cols available at http://cmgm.stanford.edu/pbrown/mguide/index.html). After printing, free amino groups of the poly-L-lysine–coated slides were acetylated with 5.5 g of succinic anhydride in 350 ml of borate-buffered 1-methyl-2-pyrrolidinone (pH 8).
9. Bacteria listed above were grown in 7H9 broth with ADC enrichment, and DNA was extracted after 14 to 21 days' growth as described [D. van Soolingen, P. W. Hermans, P. E. de Haas, D. R. Soll, J. D. van Embden, *J. Clin. Microbiol.* **29**, 2578 (1991)]. Whole genomic DNA from either H37Rv or BCG strain (2 μg) was used as templates for direct incorporation of fluorescent nucleotide analogs (Cy3 and Cy5 dUTP, Amersham) by a randomly primed polymerization reaction. In brief, 50-μl labeling reactions contained 2 μg of template DNA, 5 μl of 10× buffer, 1.5 μl of fluorescent dUTP, 0.5 μl each of 5 mM dATP, dCTP, and dGTP, 1 μl of random hexamers and decamers, and 2 μl of Klenow (*Escherichia coli* DNA polymerase 3′ to 5′exo-, New England Biolabs). Labeled DNAs from the strains being compared were mixed together to 14.75 μl total volume in a solution with final concentration of 4× SSC buffer, tRNA (0.7 μg/μl), and 0.3% SDS. The hybridization mixture was denatured at 98°C for 2 min, cooled to 65°C, applied to the microarray, and covered with a 22-mm^2 cover slip. The slide was then placed in a waterproof hybridization chamber for hybridization in a 65°C water bath for 3 hours. After hybridization, slides were washed 2 min in 1× SSC buffer with 0.06% SDS followed by 2 min in 0.06× SSC buffer.
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12. Slides were scanned with a custom-built scanning laser microscope as described [J. L. DeRisi, V. R. Iyer, P. O. Brown, *Science* **278**, 680 (1997)]. Fluorescent spot intensities were quantified using custom software as described by DeRisi and available at http://rana.Stanford.EDU/software. For each spot, background fluorescence was subtracted from the average spot fluorescence to produce a channel-specific value. Spots were excluded from analysis when background intensity was greater than the spot intensity. The ratio of Cy3/Cy5 hybridization was calculated and log-transformed for representation according to genomic location implementing ProbeBrowser software (designed and written in our laboratory and available at http://molepi.stanford.edu/free_software.html). This software integrates microarray experimental results with the genomic positions of the hybridization targets and displays corresponding ORF annotations (see Fig. 1, B and C).
13. For any single experimental spot, a mismatched hybridization could represent (i) a repeated element more numerous in H37Rv, such as IS*6110*, (ii) an element present in H37Rv and absent from BCG, or (iii) a false positive result that may occasionally occur during thousands of simultaneous hybridizations. We observed that spots representing IS*6110* reliably resulted in high ratios of H37Rv:BCG, corresponding to their known 16:1 genomic presence. For assigning deletions, we restricted our investigation to regions where probes from at least two adjacent ORFs exhibited mismatched hybridization. This strategy was invoked to maximize the likelihood of detecting true deletions at the expense of decreased resolution. For putative deletions identified by the array experiments, we performed Southern (DNA) hybridization using probes independent of those on the array to confirm their absence in BCG strains. Then, to characterize confirmed deletions, we designed PCR primers to approach the predicted edges of each deletion so that the amplified region would span the missing genomic locus for those strains in which it is deleted. These amplicons were sequenced using the PCR primers at the Protein and Nucleic Acid (PAN) Core Facility, Stanford University, by ABI dye terminator chemistry. Sequences obtained were aligned to the known sequence of H37Rv by BLAST search [S. F. Altschul *et al.*, *Nucleic Acids Res.* **25**, 3389 (1997)] to determine the precise site of each deletion (details available at http://molepi.stanford.edu/bcg). Based on the site and the predicted ORFs from the H37Rv, we documented which reading frames were partially or completely missing from BCG strains. To classify deleted regions as BCG-specific or as differences between H37Rv and virulent *M. bovis*, we repeated PCR with the primers spanning the missing genetic region using DNA from virulent *M. bovis* (four human and four bovine isolates).
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27 January 1999; accepted 8 April 1999

Broadly Protective Vaccine for *Staphylococcus aureus* Based on an in Vivo–Expressed Antigen

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Vaccines based on preferential expression of bacterial antigens during human infection have not been described. *Staphylococcus aureus* synthesized poly-*N*-succinyl β-1-6 glucosamine (PNSG) as a surface polysaccharide during human and animal infection, but few strains expressed PNSG in vitro. All *S. aureus* strains examined carried genes for PNSG synthesis. Immunization protected mice against kidney infections and death from strains that produced little PNSG in vitro. Nonimmune infected animals made antibody to PNSG, but serial in vitro cultures of kidney isolates yielded mostly cells that did not produce PNSG. PNSG is a candidate for use in a vaccine to protect against *S. aureus* infection.

Staphylococcus aureus is the most frequently isolated bacterial pathogen in hospital-acquired infections (*1*) and is a common cause of community-acquired infections, including endocarditis, osteomyelitis, septic arthritis, pneumonia, and abscesses (*1*). *Staphylococcus aureus* is also a significant pathogen in economically important animals (*2*). Staphylococcal resistance to first-line drugs such as synthetic penicillins has resulted in major problems in treating methicillin-resistant *S. aureus* (MRSA) strains, which are increasingly common, especially in hospitalized patients. Of greater concern is the recent emergence in several countries of MRSA strains with reduced susceptibility to vancomycin, the antibiotic of last resort (*3*). The appearance of these vancomycin-intermediate *S. aureus* (VISA) strains raises the specter of untreatable staphylococcal infections, necessitating a search for alternative therapies (*1*, *3*).

One potential therapeutic target for bacterial infections is products of bacterial genes activated during in vivo infections (*4*). Presumably these genes encode factors critical for infection and disease progression. Strategies designed to discover in vivo–expressed genes have, of necessity, used animal models for gene identification, and in vivo–expressed bacterial factors have not yet been detected in human infections. In addition, the usefulness in vaccines of any factors encoded

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by these genes has not been demonstrated. We now provide evidence that antibodies to a distinct surface polysaccharide antigen of *S. aureus* that is preferentially elaborated in vivo in infected humans and experimental animals are broadly protective against many *S. aureus* strains.

Lung tissue removed from two *S. aureus*–infected cystic fibrosis (CF) patients contained organisms that elaborated a surface polysaccharide that reacted positively with immune rabbit serum specific for poly-*N*-succinyl β-1-6 glucosamine (PNSG), previously determined to be the *Staphylococcus epidermidis* capsular polysaccharide adhesin (*5*) (Fig. 1A). PNSG is distinct from the serologically and structurally defined *S. aureus* capsular polysaccharides (CP), including CP5 and CP8, which are made by >70% of all *S. aureus* strains (*6*). Only background fluorescence was observed in the lung sections with antibodies to CP antigens (Fig. 1A). In addition, six of nine sputum samples from *S. aureus*–infected CF patients were positive for PNSG expression by immunofluorescent microscopy (Fig. 1B; 80 to 90% of cells fluorescent) (*7*), whereas bacteria in these samples expressed little CP antigen (<10% of cells were positive) (*8*). In vitro cultivation of the *S. aureus* lung and sputum isolates in trypticase soy broth under aerobic conditions resulted in high expression of CP antigen by all isolates as previously shown (*9*) and decreased expression of PNSG (*8*), indicating that PNSG is an in vivo–expressed bacterial factor in human infection.

A set of animal and human clinical isolates of *S. aureus* was investigated for PNSG production. With the use of a colony immunoblot assay (*10*), 29 of 82 (35%) freezer-stored *S. aureus* isolates from the milk of cows and sheep and 14 of 82 (17%) stored human isolates were PNSG-positive; the latter comprised strains from blood, wounds, and vaginas (associated with toxic shock syndrome). To determine if the low expression of PNSG among these isolates was a result of laboratory storage and passage of strains, we used an enzyme-linked immunosorbent assay (ELISA) inhibition technique (*11*) to detect PNSG production by 43 fresh clinical bloodstream isolates of *S. aureus* growing on primary blood agar plates obtained from a hospital microbiology laboratory. The geometric mean percentage of inhibition of antibody binding was only 13% (95% confidence interval of 8 to 20%), and only 8 of 43 (19%) isolates inhibited >50% of the antibody binding to PNSG. However, on some isolates, material on the cell surface bound antibody to PNSG when visualized by immunoelectron microscopy (Fig. 1C). Although in vitro expression by *S. aureus* of the immunoreactive PNSG antigen was low or absent among most strains, it was detectable as a surface polysaccharide on some strains.

Because immunoreactive PNSG antigen production appeared to undergo an in vivo–in vitro phenotypic variation in *S. aureus*, we investigated whether *S. aureus* strains had the intercellular adhesin (*ica*) locus of *S. epidermidis* made up of four genes (*icaADBC*) whose protein products synthesize PNSG (*5*, *12*). The *icaADBC* genes were detected in eight *S. aureus* strains by polymerase chain reaction (PCR) (Fig. 1D) (*13*), whereas a *Staphylococcus carnosus* strain known to lack the *ica* locus was negative. In addition, the *icaADBC* genes are present in the genomes of both *S. aureus* strains, COL and NCTC 8325-4, that are currently being sequenced (*14*).

To purify and characterize the antigen from *S. aureus* reactive with antisera to PNSG, we used strain MN8m, which was recovered from a chemostat culture of strain MN8. Strain MN8m is a constitutive, copious producer of immunoreactive material (Fig. 2). Using the method previously developed for isolating PNSG from *S. epidermidis* (*5*), we obtained from strain MN8m an antigen identical to the *S. epidermidis* PNSG as determined by proton nuclear magnetic resonance (NMR) and chemical analysis (*15*) (Fig. 2).

A mouse model of renal infection was used for active immunization with PNSG to evaluate protective efficacy against *S. aureus* infection (*16*). Because heavily infected kidneys are found in animals with *S. aureus* infections of other tissues resulting in endocarditis, arthritis, and bacteremia (*17*), the colony-forming units (CFU) of *S. aureus* in the kidneys reflect systemic infection of a variety of tissues. Mice immunized with either PNSG derived from *S. aureus* MN8m or a control, irrelevant bacterial polysaccharide developed high titers (>500) of immunoglobulin G (IgG) antibodies to the im-

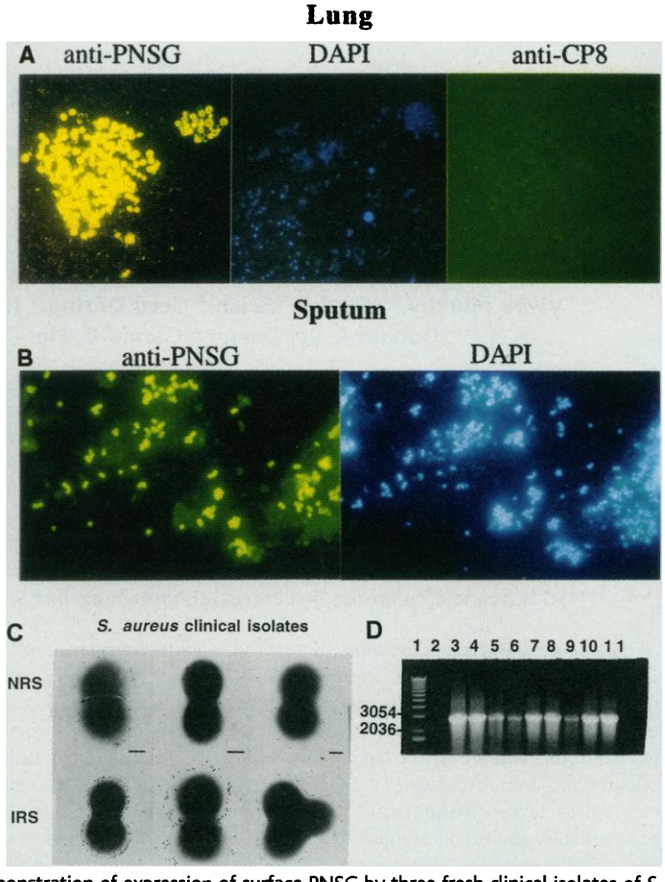

Fig. 1. PNSG is synthesized in infected human tissues, and *S. aureus* strains carry genes needed for PNSG expression. (**A**) (Left) Immunofluorescent staining of *S. aureus*–infected lung as seen by reaction with antiserum to PNSG (anti-PNSG) and CY3-indocarbocyanine–conjugated antibody to rabbit IgG (yellow fluorescence) (*27*). (Middle) DAPI staining of *S. aureus* cells in a lung section. (Right) Only background immunofluorescence is seen when antibody to the CP microcapsule (anti-CP8) is reacted with *S. aureus* cells in an infected lung. (**B**) Immunofluorescent micrographs of a sputum sample from an *S. aureus*–infected CF patient (*19*). (Left) Production of PNSG as seen by reaction with antiserum to PNSG (anti-PNSG) and fluorescein-conjugated swine antibody to rabbit IgG. (Right) DAPI staining of *S. aureus* cells in sputum sample. (**C**) Immunoelectron microscopic demonstration of expression of surface PNSG by three fresh clinical isolates of *S. aureus* (*5*). Only 8 of 43 fresh isolates elaborated detectable PNSG on primary agar plates obtained from the microbiology laboratory. When three of these positive strains were reacted with normal rabbit serum (NRS) and protein A conjugated to 20-nm gold particles, there was little binding of the gold to the extracellular surface, whereas reaction with immune rabbit serum (IRS) to PNSG and protein A–gold resulted in binding of gold particles to the cell surface. Bars, 0.5 μm. (**D**) *Staphylococcus aureus* isolates contain the *ica* locus needed for PNSG synthesis (*5*, *12*). PCR performed on chromosomal DNA from controls and eight isolates of *S. aureus* (*13*). Lane 1, molecular weight markers; lane 2, *S. carnosus* TM300, negative control; and lane 3, positive control DNA from *S. epidermidis* RP62A. *Staphylococcus aureus* strains: lane 4, Reynolds; lane 5, MN8; lane 6, 5827; lane 7, 5836; lane 8, Vas; lane 9, VP; lane 10, 265; and lane 11, Por. Primers were designed to amplify a 2.7–kilobase pair fragment [migrates between 2036–base pair (bp) and 3054-bp markers] containing the *icaA*, *icaD*, and *icaB* genes and a section of *icaC* genes (*12*).

munizing, but not heterologous, antigen. Five days after intravenous challenge with two different doses of two *S. aureus* strains (CP5 strain Reynolds and CP8 strain MN8) phenotypically negative for PNSG production in vitro, the PNSG-immunized mice had significant reductions in CFU of *S. aureus* per gram of kidney compared with the groups immunized with an irrelevant polysaccharide (Fig. 3A).

Rabbits immunized with PNSG responded by producing high titers (>2500) of PNSG-specific IgG that persisted for at least 8 months. To document that PNSG-specific antibody mediated the protection afforded by active immunization, we passively immunized mice with rabbit antibody before and again 18 hours after intravenous (IV) challenge with eight strains of *S. aureus*. Controls received comparably titered antisera to an irrelevant polysaccharide from *Pseudomonas aeruginosa*. Seven of the *S. aureus* challenge strains produced little to no PNSG at challenge (0 to 21% ELISA inhibitory activity), whereas the *S. aureus* strain 5827 inhibited PNSG antibody binding by 48%. The challenge strains had a broad range of minimal doses that resulted in kidney infections in 100% of animals (range of 10^2 to 10^6 CFU per mouse), but for all eight strains tested, passive immunization with antibodies to PNSG significantly reduced or completely eliminated bacteria from the kidneys (Fig. 3B) compared with controls. Although it was possible to overwhelm the protective effect with higher challenge doses in some cases (for example, strain Reynolds at a challenge dose of 8×10^5 CFU per mouse), it was also possible to achieve total protection against at least one challenge dose for four of the eight strains (Fig. 3B). The lower numbers of *S. aureus* in the kidneys of some control animals in the passively immunized groups compared with the numbers in control animals in the actively immunized control groups (Fig. 3A) were likely due to a modest enhancement of resistance to infection from the control antiserum in the passive protection studies.

An additional study was done comparing protection by rabbit IgG antibodies to PNSG with human IgG to CP5 and CP8. The dose of the human IgG that was used (300 μg of CP5-specific and 246 μg of CP8-specific antibody per mouse) was greater than that necessary to protect mice against lethal infection with a virulent, CP5 MRSA strain when both the antibodies and bacteria were injected intraperitoneally (IP) (*18*). However, in mice injected IP with antibodies and challenged IV with 10^6 CFU of strain 5836 (a CP5 MRSA-VISA strain), there was no reduction in bacteria in the kidneys by CP-specific antibodies compared with animals given control human antibodies to an irrelevant bacterial polysaccharide [mean $\log_{10}$ CFU per gram of kidney = 3.3 ± 1.1 (SEM) and 3.7 ± 1.1 (SEM), respectively]. In contrast, PNSG-specific rabbit antibodies significantly reduced *S. aureus* 5836 in infected kidneys [mean $\log_{10}$ CFU per gram of kidney = 0.71 ± 0.3; $P \leq 0.03$, one-way analysis of variance (ANOVA) and Fisher probable least significant difference]. CP-specific IgG also did not prevent infection by the representative CP5 and CP8 *S. aureus* strains Reynolds and MN8 (*8*).

The protective efficacy of antisera to PNSG against lethal challenge (5×10^7 CFU per mouse) with two MRSA-VISA strains was also tested. None of the 10 infected mice given antibodies to PNSG died (five mice per strain). In contrast, 80% (4/5; $P = 0.02$, Fisher exact test) of mice infected with strain 5836 and 100% (5/5; $P = 0.004$, Fisher exact test) of mice infected with strain 5827 died after administration of control antibodies spe-

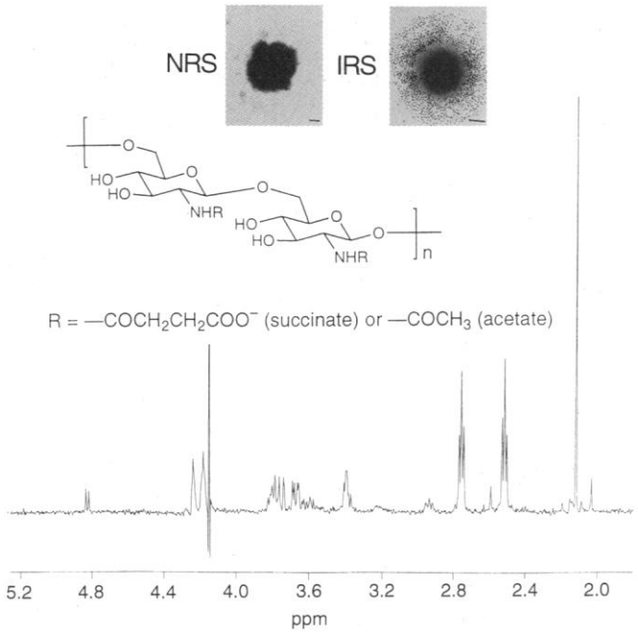

Fig. 2. PNSG elaboration by *S. aureus* strain MN8m. (Top) Probing of strain MN8m with IRS to purified PNSG followed by gold-labeled protein A showed binding of the gold particles to a thick extracellular antigen surrounding the bacterial cells in electron micrographs (*11*). NRS produced only a minimal binding of the gold particles. Bars, 0.5 μm. (Bottom) Proton NMR spectrum of the acid-hydrolyzed PNSG antigen isolated from *S. aureus* MN8m and analyzed as described (*15*). The deduced chemical structure of PNSG is drawn above the spectrum.

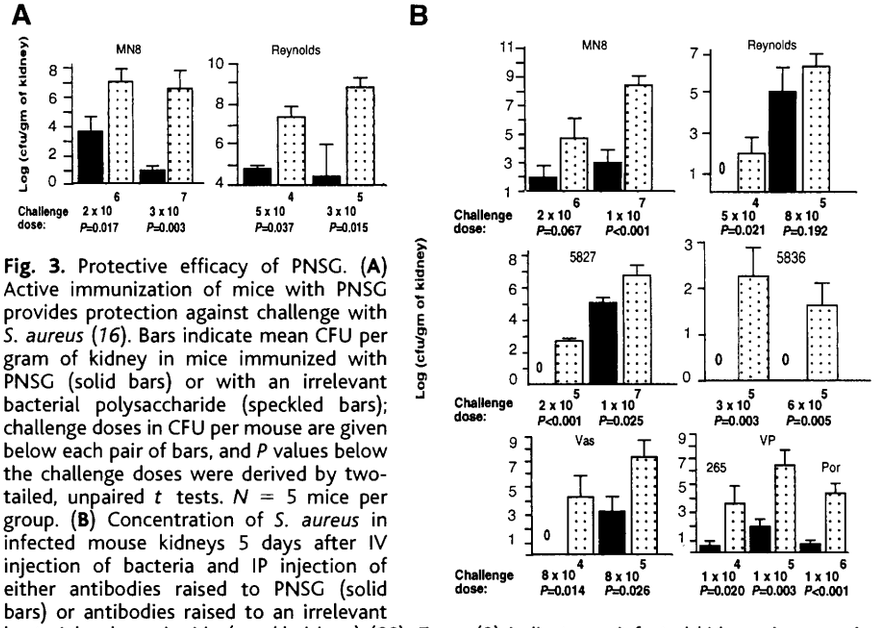

Fig. 3. Protective efficacy of PNSG. (**A**) Active immunization of mice with PNSG provides protection against challenge with *S. aureus* (*16*). Bars indicate mean CFU per gram of kidney in mice immunized with PNSG (solid bars) or with an irrelevant bacterial polysaccharide (speckled bars); challenge doses in CFU per mouse are given below each pair of bars, and *P* values below the challenge doses were derived by two-tailed, unpaired *t* tests. $N = 5$ mice per group. (**B**) Concentration of *S. aureus* in infected mouse kidneys 5 days after IV injection of bacteria and IP injection of either antibodies raised to PNSG (solid bars) or antibodies raised to an irrelevant bacterial polysaccharide (speckled bars) (*28*). Zeros (0) indicate no infected kidneys in any mice (lower limit of detection ~5 CFU per gram of kidney), bars represent geometric means, and error bars represent the upper standard deviation; challenge doses in CFU per mouse are given below each pair of bars, and *P* values given below the challenge doses were derived by two-tailed, unpaired *t* tests. *Staphylococcus aureus* strains MN8 and Reynolds represent CP8 and CP5 strains, respectively; *S. aureus* 5827 and 5836 are MRSA-VISA strains; *S. aureus* 265 is methicillin resistant; *S. aureus* Vas and VP are freezer-stored human isolates; and *S. aureus* Por is a fresh clinical isolate from a patient with bacteremia. $N = 5$ mice per group.

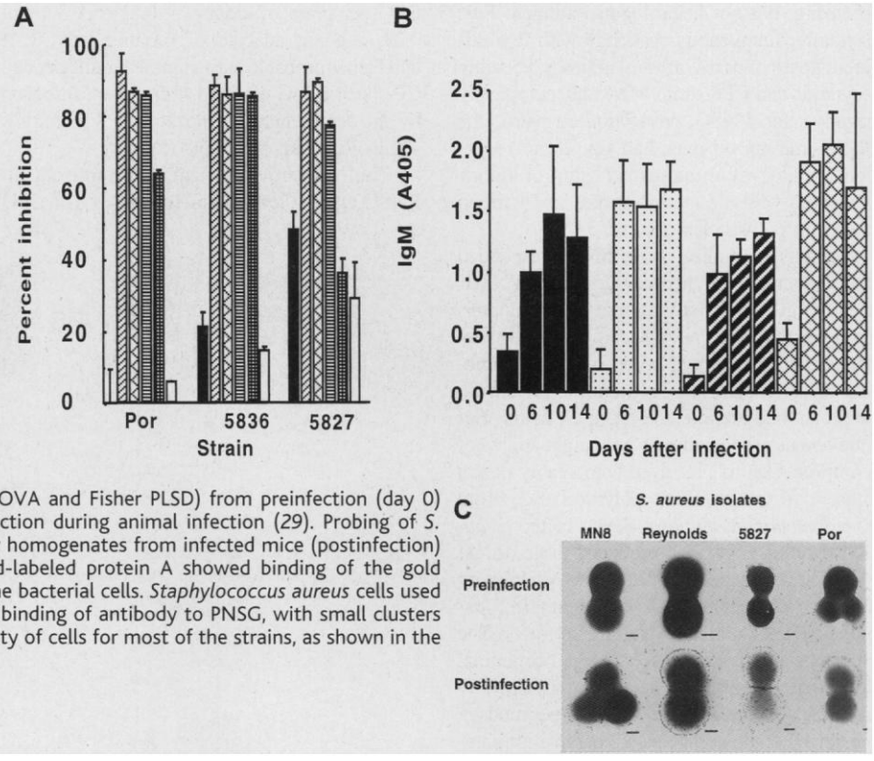

Fig. 4. Detection of PNSG expression during and after infection. (**A**) ELISA inhibitions for detection of PNSG were performed on *S. aureus* strains before infection of mice (solid bars; percentage of inhibition value of 0 is shown only with error bar) and on strains freshly isolated from kidney homogenates (diagonal-stripped bars) and after passage on TSA one (cross-hatched bars), two (horizontal-lined bars), three (speckled bars), and four (clear bars) times. *Staphylococcus aureus* production of PNSG is lost after passage on TSA. Bars depict mean percentage of inhibition of antibody binding and error bars the SE. (**B**) PNSG elicits specific antibody during *S. aureus* infection. Mice infected IV with four different strains of *S. aureus* (Reynolds, solid bars; MN8, speckled bars; 5827, diagonal-stripped bars; and Por, cross-hatched bars) developed PNSG-specific IgM antibodies on the indicated day after infection. Bars depict mean absorbance (A405) and error bars the SE achieved with a serum dilution of 1:100. All postinfection values were significantly different ($P < 0.01$, ANOVA and Fisher PLSD) from preinfection (day 0) values. (**C**) Microscopic evidence for PNSG production during animal infection (*29*). Probing of *S. aureus* cells growing on primary platings of kidney homogenates from infected mice (postinfection) with antisera to purified PNSG followed by gold-labeled protein A showed binding of the gold particles to an extracellular antigen surrounding the bacterial cells. *Staphylococcus aureus* cells used to infect the mice (preinfection) showed minimal binding of antibody to PNSG, with small clusters of surface-localized gold particles seen on a minority of cells for most of the strains, as shown in the figure. Bars, 0.5 μm.

cific to an unrelated bacterial polysaccharide from *P. aeruginosa*.

Evidence for in vivo production of PNSG after injection of mice with PNSG-low *S. aureus* challenge strains was investigated. As PNSG is only soluble at pH < 4 (*5*), kidney homogenates were extracted with 0.1 M HCl to solubilize PNSG, which was then precipitated at neutral pH for probing with PNSG-specific antibodies. Only extracts from kidneys infected with *S. aureus* reacted with antibodies to PNSG (*8*). ELISA inhibition assays confirmed that PNSG elaboration was induced during in vivo infection, but expression was reduced or lost after in vitro passage of these bacterial cells on trypticase soy agar (TSA) (Fig. 4A) (*19*). Increased detection of PNSG expression on the mouse-kidney isolates compared with the fresh human blood isolates was attributed to environmental differences in these tissues that affect induction and stabilization of PNSG expression in vivo. Serum antibody responses to PNSG after *S. aureus* infection were also analyzed. Between 6 and 14 days after infection, specific IgM antibody responses to PNSG were detected (Fig. 4B), and all of the mice had high concentrations ($>10^5$ CFU per gram of kidney) of *S. aureus* in their kidneys on day 14. In contrast to the vigorous IgG responses elicited in PNSG-immunized mice and rabbits, no IgG responses to PNSG were detected by day 14 in any of the infected animals, possibly explaining the persistence of *S. aureus* infection in spite of the immune response to PNSG. Finally, the results of immunoelectron microscopy revealed that *S. aureus* cells cultivated directly from infected kidneys produced extracellular PNSG (Fig. 4C).

Our findings indicate that all *S. aureus* strains examined had the *ica* genes needed for PNSG synthesis but that PNSG was expressed as a surface polysaccharide antigen principally in vivo during human and animal infection where it was a target for protective antibodies. Several in vivo expression strategies have been specifically used with *S. aureus* (*20*), although none identified the *ica* locus in the screens. This may be due to the small number of genes characterized against a background of a large number of candidate genes identified by these strategies. In addition, PNSG may not be made under the conditions used in the various in vivo expression screening approaches. In *S. epidermidis*, expression of PNSG potentiates infection but PNSG-negative strains can still cause infections if a high enough challenge dose is used (*21*).

Development of a vaccine for *S. aureus* is considered a high priority, and current candidates include the CP5 and CP8 microcapsules (*18*, *22*), the recently described RNAIII activating protein that regulates the production of many *S. aureus* virulence factors (*23*), and native and recombinant fragments of *S. aureus* protein adhesins for host extracellular matrix proteins (*24*). However, an advantage of a staphylococcal PNSG vaccine would be that PNSG is also elaborated by the majority of clinically important isolates of coagulase-negative staphylococci (CoNS) (*25*). Together, *S. aureus* and CoNS account for 40 to 60% of bacterial blood isolates from hospitalized patients (*1*). PNSG is effective in laboratory animals as a vaccine against CoNS infections (*26*). Thus, PNSG has potential as a vaccine for protection against hospital-acquired staphylococcal infections, community-acquired *S. aureus* infections, and infections in farm animals, where staphylococcal diseases have substantial economic impact (*2*).

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swine antibody to rabbit IgG (diluted 1:40) was used to visualize PNSG. Cover slips were mounted with Permafluor and analyzed with a fluorescence microscope as described (9). Each sputum sample was analyzed twice.
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11. For detection of PNSG expression by fresh clinical isolates, we used a sensitive ELISA inhibition that was slightly modified from the previously described procedure (5), as well as immunoelectron microscopy (5). Bacterial cells were suspended to an optical density at 650 nm of 2.0 in 0.1 M phosphate and 0.15 M NaCl [phosphate-buffered saline (PBS)] and then treated with trypsin (0.65 mg/ml for 30 min at 37°C) to destroy antibody-binding structures such as protein A present on the surface of most *S. aureus* strains [B. F. King and B. J. Wilkinson, *Infect. Immun.* **33**, 666 (1981)]. After washing, the bacterial pellet was suspended in a 1:500 dilution of antiserum to PNSG and incubated at 4°C overnight, after which cells were removed and adsorbed sera were tested for residual binding activity in a PNSG-specific ELISA as described (5).
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13. Primers were designed to amplify a gene product of 2.7 kb encompassing a region of the *icaADBC* genes of the staphylococcal *ica* locus (12). The PCR forward primer was TGCACTCAATGAGGGAATCA, corresponding to nucleotides 409 to 428 in the *icaA* gene; the reverse primer was AATCACTACCGGAAACAGCG, complementary to nucleotides 3114 to 3133 in the *icaC* gene. PCR was carried out with Platinum PCR Supermix and 200 nM primers. DNA melting was at 95°C for 30 s, annealing was at 60°C for 60 s, and elongation was at 72°C for 60 s; repeat cycles decreased the annealing temperature by 0.5°C each cycle until 28 cycles were completed. Amplified DNA was visualized after separation in a 0.7% agarose gel and staining with ethidium bromide.
14. With the use of the blastN and blastT search programs [S. F. Altschul *et al.*, *J. Mol. Biol.* **215**, 403 (1990)] on the unfinished nucleotide sequences of the *S. aureus* NCTC 8325-4 genome (University of Oklahoma's Advanced Center for Genome Technology) and the *S. aureus* COL genome (Institute for Genome Research), there was 71 and 74% identity, respectively, with the *ica* locus of *S. epidermidis* RP62A (accession number U43366). The predicted protein sequences from *S. aureus* shared 72% identity and 80% (NCTC 8325-4) and 87% (COL) similarity to the *S. epidermidis* icaADBC proteins. The matches were on three unassembled fragments (contigs 1441, 1348, and 1147) of the NCTC 8325-4 genome sequence and in the proper order on a single fragment (gsa–76) of 9459 base pairs of the COL genome sequence. The GCG suite of programs (Wisconsin Package 9.1; Genetics Computer Group, Madison WI) was used to assemble the fragments of the *S. aureus* NCTC 8325-4 genome for translation and analysis.
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16. Swiss Webster mice were actively immunized IP with three 100-μg doses of PNSG 5 to 6 days apart or identically with a control polysaccharide antigen from *P. aeruginosa*. Five days after the last dose, the mice were challenged with *S. aureus*, and infection was allowed to proceed for 5 days, after which the mice were killed and bacterial counts on kidney homogenates were performed. The significance of the differences in CFU per gram of kidney between PNSG-immune and control mice was determined by a *t* test. All mice were treated in accordance with institutional guidelines for the humane care and treatment of animals.
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19. Three examples are shown of eight tested, representative of five PNSG-negative, two PNSG-low, and one PNSG-intermediate strain at challenge. Baseline values for percentage of inhibition of antibody binding ± the standard error for *S. aureus* are as follows: strain Por = 0 ± 9%, strain 5836 = 21 ± 4%, and strain 5827 = 48 ± 5%.
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27. For characterization of PNSG production in *S. aureus*–infected lungs, bronchial tissue pieces (2 mm by 2 mm) from right upper lobes of two 8-year-old female CF patients who underwent lobectomy because of chronic *S. aureus* infection were embedded in agarose and thereafter in K11M for sectioning. Ultrathin sections (0.1 to 0.2 μm) were fixed on glass slides, and nonspecific binding of antibodies to Protein A was blocked with swine serum diluted 1:10 in PBS (pH 7.4) supplemented with 0.1% Tween 20 for 1 hour at room temperature. After washing with PBS–Tween 20, sections were incubated with rabbit antibody to *S. aureus* PNSG for 1 hour at room temperature in a wet chamber, followed by incubation with a mouse monoclonal IgG antibody to the CP5 or CP8 antigen of *S. aureus* for 1 hour, and then washed with PBS–Tween 20. For detection of PNSG expression, sections were incubated for 40 min with CY3-indocarbocyanine–conjugated antibody to rabbit IgG diluted 1:500 in PBS–Tween 20. For detection of CP antigens, sections were incubated with FITC-conjugated antibody to mouse IgG diluted 1:200 in PBS–Tween 20. After washing, DNA was stained with 1 μg of 4′,6-diamidino-2-phenylindole, dilactate (DAPI) per milliliter for 5 min, and sections were washed again with distilled water; the sections were embedded in Permafluor and analyzed with a fluorescence microscope (9).
28. PNSG was used to immunize rabbits to obtain specific antibodies. After an antibody titer >1000 was detected by ELISA, immune rabbit sera were used for protection studies in the mouse renal abscess model [A. Albus, R. D. Arbeit, J. C. Lee, *Infect. Immun.* **59**, 1008 (1991)]. Control sera were from rabbits immunized with the irrelevant *P. aeruginosa* polysaccharide. Six- to eight-week-old Swiss Webster mice were treated with 0.5 ml of rabbit serum IP 4 hours before challenge with *S. aureus* strains and again 18 hours later. Infection was allowed to proceed for 5 days, after which the mice were killed and bacteria were counted in kidney homogenates (16).
29. *Staphylococcus aureus* cells growing on primary cultures from infected mouse kidneys were scraped directly from TSA plates into PBS. A 1-ml volume of the cells was centrifuged (15,000*g*, 5 min), washed in sterile PBS, and treated with trypsin (0.65 mg/ml for 30 min at 37°C). Electron microscopic grids were prepared and processed for viewing as described (5). The grids were examined with a transmission electron microscope at magnifications of 6000 to 25,000.
30. We thank J. Hübner and E. Muller for helpful input, F. Tenover for MRSA-VISA strains, A. Onderdonk for clinical isolates, R. Ross for *S. aureus* strain MN8m, L. Almeida for assistance with colony immunoblots, A. Fattom for human antibodies to CP5 and CP8, M. Coyne for assistance with analysis of *S. aureus* genome sequences, J. M. Fournier for monoclonal antibodies to CP5 and CP8, S. Campana and L. Marianelli for supplying CF sputum samples, and G. Bellon for CF lung tissue. Supported by NIH grant AI23335.

13 January 1999; accepted 20 April 1999

Spatiotemporal Dynamics of Inositol 1,4,5-Trisphosphate That Underlies Complex Ca^{2+} Mobilization Patterns

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Inositol 1,4,5-trisphosphate (IP_3) is a second messenger that elicits complex spatiotemporal patterns of calcium ion (Ca^{2+}) mobilization and has essential roles in the regulation of many cellular functions. In Madin-Darby canine kidney epithelial cells, green fluorescent protein–tagged pleckstrin homology domain translocated from the plasma membrane to the cytoplasm in response to increased concentration of IP_3. The detection of translocation enabled monitoring of IP_3 concentration changes within single cells and revealed spatiotemporal dynamics in the concentration of IP_3 synchronous with Ca^{2+} oscillations and intracellular and intercellular IP_3 waves that accompanied Ca^{2+} waves. Such changes in IP_3 concentration may be fundamental to Ca^{2+} signaling.

IP_3 production by phospholipase C (PLC)–mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) is an early intracellular event after stimulation by hormones, autacoids, and neurotransmitters. IP_3 mobilizes Ca^{2+} from intracellular stores through the IP_3 receptor, resulting in activation of Ca^{2+}-dependent cellular events such as contraction, secretion, gene expression, and synaptic plasticity (*1*, *2*). Ca^{2+} mobilization occurs in complex temporal and spatial patterns, including Ca^{2+} oscil-

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