261.
10. **Hunter, W. M.** 1978. Radioimmunoassay, p. 14.1-14.40. *In* D. M. Weir (ed.), Handbook of experimental immunology. Blackwell Scientific Publications, Ltd., Oxford.
11. **Kreiswirth, B. N., S. Löfdahl, M. J. Betley, M. O'Reilly, P. M. Schlievert, M. S. Bergdoll, and R. P. Novick.** 1983. The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. Nature (London). **305:**709–712.
12. **Lantz, M. S., L. M. Switalski, K. S. Kornman, and M. Höök.** 1985. *Bacteroides intermedius* binds fibrinogen. J. Bacteriol. **163:**623–628.
13. **Lee, C. Y., S. L. Buranen, and Z. H. Ye.** 1991. Construction of single-copy integration vectors for *Staphylococcus aureus*. Gene **103:**101–105.
14. **Lee, C. Y., and J. J. Iandolo.** 1986. Integration of staphylococcal phage L54a occurs by site-specific recombination: structural analysis of the attachment sites. Proc. Natl. Acad. Sci. USA **83:**5474–5478.
15. **Luchansky, J. B., A. K. Benson, and A. G. Atherly.** 1989. Construction, transfer properties of a novel temperature-sensitive integrable plasmid for genomic analysis of *Staphylococcus aureus*. Mol. Microbiol. **3:**65–78.
16. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3:**208–218.
17. **McGavin, M. H., D. Krajewska-Pietrasik, C. Rydén, and M. Höök.** 1993. Identification of a *Staphylococcus aureus* extracellular matrix-binding protein with broad specificity. Infect. Immun. **61:**2479–2485.
18. **McGavin, M. J., G. Raucci, S. Gurusiddappa, and M. Höök.** 1991. Fibronectin binding determinants of the *Staphylococcus aureus* fibronectin receptor. J. Biol. Chem. **266:**8343–8347.
19. **Murphy-Ullrich, J. E., S. Shultz-Cherry, and M. Höök.** 1992. Transforming growth factor-β complexes with thrombospondin. Mol. Biol. Cell **3:**181–188.
20. **O'Reilly, M., J. C. S. de Azavedo, S. Kennedy, and T. J. Foster.** 1986. Inactivation of the alpha-haemolysin gene of *Staphylococcus aureus* 8325-4 by site-directed mutagenesis and studies on the expression of its haemolysins. Microb. Pathog. **1:**125–138.
21. **Pattee, P. A.** 1987. *Staphylococcus aureus*. Genet. Maps **4:**148–154.
22. **Patti, J. M., H. Jonsson, B. Guss, L. M. Switalski, K. Wiberg, M. Lindberg, and M. Höök.** 1992. Molecular characterization and expression of a gene encoding a *Staphylococcus aureus* collagen adhesin. J. Biol. Chem. **267:**4766–4772.
23. **Rydén, C., A. I. Yacoub, I. Maxe, D. Heinegård, Å. Oldberg, A. Franzén, Å. Ljungh, and K. Rubin.** 1989. Specific binding of bone sialoprotein to *Staphylococcus aureus* isolated from patients with osteomyelitis. Eur. J. Biochem. **184:**331–336.
24. **Switalski, L. M., J. M. Patti, W. G. Butcher, A. G. Gristina, P. Speziale, and M. Höök.** 1993. A collagen receptor on *Staphylococcus aureus* strains isolated from patients with septic arthritis mediates adhesion to cartilage. Mol. Microbiol. **7:**99–107.
25. **Switalski, L. M., P. Speziale, and M. Höök.** 1989. Isolation and characterization of a putative collagen receptor from *Staphylococcus aureus*. J. Biol. Chem. **264:**21080–21086.
26. **Voytek, A., A. G. Gristina, E. Barth, Q. Myrvik, L. M. Switalski, M. Höök, and P. Speziale.** 1988. Staphylococcal adhesion to collagen in intraarticular sepsis. Biomaterials **9:**107–110.
27. **Waldvogel, F. A., and H. Vasey.** 1980. Osteomyelitis: the past decade. N. Engl. J. Med. **303:**360–369.