Protection against *Escherichia coli* infection by antibody to the *Staphylococcus aureus* poly-*N*-acetylglucosamine surface polysaccharide

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Poly-*N*-acetylglucosamine (PNAG) is a surface polysaccharide produced by *Staphylococcus aureus* and *Staphyloccus epidermidis* and is an effective target for opsonic and protective Ab for these two organisms. Recently, it has been found that *Escherichia coli* produces an exo-polysaccharide, designated polyglucosamine, that is biochemically indistinguishable from PNAG. We analyzed 30 *E. coli* strains isolated from urinary tract and neonatal bloodstream infections for the *pga locus*, PNAG antigen production, and susceptibility to opsonic killing and protection from lethal infection by Ab to PNAG. Twenty-six of 30 strains carried the *pga* locus, 25 of 30 expressed immunologically detectable PNAG, and 21 of 30 could be killed by rabbit IgG specific for the deacetylated form of the staphylococcal PNAG. Ab to staphylococcal PNAG protected mice against lethality from five different *E. coli* strains expressing PNAG. PNAG expression by both Gram-negative and Gram-positive organisms could make this antigen a conserved vaccine target for multiple pathogenic species of bacteria.

vaccine | polyglucosamine | *pga* locus | *ica* locus | biofilms

Staphylococcus aureus and coagulase negative staphylococci are the most frequent causes of nosocomial bloodstream infections (1). One virulence factor in many such infections is the ability to form a biofilm on synthetic materials (2–4). Most biofilm-forming strains of *S. aureus* secrete a large exo-polysaccharide, poly-*N*-acetylglucosamine (PNAG) that is involved in intercellular adhesion and is sometimes referred to as polysaccharide intercellular adhesin or PIA (5–7). PNAG also protects planktonic cells *of S. aureus* and *Staphylococcus epidermidis* from Ab-independent phagocytic killing by the host immune system (8–10). PNAG is synthesized by proteins encoded by the *icaADBC* genes of the intercellular adhesin locus (*ica*) (6, 11, 12). PNAG is also a promising vaccine candidate for staphylococcal infections (5, 13–17).

Wang *et al*. (18) recently found that *Escherichia coli* has a genetic locus homologous to the staphylococcal *ica* locus, which they termed *pgaABCD*, although it was originally designated as the *ycdSRQP* locus in the annotated *E. coli* K12 genome. *pgaABCD* encodes proteins able to synthesize an exo-polysaccharide that they designated polyglucosamine (PGA), which they found to be chemically nearly identical to PNAG (18). They also reported that homologues of the Gram-negative bacterial *pga* locus have been identified in a number of other important pathogens, including *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica* (18), but they did not demonstrate PNAG production by these species. However, Kaplan *et al.* (19) showed that *Actinobacillus actinomycetemcomitans* and *Actinobacillus pleuropneumoniae* have a *pga*-homologous locus and synthesize PNAG, as do *Bordetella* spp. (20). Thus, there is increasing evidence that PNAG can be synthesized as a surface polysaccharide by a variety of pathogenic bacteria.

In this work, we analyzed whether 30 clinical isolates of *E. coli* from urinary tract and neonatal blood stream infections contained the *pga* locus, expressed an antigen reactive with antibodies raised to staphylococcal PNAG, could be killed in an opsonophagocytosis assay with the antibodies to the staphylococcal PNAG and whether mice could be protected by passively administering Ab to PNAG when challenged with lethal doses of *E. coli* by i.p. injection.

Results

***Presence of pga* Locus and Production of PNAG by *E. coli* Clinical Strains.** Table 1 summarizes the characteristics of the 30 *E. coli* clinical isolates, comprising 18 urinary tract infection (UTI) isolates and 12 isolates from neonatal bacteremia for the presence of the *pgaA* and *pgaC* loci, which are not always present in the same operon in Gram-negative bacteria (18). We detected both of these *pga*-associated genes in 26 of the 30 clinical *E. coli* isolates. None had only one of the genes.

Fig. 1 *Left* shows the specificity of the PNAG immunoblot reagents when tested against *S. aureus* and *S. epidermidis* strains known to produce PNAG or deleted for the staphylococcal *ica* genes (Δ*ica*), as well as the sequenced *E. coli* UTI strain CFT073, known to have a *pga* locus (18, 21). We also found that the clinical *E. coli* UTI isolate UTI-J expressed detectable PNAG, whereas UTI-JΔ*pga* did not; the UTI-JΔ*pga* strain complemented in trans with pPGA reacquired the ability to produce PNAG. Identical results were obtained with the *E. coli* BW25113 parental, Δ*pga*, and transcomplemented strains (data not shown).

When EDTA extracts prepared from 18 *E. coli* UTI isolates and 12 *E. coli* isolates from neonatal bacteremia were probed for expression of PNAG, 25 of 30 (83%) produced detectable levels of PNAG (Fig. 1 *Center* and *Right*). All 7 of the *E. coli* isolates from neonates known to produce the K1 capsular polysaccharide also produced PNAG (Fig. 1 *Right*, lanes H–K, rows 1 and 3). A

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Abbreviations: dPNAG, deacetylated PNAG; DT, diphtheria toxoid; *ica*, intercellular adhesin; NICU, neonatal intensive care unit; PGA, polyglucosamine; PNAG, poly-*N*-acetylglucosamine; UTI, urinary tract infection.

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Table 1. Molecular and immunological characterization of all *E. coli* clinical isolate strains (n = 30)

Group	No. of strains	DNA*		PNAG blot†			Opsonophagocytic killing‡		
		−	+	−	+	++	<10%	11–30%	>31%
UTI	18	2§	16	3§	6	9	4 (13%)	5 (17%)	9 (30%)
NICU	12	2§	10	2§	2	8	5 (17%)	0 (0%)	7 (23%)

*No. of strains without (−) or with (+) *pga* locus, determined by PCR amplification of *pgaA* and *pgaC* genes.
†No. of strains producing PNAG detected by immunoblot: −, no signal detected; +, weak signal detected; ++, strong signal detected.
‡No. of strains falling into each category; the percentage of all strains (UTI plus NICU groups) is shown in parentheses.
§All four strains negative for the *pga* locus were also negative for PNAG by immunoblotting.

summary of the semiquantitative detection of PNAG production determined by immunoblot is given in Table 1. The 5 strains producing no detectable PNAG included the 4 strains negative for the *pga* locus. Lack of detectable PNAG in the other strain could be due to low-level synthesis of PNAG or mutations in the *pga* locus preventing production of functional biosynthetic genes.

Opsonic Activity of Ab Raised to *S. aureus* PNAG Glycoforms Against Clinical Isolates of *E. coli*. Fig. 2 presents the opsonic killing activity against 4 different E. coli clinical isolates using dilutions of rabbit polyclonal antibodies raised to either a poorly acetylated glycoform of PNAG designated dPNAG (deacetylated PNAG) or raised to highly acetylated (>95% substitution) native PNAG. Prior results with *Staphylococcus* showed that antibodies raised to dPNAG had superior opsonic killing activity and protective efficacy in animals (14). Although many *E. coli* strains can be killed in a bactericidal assay using only Ab to capsular polysaccharides and complement, we found that after absorption of the complement source with *E. coli* cells to remove any antibody from this reagent, the Ab to PNAG could not mediate bacterial killing in the absence of phagocytes. For all 4 of the strains at essentially all of the serum dilutions tested, dPNAG Ab mediated superior opsonic killing compared with Ab raised to native PNAG. Thus, as we found for *S. aureus* (14), Ab raised to the dPNAG antigen mediated better killing than Ab raised to then native PNAG antigen. Superior opsonic killing activity correlates with a better ability to deposit opsonically active fragments of the third component of complement on the bacterial surface (22).

When we evaluated the opsonic killing of the 30 *E. coli* clinical isolates by the rabbit polyclonal Ab raised to dPNAG, we found that 9 of the *pga*-positive strains were poorly killed *in vitro* (<10%), 5 (all UTI isolates) were modestly killed (11–30%) and 16 were killed fairly well (>30%) (Fig. 3). Results are also summarized in Table 1. None of the *E. coli* strains lacking the *pga* locus was killed >10%. Several of the strains (indicated on the graph in Fig. 3) were then used in protection studies.

The specificity for the PNAG antigen of the opsonically active Ab in antisera raised to dPNAG conjugated to diphtheria toxin (dPNAG-DT) was shown by the loss of killing activity in the immune sera, initially diluted to have 40–70% killing activity, after adsorption with *E. coli* J. When the sera were adsorbed with the Δ*pga* strain, no diminution in opsonic killing was observed [supporting information (SI) Fig. 4].

Protection Studies. To determine the specificity of the goat antisera used in the protection studies, we first evaluated binding of pre- and postimmunization antisera to *E. coli* J and *E. coli* JΔ*pga* whole cells by ELISA. Although there was binding of both the preimmune and immune goat antisera to both strains, there was no difference in binding of pre or postimmune sera when using the *E. coli* JΔ*pga* strain (SI Fig. 5), whereas the PGA-positive *E. coli* J strain bound the immune serum noticeably better. Thus, if the preexisting Ab could protect against *E. coli* infection, it should be manifest in mice given preimmune serum. Table 2 summarizes the protective activity in a murine i.p. infection model of these goat antisera against a selection of *E.*

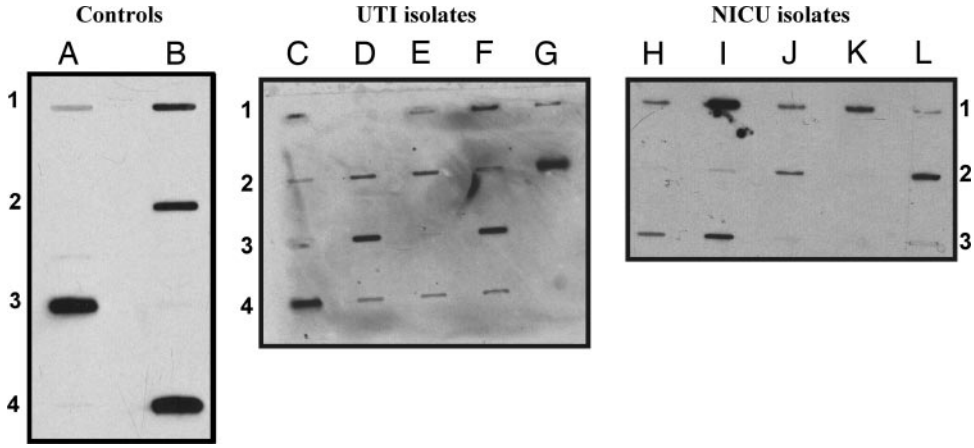

Fig. 1. PNAG expression in control strains and *E. coli* clinical isolates. Cell surface extracts were probed with affinity-purified goat Ab to PNAG. (*Left*) Control strains. Lanes: A1, *S. aureus* MN8; A2, *S. aureus* MN8Δ*ica*; A3, *S. epidermidis* M187; B1, *E. coli* CTF073; B2, *E. coli* UTI-J; B3, *E. coli* UTI-JΔ*pga*; B4, *E. coli* UTI-JΔ*pga*+pPGA. (*Center*) Clinical UTI isolates from different patients collected in the U.S. Lanes: C1, UTI-D; C2, UTI-E; C3, UTI-F; C4, UTI-G; D1, UTI-H; D2, UTI-I; D3, UTI-J; D4, UTI-K; E1, UTI-L; E2, UTI-MI; E3, UTI-N; E4, UTI-O; F1, UTI-P; F2, UTI-Q; F3, UTI-R; F4, UTI-S; G1, UTI-T; G2, UTI-U. (*Right*) Neonatal isolates from different patients in Europe and the U.S. Lanes: H1, NICU-1; I1, NICU-2; J1, NICU-3; K1, NICU-4; L1, NICU-5; H2, NICU-6; I2, NICU-7; J2, NICU-8; K2, NICU-9; L2, NICU-10; H3, NICU-11; I3, NICU-12. Extracts from isolates in the first and last rows (rows 1 and 3) are from *E. coli* strains also positive for the K1 capsular antigen.

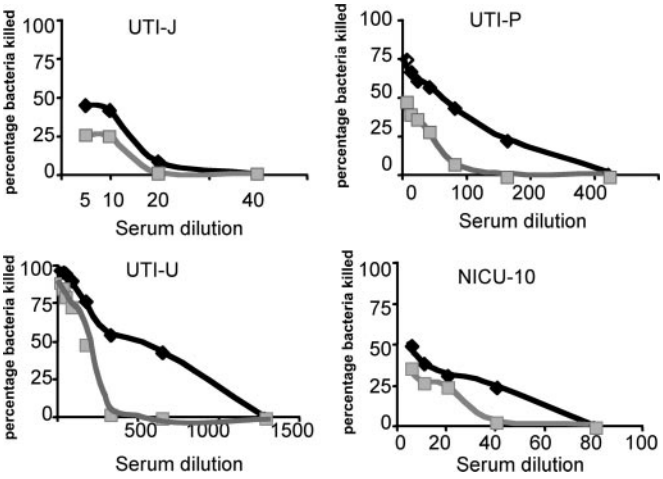

Fig. 2. Opsonophagocytic killing activity of goat Ab raised to either dPNAG (black diamonds) or PNAG (gray squares) against *E. coli* strains UTI-J, UTI-P, UTI-U, and NICU10. Points represent means of triplicates; SE bars are <5% and are contained within each point.

Table 2. Protection studies in an intraperitoneal murine model

Strain	Dose per mouse, cfu*	Preimmune goat serum	Immune serum
	Anti-dPNAG-DT goat		
UTI-J	2.5×10^8	0/8 (0%)†	5/8 (63%)‡
UTI-JΔ*pga*§	1.0×10^9	2/8 (25%)	2/8 (25%)
UTI-H§	5.0×10^8	0/8 (0%)	0/8 (0%)
UTI-P	2.5×10^8	0/8 (0%)	7/8 (88%)‡
UTI-U	5.0×10^8	1/8 (13%)	7/8 (88%)‡
NICU-3	3.9×10^8	0/8 (0%)	7/8 (88%)‡
NICU-10	1.25×10^8	4/24 (17%)	14/24 (58%)‡
	Anti-human IgG		
UTI-J	1.0×10^8	1/8 (13%)†	2/8 (25%)
UTI-JΔ*pga*§	2.0×10^9	1/8 (13%)	1/8 (13%)

*Minimal bacteria concentration necessary to kill at least 75% of the mice in each group.
†No. of surviving mice over the total mice used; percentage survival is in parentheses.
‡Survival ≥58% from immune goat serum significant at $P \leq 0.01$, Fisher's exact test compared with normal goat serum giving survival of ≤25%.
§Lacks *pga* locus and PNAG production.

coli strains. For each *E. coli* strain, we selected a challenge dose that would kill at least 75% of the mice. Goat Ab was used because of requirements for significant volumes of sera in these experiments, but this serum has been shown to have opsonic killing activity to *S. aureus* strains comparable with that of the rabbit antiserum used in the opsonic assays (Fig. 3) (14). This conclusion was verified by using 3 strains of *E. coli* (data not shown). As can be seen (Table 2), for the 5 *E. coli* strains expressing PNAG, there was significant protection ranging from 58% to 88% of animals given dPNAG-specific immune serum, whereas animals given preimmune goat serum had survival rates of 0–25% despite the presence of non-PNAG-specific Ab to *E. coli* in the preimmune serum. Furthermore, although both the preimmune and immune antisera bound to the *E. coli* JΔ*pga* cells, there was no protective efficacy against this strain or against the *E. coli* UTI-H strain, which is negative for the *pga* locus.

When an antiserum raised to an irrelevant antigen in conjunction with the same adjuvants as used with the dPNAG-DT conjugate was given to mice, no protection against either *E. coli* J or *E. coli* JΔ*pga* was observed (Table 2). All dead mice had bacterial dissemination into their spleens, indicative of bacteremic spread from the initial focus of infection, whereas survivors had spleens lacking detectable bacteria as determined after the mice were killed.

Discussion

E. coli is an important cause of gastrointestinal diseases (23–25), UTIs (26–28), and neonatal meningitis and sepsis (29, 30), but the potential for vaccination against these strains has been deemed low because of the chemical and serologic variability of the most efficacious target antigens, lipopolysaccharide O antigens and capsular polysaccharides. Finding a conserved surface polysaccharide among diverse *E. coli* strains would raise the potential for use of such an antigen as a vaccine. Furthermore, finding that such an antigen was also expressed by other common but phylogenetically diverse pathogens, such as staphylococci, further raises interest in the vaccine potential of such an antigen. Given that vaccines injected into humans to prevent *Streptococcus pneumoniae* invasive disease, as well as *Neiserria meningitidis*, *Haemophilus influenzae* type b, and *Salmonella enterica* serovar Typhi infections are all composed of capsular polysaccharides as the major vaccine component, finding a broadly expressed surface polysaccharide such as PNAG among diverse pathogens that is a target for opsonic and protective Ab represents a potential means to have a vaccine effective against a wide number of important human bacterial pathogens.

As a first step in determining whether PNAG has such broad vaccine potential, we followed up on the recent findings of Wang *et al*. (18), who showed that strains of *E. coli* for which a sequenced genome has been produced all contain a genetic locus

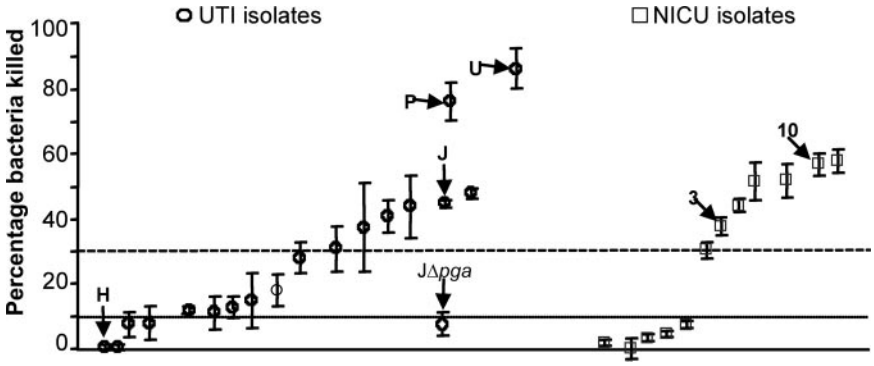

Fig. 3. Opsonophagocytosis of the *E. coli* clinical isolates. Circles, UTI isolates. Squares, isolates from neonates in the NICU. A symbol indicates mean of triplicates, error bars the SD. Six isolates used in the protection studies are noted, as is *E. coli* UTI-JΔ*pga*, for which only background levels of killing of were achieved. UTI strain H lacks the *pga* locus.

homologous to the staphylococcal *icaADBC* locus, designated *pgaABCD* by them (18). The presence of a *pga* locus is associated with the production of PNAG. In both staphylococci and *E. coli*, the PNAG polymer promotes biofilm formation and is cell surface-associated (5, 6, 18). In staphylococci, PNAG has been found to be a virulence factor (8, 31–33) and target for protective Ab (14–16). In this work, we report that a high percentage of *E. coli* UTI and neonatal bacteremia isolates contain the *ica*-homologous *pga* genetic locus and synthesize PNAG that is immunologically cross-reactive with PNAG isolated from *S. aureus*, and the production of PNAG by *E. coli* allows opsonically active antibodies raised to the *S. aureus* dPNAG antigen to mediate *in vitro* killing of these organisms. When the *pga* locus was deleted from *E. coli* strains BW25113 and clinical UTI isolate strain J, they no longer synthesized PNAG, but when the *pga* locus was added back in trans, PNAG synthesis was restored. In addition, clinical isolates lacking detectable *pgaABCD* also failed to produce PNAG. One isolate contained the *pgaA* and *pgaC* genes but nonetheless failed to produce detectable PNAG, potentially as a result of a mutation or gene repression. Overall, production of immunologically cross-reactive PNAG antigen was detected in 83% of 30 clinical isolates of *E. coli*.

To explore the potential of PNAG to vaccinate against *E. coli* infections, we initially compared the opsonic killing activity of antibodies raised with a mostly deacetylated form of PNAG (dPNAG, ≈15% residual *N*-acetyl groups) with that of antibodies raised to native PNAG (>95% *N*-acetyl groups). Prior results with staphylococci have shown that the Ab to the dPNAG antigen has greater activity in opsonizing *S. aureus* and *S. epidermidis* for phagocytic killing (14, 22, 34) and in mediating protective efficacy in experimental animal infections (14, 34). Ab to dPNAG did not mediate killing of *E. coli* in the absence of phagocytes. As we found with staphylococci, Ab raised to the dPNAG had superior opsonic activity against *E. coli*. The molecular basis for this phenomenon has been elucidated by the studies of Vuong *et al.* (10) by using *S. epidermidis*, wherein partial deacetylation of PNAG by the IcaB protein is needed to retain the antigen on the bacterial cell surface. We have found that the IcaB protein in *S. aureus* behaves identically (N.C., K.K.J., T.M.-L., D. B. Pier, C. Kelly-Quintos, D.A.G., J. Azeredo, and G.B.P, unpublished observations). Therefore, the epitopes recognized by the antibodies raised to dPNAG are present at a higher density on the bacterial cell surface, which is needed for efficient opsonic killing.

We next analyzed the protective efficacy of Ab raised to dPNAG conjugated to DT (14) against several *E. coli* strains expressing high or moderate levels of PNAG, as well as some negative controls comprising *E. coli* strains lacking the *pga* locus. Compared with normal goat serum, the goat serum raised to dPNAG-DT effectively protected against lethal sepsis after i.p. infection, except with the *pga*-negative controls, as expected. Although the i.p. infection model might be considered to mimic aspects of *E. coli* peritonitis, including bacteremic spread as evidenced by the presence of bacteria in the spleens of nonprotected animals, it clearly does not mimic the various manifestations of *E. coli* UTI or neonatal bacteremia. Nonetheless, a high-dose challenge model is a robust initial test of a vaccine's potential efficacy, primarily evaluating the *in vivo* activities of the antibodies and expression of the antigen. Although additional studies are needed to determine whether Ab to dPNAG can protect against experimental *E. coli* bladder infections (35, 36) or bacteremia emanating from the gastrointestinal tract (37, 38), these initial studies validate the potential of dPNAG as a protective vaccine for diverse and common pathogens such as *E. coli*, *S. aureus*, and coagulase-negative staphylococci. In addition, if pathogens such as *Bordetella* spp., *Yersinia* spp., *A. actinomycetemcomitans*, and others known to have a *pga*-homologous genetic locus (18, 19) also produce PNAG in a manner and form rendering antibodies to the antigen opsonic and protective, then a properly constructed PNAG-based vaccine could be protective against a broad array of human and animal pathogens. Currently, no such broadly expressed, protective surface polysaccharide antigen, the basis for most successful vaccines used in humans to elicit antibodies that kill bacterial pathogens, has been identified.

Materials and Methods

Strains and Media. To evaluate the expression of PNAG, we used several strains of staphylococci, including PNAG-positive *S. aureus* MN8 (15) and *S. epidermidis* M187 (39) and PNAG-negative *S. aureus* MN8Δ*ica* (8). *E. coli* strains used included CFT073, a urinary tract isolate previously shown to contain the *pga* locus (18), and 30 clinical isolates of *E. coli* collected over different periods of time and also geographically distant. These isolates included 18 obtained from 18 different patients hospitalized at Brigham and Women's Hospital, Boston, MA, and were provided by the Clinical Microbiology Laboratory, courtesy of Andrew Onderdonk, and 12 blood isolates from patients in neonatal intensive care units (NICU) in Europe and the U.S. which were kindly provided by Kwang-Sik Kim, Baltimore, MD, or Peter W. Taylor, London, U.K. Seven of the *E. coli* isolates from neonates were known to express the K1 capsular polysaccharide frequently found among isolates from such patients (40). *S. epidermidis*, *S. aureus*, and *E. coli* strains were grown for 16 h at 37°C in tryptic soy broth/1% (gram percent) glucose (TSBG).

Construction of *E. coli pga*-Deleted and Complemented Strains. The *pga* locus was replaced with a chloramphenicol acetyltransferase cassette by the PCR-mediated one-step method of gene inactivation described by Datsenko and Wanner (41). To accomplish this goal, *E. coli* BW25113 and the clinical UTI isolate strain J were first transformed with the red recombinase expression plasmid pKD46 then grown overnight at 25°C in SOC medium (2×10^{-2} g/ml Bacto-tryptone/5×10^{-3} g/ml yeast extract/5.84×10^{-4} g/ml NaCl/1.86×10^{-4} g/ml KCl/20 mM $MgCl_2$/20 mM Glc, pH 7.0) containing 100 μg of ampicillin per ml and 10 mM L-arabinose. The next day, the cells were collected by centrifugation, washed three times with deionized H_2O containing 10% glycerol, and resuspended at a 50-fold concentration over the original volume. A PCR product of the chloramphenicol acetyltransferase cassette was generated from plasmid pKD3 by using the primer pair pgadeleteFWD (5′-CCGGACTAGCGCTTTTTCTGAAACCACCATTTTTATTTGCCCCTGGCTGGGTGTAGGCTGGAGCTGCTTC-3′) and pgadeleteREV (5′-AAGTAGCAGAAAAAGGTGCCCGAAAACCAAATGGGCTTTGAAACTTCTTACATATGAATATCCTCCTTAG-3′). The PCR product had 5′ and 3′ ends homologous to the 5′ and 3′ ends of the *E. coli pga* locus and was gel-purified by using the QIAquick gel extraction kit (Qiagen, Valencia, CA). Electrocompetent *E. coli* cells were transformed with the purified PCR product by electroporation, and transformants were selected on LB agar containing 30 μg of chloramphenicol per ml. Chloramphenicol-resistant bacteria were analyzed by PCR to ascertain that the chromosomal *pga* locus was deleted (Δ*pga* strains) and were then cured of the pKD46 plasmid by two rounds of growth at 42°C in the absence of ampicillin. The *E. coli* BW25113 parental and Δ*pga* strains were used to confirm the specificity of the immunoblot, and the Δ*pga* strain was used to absorb antisera used in the opsonic killing assay.

To transcomplement the *pga*-deletion mutants, the entire *pga* locus was amplified from *E. coli* BW25311 DNA by PCR using primers pgaexpressFWD (5′-ATGTATTCAAGTAGCAGAAAAAGGTGC-3′) and pgaexpressREV (5′-GTGTTTACGCCCGGACTAGC-3′) and cloned into the arabinose-inducible pBAD-TOPO vector (Invitrogen, Carlsbad, CA). The DNA sequence of the insert in the resulting construct, pPGA, was determined to confirm identity to the known *pga* DNA

sequence and then used to transform the *pga*-deletion mutant strains by electroporation. Transformants were selected on LB agar containing 100 μg ampicillin per ml. Addition of arabinose to the growth medium was not necessary for sufficient expression levels in pPGA-complemented strains.

Detection of PNAG Production by Bacteria. Immunoblots for production of PNAG were performed essentially as described previously (7, 42) with minor modifications. Bacteria were grown to stationary phase in TSBG, and the cultures were diluted in this growth medium to produce an A_{600} = 1.5. Bacterial cells were pelleted by centrifugation, and equivalent amounts of each of the different bacterial preparations were resuspended in 300 μl of 0.5 M EDTA (pH 8.0) and incubated for 5 min at 100°C. Cells were removed by centrifugation at 10,500 $\times$ *g* for 6 min, and 100 μl of the supernatant was then incubated with 10 μl of proteinase K (20 mg/ml; Qiagen) for 60 min at 60°C. Proteinase K was heat-inactivated by incubating for 30 min at 80°C. This solution was diluted 3-fold in Tris-buffered saline (20 mM Tris·HCl/150 mM NaCl, pH 7.4), except for the extract from *S. epidermidis* M187, which was diluted 1,000-fold because of hyperproduction of PNAG by this strain, and 200 μl of each dilution was immobilized on a nitrocellulose filter attached to a slot-blot vacuum manifold. The filter was blocked with a solution of 1% BSA, then incubated for 2 h with a rabbit Ab raised to *S. aureus* PNAG conjugated to DT, produced as described previously (14) and further purified on an affinity column, also as described previously (22). The secondary Ab used was a horseradish peroxidase-conjugated anti-rabbit IgG Ab (Southern Biotech, Birmingham, AL) diluted 1:6,000, and detection of the PNAG antigen used the enhanced chemiluminescence (Amersham, Piscataway, NJ) Western blotting system.

***Detection of the pga* Locus in *E. coli* Clinical Isolates by PCR.** Genomic DNA from *E. coli* strains was extracted by using a DNeasy tissue kit (Qiagen). Primers used for the detection of the *pgaA* (5′-GGCTTTGAAACTTCTTACTGC-3′ and 5′-CCTGTTTATCT-TGCCCGGCC-3′) and *pgaC* (5′-ATGATTAATCGCATCG-TATCG-3′ and 5′-CATCGGTTCCACAATATATGC-3′) were custom synthesized by Qiagen Operon (Alameda, CA). For the PCR we added 25 μl of PCR supermix high-fidelity (Invitrogen), 0.5 μl of each primer, and 2 μl of DNA from the bacterial strains. PCR conditions comprised an initial 5-min denaturation step at 94°C followed by 32 cycles of 30 s at 94°C, 30 s at 50°C, and 45 s at 72°C, and a final extension step of 5 min at 72°C.

Antisera. We used antibodies raised to either native *S. aureus* PNAG, wherein the glucosamine monosaccharides contained ≈95% acetate groups, or dPNAG, wherein the level of substitution with acetate groups was ≈15% (29). Both polysaccharides were conjugated to DT as described (14) and used to raise antibodies in rabbits, also as described previously (14). The dPNAG-DT conjugate was also used to immunize a goat as described previously (20). Specificity of the rabbit antiserum used in the opsonophagocytic assay was evaluated by adsorbing the sera with either PNAG-expressing *E. coli* J or the isogenic PNAG-negative *E. coli* Δ*pga* strain. Specificity of the goat antisera for PNAG expression by *E. coli* was evaluated by whole-cell ELISA using the *E. coli* J and JΔ*pga* strains. For some control experiments, we used a goat antiserum to human IgG raised by using the same adjuvants and immunization schedule as was used for the dPNAG-DT conjugate vaccine and was supplied by Lampire Biological Laboratories, Pipersville, PA. We have shown previously that the goat antiserum has comparable binding and opsonic activity against PNAG-producing *S. aureus* as does the rabbit antiserum raised to the dPNAG-DT conjugate (14).

Opsonic Killing of *E. coli* Clinical Isolates. WBC were prepared from fresh human blood collected from healthy adult volunteers. Twenty-five milliliters was mixed with an equal volume of dextran/heparin buffer and incubated at 37°C for 1 h. The upper layer containing the leukocytes was collected, the cells were pelleted by centrifugation, and the remaining erythrocytes were removed by hypotonic lysis after resuspension of the cell pellet in 1% NH_4Cl with incubation for 10 min at room temperature. WBC were then washed three times and resuspended with RPMI containing 15% heat-inactivated (56°C, 30 min) FBS (RPMI/FBS). Trypan blue staining was used to determine the number of viable leukocytes, then the final WBC count was adjusted to 2.5 $\times$ 10^7 WBC per ml. The complement source (1 ml of baby rabbit serum diluted 1:10 in RPMI/FBS) was adsorbed twice at 4°C for 45 min with continual mixing using bacteria resuspended from a pellet containing ≈10^9 cfu of *E. coli* BW25113 to remove natural Ab that mediated bactericidal killing of some of the target *E. coli* strains. The rabbit antisera were diluted 1:10 in RPMI/FBS and absorbed twice at 4°C for 45 min with continual mixing using bacteria resuspended from a pellet containing ≈10^9 cfu of *E. coli* BW25113Δ*pga*. After absorption, the complement solution and the Ab were centrifuged and filter-sterilized. The bacterial strains to be evaluated for phagocyte-dependent killing activity of the rabbit Ab to PNAG were grown overnight in TSBG and then adjusted to an A_{650} = 0.4. A 1:100 dilution (3–6 $\times$ 10^7 cfu/ml) was then made in RPMI/FBS for use in the killing assay.

The opsonophagocytic assay was performed with 100 μl of leukocytes/100 μl of bacteria/100 μl of the complement solution/100 μl of a 1:10 or higher dilution of the Ab. Several controls were used: each component individually and all of the possible combinations of the individual components with one missing. The missing volume was made up with 100 μl of RPMI/FBS. The reaction mixture was incubated on a rotor rack at 37°C for 90 min. The tubes were vortexed for 15 s, and then dilutions were made in TSB with 0.025% Tween (to prevent bacterial sticking to pipette tips and the walls of dilution vessels); samples were than plated onto tryptic soy agar plates. The percentage of killing was calculated by determining the ratio of the cfu surviving in the tubes with bacteria, leukocytes, complement and Ab, to the cfu surviving in the tubes with bacteria, complement, leukocytes, and RPMI/FBS. Each assay was done in triplicate and repeated two or three times.

Murine Lethality Model. To evaluate the *in vivo* protective efficacy of Ab to PNAG, we used an i.p. infection model in mice, as described previously (8, 14). Briefly, groups of eight mice (FVB, female, 6–8 weeks of age) were immunized i.p. 24 h before and 4 h after infection with 0.3 ml of normal goat serum or immune goat serum raised against staphylococcal dPNAG conjugated to DT. Some mice received a heterologous immune serum raised to human IgG with the same adjuvants and schedule as used with the dPNAG-DT conjugate. Bacteria were grown overnight in TSBG and then resuspended in sterile PBS to ≈5 $\times$ 10^8 to 5 $\times$ 10^9 cfu/ml, depending on the strain. For each strain, we selected a minimal dose that would kill at least 75% of the mice. Mice were challenged i.p. with a dose of 1.25 $\times$ 10^8 to 1 $\times$ 10^9 cfu in 0.2 ml of PBS and monitored at least twice daily. When mice became moribund, as defined by hunched appearance, markedly increased respiration rate, piloerection, and lack of an ability to move in response to being touched, they were considered moribund; they were killed and counted as dead for these experimental outcomes. After death, the spleens of the mice were removed, homogenized in RPMI, and cultured on tryptic soy agar and MacConkey agar to determine that the *E. coli* had disseminated from the initial site of infection.

Statistical Analysis. Differences in outcomes in the protection studies were determined by Fisher's exact test.

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