# Antibodies to Cell Wall Peptidoglycan of Staphylococcus aureus in Patients with Serious Staphylococcal Infections

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An enzyme-linked immunoassay was used to detect antibodies to the cell wall peptidoglycan of Staphylococcus aureus in human sera. All 170 sera from donors and patients with staphylococcal and nonstaphylococcal infections contained IgG antibodies to peptidoglycan; antibody levels varied with age, and transplacental transfer occurred. IgM antibodies to peptidoglycan were not found in donors and were present in only one patient with serious staphylococcal infection. Significantly elevated levels of IgG antibodies to peptidoglycan were observed in 20 (80%) of 25 patients with deep tissue infection with S. aureus but in only two (9%) of 22 patients with superficial staphylococcal infection. An increase in levels of antibodies to peptidoglycan generally coincided with an increase in level of IgG antibodies to teichoic acid. No cross-reactivity between peptidoglycan and teichoic acid was observed. Thus, staphylococcal peptidoglycan is immunogenic in humans, and testing for IgG antibodies to peptidoglycan may be useful in the diagnosis and follow-up of serious staphylococcal infections.

The major cell wall component of most grampositive bacteria, including Staphylococcus aureus, is an insoluble heteropolymer termed peptidoglycan. This structure forms the basic matrix of the cell wall, representing about 50% of its weight, and is composed of chains of alternating N-acetylglucosamine and N-acetylmuramic acid residues which are cross-linked through short peptide side chains [1]. In addition to providing shape and rigidity to the bacterial cell, peptidoglycans of streptococcal and staphylococcal origin have been shown to exhibit various biologic activities of potential importance in the pathogenesis of bacterial infections. Purified staphylococcal peptidoglycan

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may activate both pathways of the human complement system [2, 3] and induce fever when injected iv into animals [4]. The endotoxin-like potential of peptidoglycan is further exemplified by its capacity to produce a local Shwartzman reaction [4] and to inhibit the migration of leukocytes [5]. Antibodies to staphylococcal peptidoglycan, raised in laboratory animals, have been shown to enhance the phagocytosis of staphylococci [3, 6] and to block the inhibition of leukocyte migration by peptidoglycan [7]. The heat-stable opsonic activity for phagocytosis of staphylococci that is present in normal human sera has been proposed to be directed against the peptidoglycan moiety of the staphylococcal cell wall [8].

In contrast to the numerous reports on antibodies to staphylococcal teichoic acid [9], information about the levels and classes of antibodies to peptidoglycan in sera from patients with staphylococcal disease is lacking. The teichoic acid component, however, is believed to be relatively nontoxic and does not contribute significantly to the biologic activities of the staphylococcal cell wall [10]. In the present study we determined the levels and classes of antibodies to the biologically highly active peptidoglycan of *S. aureus* in healthy individuals and in patients with staphylococcal and nonstaphylococcal infections with a sensitive enzyme-linked immunosorbent assay (ELISA). The concentration of antibodies to peptidoglycan

was compared with that of antibodies to teichoic acid.

#### Materials and Methods

Healthy donors. Serum samples from screened healthy individuals, aged one to 65 years, were donated by Dr. J. H. T. Wagenvoort, University of Utrecht, Utrecht, the Netherlands. These sera had been collected during a mumps epidemiologic survey and were stored at -20 C until use. The male to female ratio was 1:1. Three pairs of sera prepared from maternal and neonatal cord blood, taken immediately after delivery, were obtained from the same serum bank. In addition, sera from 20 healthy donors were collected, pooled, and stored as normal pooled human serum at -70 C.

Patients with deep tissue staphylococcal infection. Twenty-five patients had clinical and bacteriologic evidence of S. aureus infection: endocarditis, four patients; osteomyelitis, 14 patients; arthritis, two patients; abscesses of internal organs, two patients; and abscesses of deep soft tissue sites, three patients. The underlying diseases were diabetes mellitus (three patients), traumatic fracture of long bones (three patients), soft tissue trauma without fracture (two patients), chronic granulomatous disease (two patients), heroin abuse (one patient), and chronic hemodialysis (two patients). Twenty-one patients had one or more blood cultures positive for S. aureus during the course of their disease. The male to female ratio was 11:14, and the age range was three to 73 years. Serum samples were obtained at least seven days after clinical symptoms of disease had developed. Serial serum samples were collected from four patients.

Patients with staphylococcal infection of body surfaces. Nineteen and three patients had culture-proven staphylococcal infection of the skin and paranasal sinuses, respectively. Fifteen of the skin-infected patients were diagnosed as having recurrent furunculosis, which required surgical drainage and/or antibacterial chemotherapy. One female patient developed recurrent sc abscesses after childhood immunizations. Staphylococcal bacteremia was never detected in these 22 patients. The male to female ratio was 9:13, and the age

range was six to 33 years. Sera were obtained at the time patients visited the clinic with active disease.

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Patients with infections not due to S. aureus. Forty-seven patients were infected with gramnegative Enterobacteriaceae, 12 patients were infected with gram-positive species other than S. aureus, and one patient had tuberculous discitis of the lumbar spine. Septicemia, septic arthritis, osteomyelitis, and pneumonia were diagnosed in 22, five, three, and four patients with gram-negative bacterial infections, respectively. Gram-positive bacterial infections were all complicated by bacteremia and included five patients with endocarditis due to Staphylococcus epidermidis (one patient), Streptococcus viridans (two patients), and enterococci (two patients). Pneumococcal pneumonia was present in six patients; four of these patients had concomitant pneumococcal meningitis. One patient had bilateral otitis media, bacteremia, and meningitis due to Streptococcus pneumoniae. The male to female ratio was 24:23, and the age range was one to 74 years. All serum samples were obtained at least 10 days after the first symptoms of disease were noted.

Miscellaneous. Immunoglobulin-deficient serum from a 12-year-old male patient with primary X-linked (Bruton type) agammaglobulinemia was provided by D. Houwert, University Hospital of Utrecht, Utrecht, the Netherlands. This serum contained <5 g of IgG/100 ml and levels of IgM, IgA, IgE, and IgD undetectable by radial immunodiffusion.

Preparation of peptidoglycan and teichoic acid antigens. S. aureus strain H was used to prepare the peptidoglycan antigen. The cell wall structure of this organism has been well characterized [11]. Peptidoglycan was obtained from purified cell walls by extraction with hot 10% trichloracetic acid [3]. The isolation of crude cell walls from S. aureus strain H and their purification by treatment with sodium dodecyl sulfate, nuclease, trypsin, and phenol were as described [8]. More than 97% of the phosphorus present in purified cell walls was removed by the treatment with hot trichloracetic acid, a result which indicated that the insoluble peptidoglycan residue was free of contaminating teichoic acids. Peptidoglycan was solubilized by digestion with egg white lysozyme (Fluka Aktein Gesellschaft, Buchs, Switzerland) [12] and stored at -70 C until use. Cell wall teichoic acid was isolated from S. aureus strain

<sup>&</sup>lt;sup>1</sup> J. H. T. Wagenvoort, "Epidemiology of Mumps in the Netherlands," Ph.D. thesis, University of Utrecht, Utrecht, the Netherlands, 1979.

Lafferty by mild extraction with 5% trichloracetic acid of purified cell walls for 72 hr at 4 C [13]. The solubilized teichoic acids were extensively dialyzed against distilled water and lyophilized.

ELISA studies. Antibody to staphylococcal peptidoglycan was quantitated using an ELISA modified from that of Engvall and Perlmann [14]. Wells of polyvinyl microtiter trays (Dynatech, Alexandria, Va.) were coated with solubilized peptidoglycan at an optimally titrated concentration of  $2 \mu g/ml$  in phosphate-buffered saline, pH 7.4. The wells were incubated with 200  $\mu$ l of the antigen solution for 1 hr at 37 C, incubated overnight at 4 C, and then washed extensively with tap water containing 0.05% Tween 20 (Merck, Darmstadt, West Germany).

Serum samples were serially diluted in phosphate-buffered saline containing 0.05% Tween 20, 0.01 M EDTA (Merck), and 4% bovine serum albumin (Organon, Oss, the Netherlands). Antigen-coated wells were incubated with 200  $\mu$ l of diluted serum, incubated for 2 hr at 37 C, and washed. Antigen-antibody complexes were detected with horseradish peroxidase-conjugated rabbit antiserum to human IgG or goat antiserum to human IgM (Miles-Yeda, Rehovot, Israel). A substrate solution containing hydrogen peroxide and 5-amino-2-hydroxybenzoic acid (Merck) was used that produced a brown color in the presence of bound conjugate. The reaction was stopped after incubation for 60 min at 37 C by adding 50  $\mu$ l of 0.1 N NaOH to each well. The intensity of the color reaction was measured in a spectrophotometer, and results are given as OD at 450 nm at indicated dilutions of serum. When antibodies to S. aureus peptidoglycan isolated from rabbit immune serum [3] were used, the ELISA could detect as little as 0.5  $\mu$ g of antibody protein/ml. Because of the well-established influence of antibody affinity on measurements made by ELISA [15], however, the results were expressed as ELISA OD at 450 nm rather than as  $\mu g$  of antibody/ml.

In some experiments, antibodies to S. aureus teichoic acid were detected by ELISA using a solution of 10  $\mu$ g of antigen/ml to coat the wells.

### Results

Antibodies to peptidoglycan in normal human sera. IgG antibodies to staphylococcal cell wall peptidoglycan were detected by ELISA in serial twofold dilutions of pooled normal human serum

(figure 1). The color reactions showed maximal A with serum diluted up to 1:32. At lower concentrations of serum the OD at 450 nm decreased accordingly, and no A of greater than the background value was recorded with serum diluted 1:512. Titration of serum from a patient with S. aureus endocarditis showed a much higher level of antibodies to peptidoglycan. An OD at 450 nm of greater than the background value was still found in serum diluted 1:8,192, and maximal A values were obtained in this patient's serum diluted up to 1:512. Human agammaglobulinemic serum was used as a negative control, and no significant A was detected with any dilution of this serum (figure 1).

Similar serum titrations revealed that IgG antibodies to staphylococcal peptidoglycan were present in each of the serum specimens from 81 healthy donors. The A values at a 1:128 dilution of these sera are given in table 1. Mean A values showed an age-related variation. Relatively high levels of antibodies to peptidoglycan were recorded in sera from donors aged six to 25 years and in individuals older than 60 years of age. Relatively low levels were found in donors 41-55 years old and in children one and five years of age. The level of antibodies to staphylococcal peptidoglycan, how-

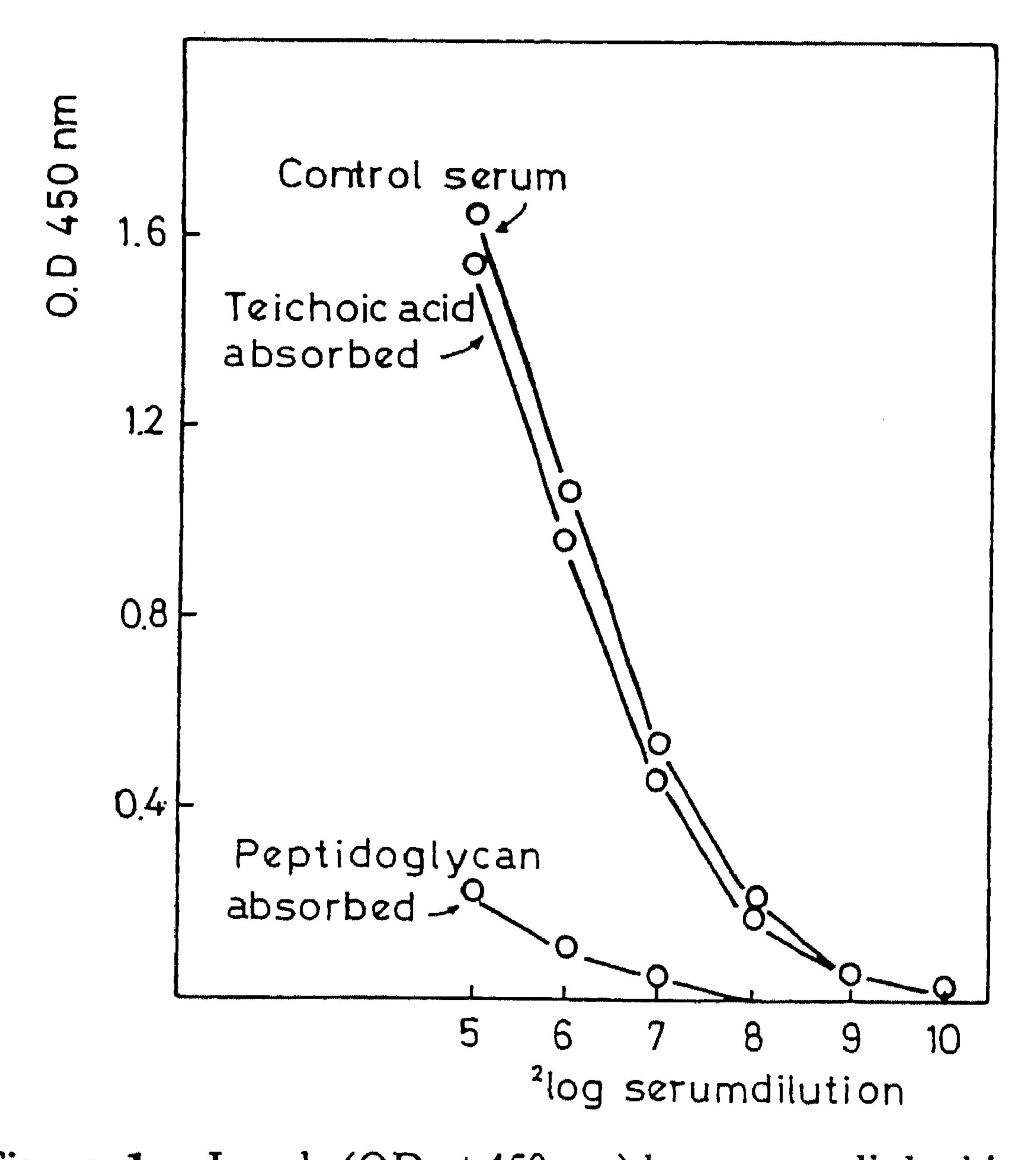


Figure 1. Levels (OD at 450 nm) by enzyme-linked immunosorbent assay of IgG antibodies to Staphylococcus aureus peptidoglycan in serial dilutions of normal pooled human serum (O—O), in serum from a patient with S. aureus endocarditis (•—•), and in serum from a patient with primary X-linked (Bruton type) agammaglobulinemia ( $\Delta$ — $\Delta$ ).

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Table 1. Levels of IgG antibodies to Staphylococcus aureus peptidoglycan measured by enzyme-linked immunosorbent assay (ELISA) in sera from healthy individuals aged one to 65 years.

Age group	No. of	Level of IgG antibodies to peptidoglycan	
(years)	individual sera	Mean ± sem	Range
1-5	6	$0.31 \pm 0.04$	0.06-0.72
6-10	10	$0.76 \pm 0.03$	0.28 - 1.12
11-15	10	$0.73 \pm 0.03$	0.41 - 1.13
16-20	5	$0.94 \pm 0.09$	0.34 - 1.40
21-25	5	$0.92 \pm 0.07$	0.51 - 1.52
26-30	5	$0.62 \pm 0.05$	0.36 - 1.03
31-35	5	$0.51 \pm 0.11$	0.01 - 1.32
36-40	5	$0.68 \pm 0.09$	0.18 - 1.14
41-45	5	$0.30 \pm 0.02$	0.15 - 0.43
46-50	5	$0.35 \pm 0.03$	0.12 - 0.49
51-55	5	$0.31 \pm 0.04$	0.07-0.65
56-60	5	$0.66 \pm 0.03$	0.46-0.94
61-65	10	$0.73 \pm 0.06$	0.13-1.03

NOTE. Full titrations of IgG antibodies to peptidoglycan in each serum specimen were performed in duplicate on two separate days, and the mean value for each individual serum specimen was used to calculate the mean  $\pm$  SEM level of the age groups. The data given are the values of OD at 450 nm measured by ELISA with the sera diluted 1:128.

ever, differed considerably among individuals within the same age group. In addition, the slope of the linear part of the individual titration curves showed variations, an observation suggesting that the affinity of antibodies to peptidoglycan may differ in healthy donors (data not shown).

IgG antibodies to peptidoglycan were present in cord blood samples of three neonates. The titration curves of these sera closely paralleled the titration curves of the sera of their respective mothers, a similarity indicating that the level and affinity of antibodies to peptidoglycan in neonates reflect those in the serum of their mothers at the time of delivery.

Attempts to demonstrate IgM antibodies specific for staphylococcal peptidoglycan in normal human sera were unsuccessful. A of greater than the background value was not obtained (data not shown).

Antibodies to peptidoglycan in sera from patients. Sera from patients with staphylococcal and nonstaphylococcal infections were screened for the presence of IgG antibodies to peptidoglycan. Because significantly increased antibody levels were found in some of these patients, the A values for sera diluted 1:512 are given in

figure 2. Sera from 44 age-matched uninfected control subjects showed a mean ( $\pm$  sp) A of 0.12  $\pm$ 0.12 at a 1:512 dilution (figure 2, left). Significantly higher levels of antibodies to peptidoglycan were present in the sera of 25 patients with staphylococcal infection of deep tissue sites; A values ranged from 0.23 to 1.46 in the sera of these patients, and the mean OD at 450 nm was 0.84 (figure 2). No such increase in level of antibodies to peptidoglycan was found in 22 patients with staphylococcal infection of the skin or mucosal surfaces; the mean of the A values was 0.18 (range, 0.01-0.68) in this patient group. A control group of 47 patients with serious infections not due to S. aureus showed normal levels of antibodies to peptidoglycan; the mean of the Avalues in these patients was 0.10 (range, 0-0.69) (figure 2). When an OD at 450 nm of 0.48 was taken as the upper limit of the normal range (corresponding to the mean + 3 sp of values for uninfected control subjects), an increased level of antibodies to peptidoglycan was found in 20 (80%) of 25 patients with deep tissue S. aureus infection, in two (9.1%) of 22 patients with super-

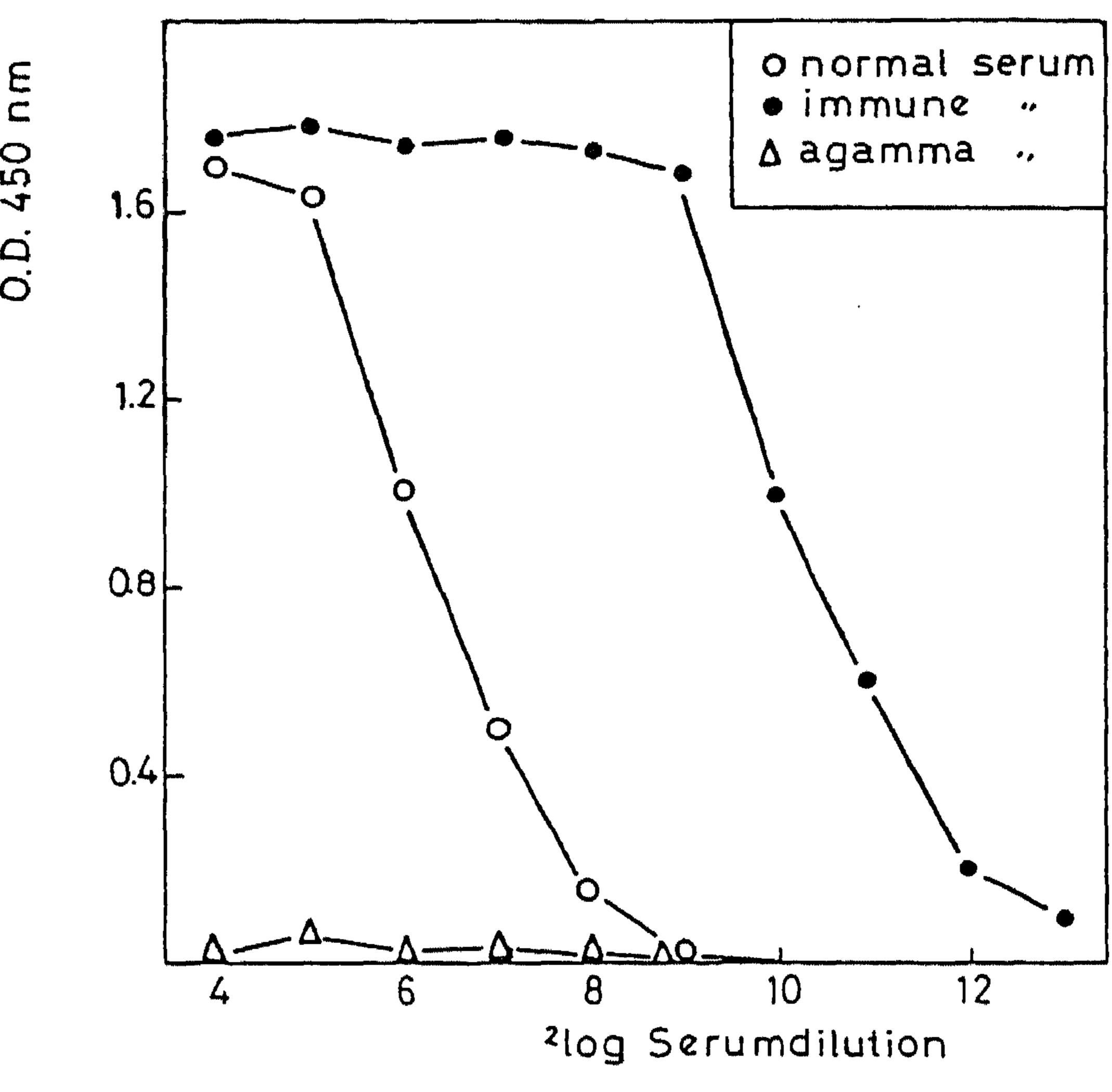


Figure 2. Levels (OD at 450 nm) by enzyme-linked immunosorbent assay of IgG antibodies to Staphylococcus aureus peptidoglycan in sera from patients with various staphylococcal infections and from infected and uninfected control subjects. The mean (O)  $\pm$  sD (vertical bars) is given for the uninfected control group. The horizontal bars in the infected patient groups indicate the respective mean values. In the infected control group, patients with gram-negative bacterial infections = ( $\bullet$ ) and those with gram-positive bacterial infections = (O), including one patient with tuberculous discitis. All sera were tested at a 1:512 dilution.

ficial staphylococcal infections, in one (2.3%) of 44 uninfected control subjects, and in one (2.1%) of 47 infected control patients. The infected control patient with an elevated antibody level had enterococcal endocarditis.

Five patients with deep tissue infection due to S. aureus did not have significantly elevated levels of antibodies to peptidoglycan. Their levels of antibodies to peptidoglycan, however, were at least 1 sp above the mean A of the uninfected control subjects. The diseases in these five patients were osteomyelitis (three patients), arthritis (one patient), and endocarditis (one patient).

IgM antibodies to peptidoglycan were undetectable in all patients except one. A 1:4 dilution of serum from a diabetic patient with acute S. aureus arthritis of the right knee gave an A value for IgM antibodies of 0.32. The serum sample was obtained at the time that culture of a joint aspirate grew S. aureus, seven days after clinical symptoms of ar-

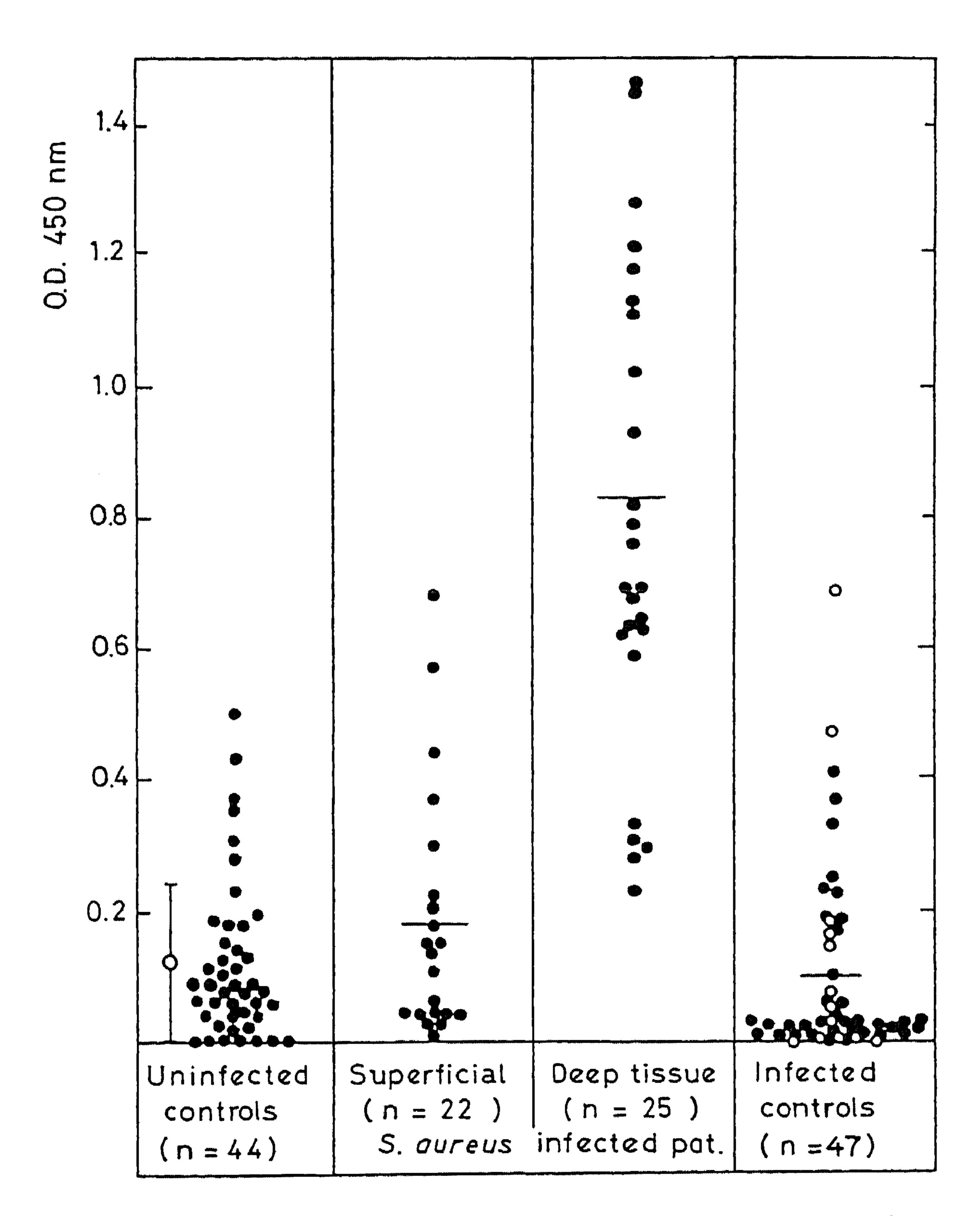


Figure 3. Levels (OD at 450 nm) by enzyme-linked immunosorbent assay of IgG antibodies to Staphylococcus aureus peptidoglycan in sera (diluted 1:512) from a patient with S. aureus endocarditis associated with drug abuse. ASta = antibodies to S. aureus  $\alpha$ -hemolysine; I.U. = international units.

thritis had developed. The A value for IgG antibodies was 1.21 in the same serum diluted 1:512.

Serial determinations of IgG antibodies to peptidoglycan. Follow-up tests were performed in four patients with serious staphylococcal disease. The results for one patient are given in figure 3. This adolescent female was admitted with a diagnosis of heroin abuse, pneumonia, tricuspid heartvalve murmurs, and multiple blood cultures positive for S. aureus. Her level of antibodies to peptidoglycan was already significantly elevated on day 7 after admission, peaked on day 11 during iv antibiotic therapy, and steadily declined thereafter; normal values were reached between two and three months after admission. The level of antibodies to staphylolysine was not elevated in the first week after admission, but it was strikingly elevated (12 international units/ml) at day 65, when the level of antibodies to cell wall peptidoglycan had dropped to almost within the normal range.

One patient with acute *S. aureus* osteomyelitis showed normal values for antibodies to peptidoglycan in three tests throughout the course of his disease. The two patients with chronic granulomatous disease had chronically elevated levels of IgG antibodies to peptidoglycan; one patient was tested nine times during a 15-month period, and all values were ≥0.63.

Correlation between levels of antibodies to S. aureus peptidoglycan and teichoic acid. A possible relationship between levels of antibodies to peptidoglycan and teichoic acid was investigated using 51 sera selected from patients and donors. A positive correlation was found (figure 4). Whereas the OD at 450 nm for IgG antibodies to peptidoglycan in these sera ranged from 0 to 1.20, the values for teichoic acid by ELISA in the same sera varied from 0.07 to 0.82. In general, the level of antibodies to teichoic acid was high in sera containing an elevated level of antibodies to peptidoglycan.

Because the observed correlation between levels of antibodies to peptidoglycan and teichoic acid could be based on cross-reactive antigenic sites within the two cell wall structures, the effect of absorption of pooled normal human serum with either peptidoglycan or teichoic acid was studied. The residual level of antibodies to peptidoglycan or teichoic acid in the absorbed sera was quantitated by ELISA (figure 5). No significant cross-absorption of antibodies to peptidoglycan by

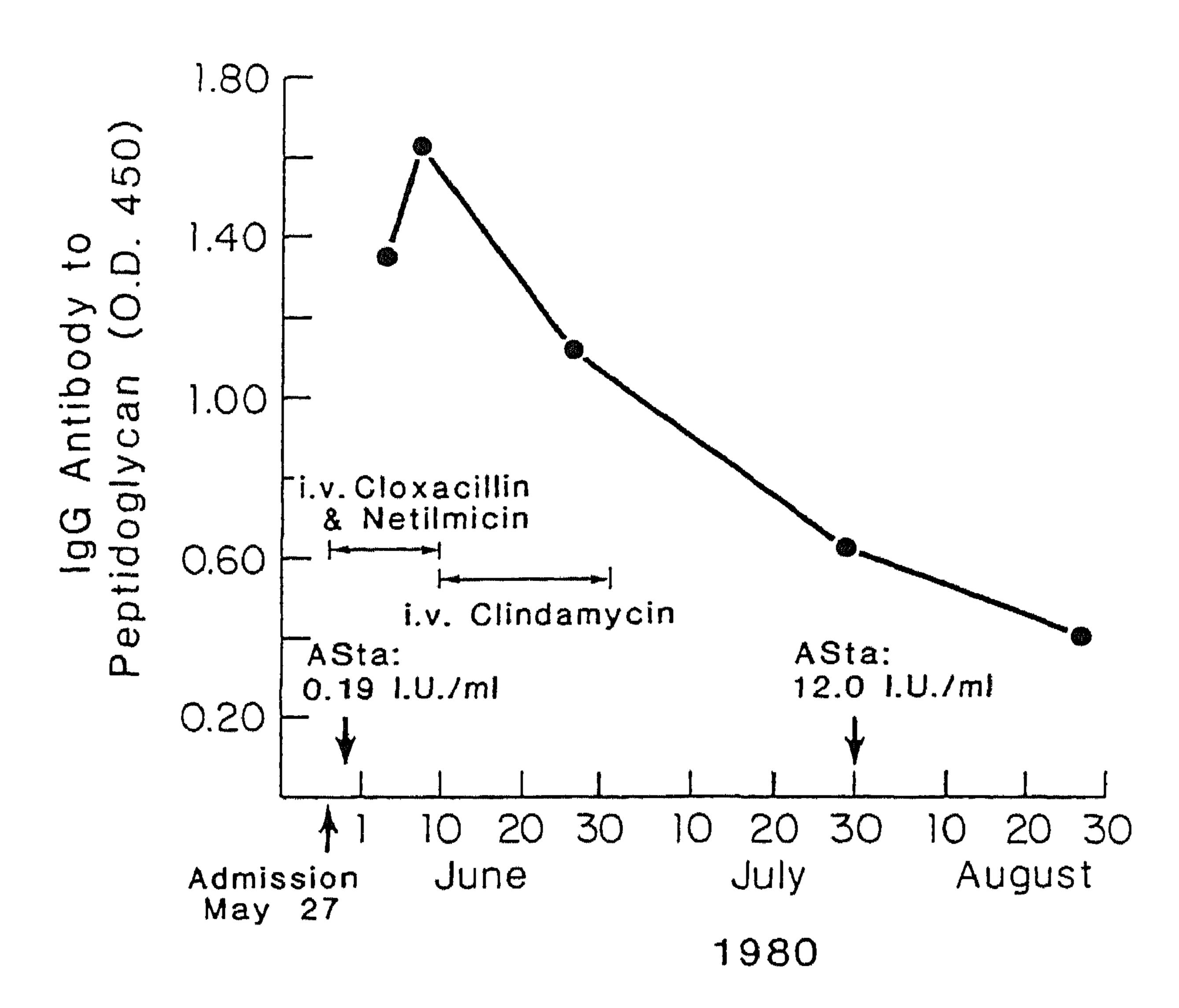


Figure 4. Correlation between levels (OD at 450 nm) by enzyme-linked immunosorbent assay of IgG antibodies to *Staphylococcus aureus* peptidoglycan and teichoic acid in 51 selected human sera at a 1:512 dilution.

teichoic acid occurred; likewise, peptidoglycan failed to absorb antibodies to teichoic acid (data not shown), results which indicated that IgG antibodies to staphylococcal cell wall peptidoglycan and teichoic acid do not recognize similar antigenic determinants and are both present in normal human serum.

## Discussion

Peptidoglycan, formerly termed mucopeptide, represents the basic structure of the cell wall of gram-positive bacteria [1]. This cell wall component was long considered to be of no biologic importance, except for providing shape and rigidity to microorganisms and as the site of action of various antibiotics. Over the past two decades, however, it has become increasingly clear that bacterial peptidoglycans, especially those of streptococcal and staphylococal origin, possess numerous immunologic and nonimmunologic properties. Important nonimmunologic phenomena elicited by staphylococcal peptidoglycans include the induction of fever and chronic inflammatory lesions of various organs in animals [4, 16] and the ability to inhibit leukocyte migration in vivo as well as in vitro [5, 17].

The role of antibodies to peptidoglycan in human staphylococcal disease is largely unknown.

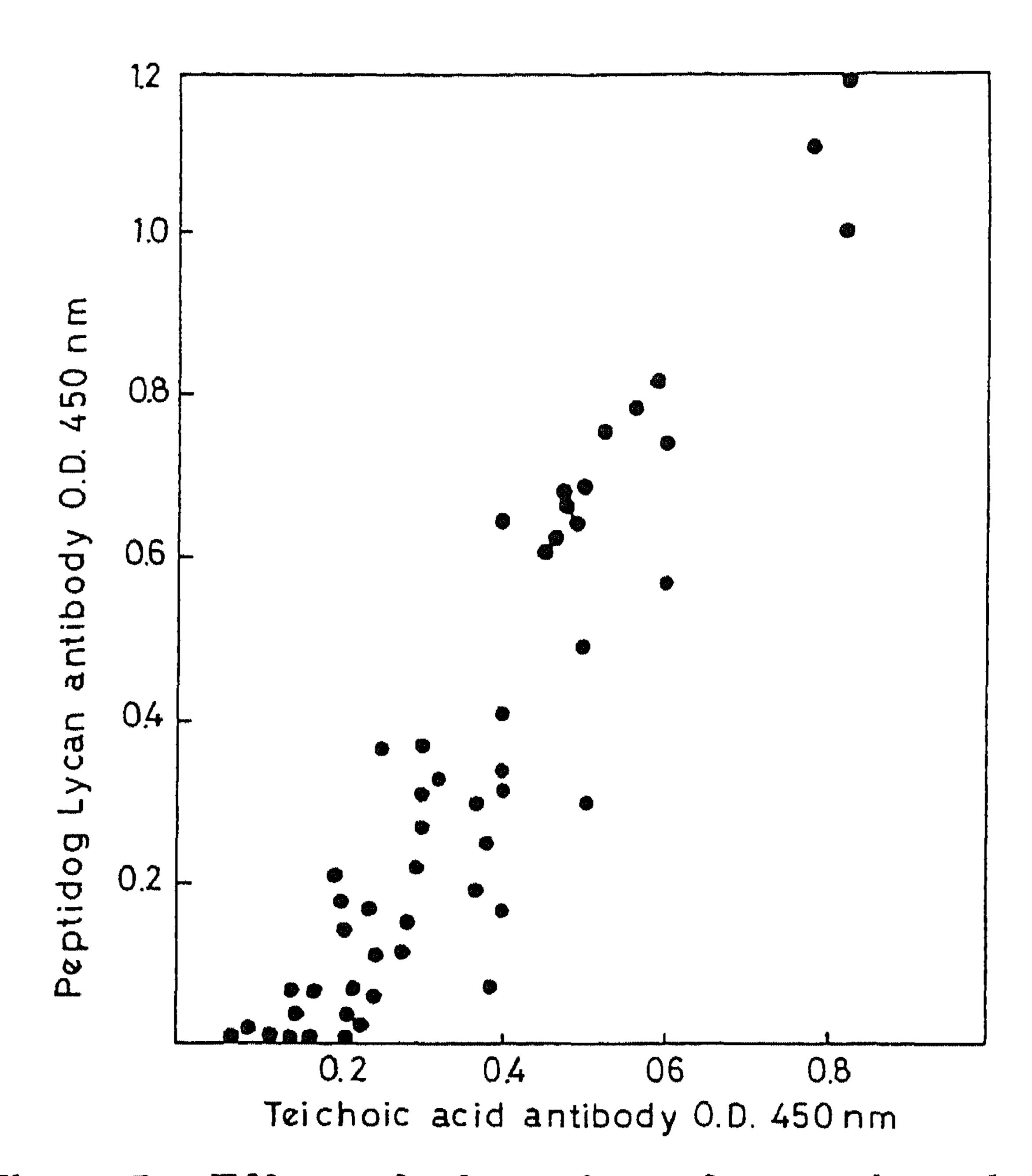


Figure 5. Effect of absorption of normal pooled human sera with purified teichoic acid or peptidoglycan on the level (OD at 450 nm) by enzyme-linked immunosorbent assay of IgG antibodies to Staphylococcus aureus peptidoglycan. The sera were absorbed for 16 hr at 4 C with either of the two cell wall components (0.5 mg/ml), and the residual level of antibodies to peptidoglycan was titrated. The control serum was unabsorbed, incubated normal pooled human sera.

Occasional reports have indicated that antibodies to staphylococcal peptidoglycan may be present in normal human serum and in sera from patients with staphylococcal infection [18-20]. A sensitive radioimmunoassay for antibodies to peptidoglycan has been described [21], and antibodies to synthetic pentapeptide side chains of streptococcal peptidoglycan were found in sera from 55 of 105 healthy donors [22]. Helgeland and Grov [18] found elevated levels of IgG antibodies to peptidoglycan in 10 human sera with high titers of antibodies to staphylococcal  $\alpha$ -hemolysine (staphylolysine). They also reported the presence of IgA antibodies to peptidoglycan in normal human colostrum. No such correlation between level of antibodies to staphylolysine and that of antibodies to peptidoglycan was found by Schachenmayer et al. [20]. IgE antibodies in patients with recurrent staphylococcal infection and hyperimmunoglobulinemia E have recently been suggested to be specific for staphylococcal peptidoglycan [23].

In the present study a sensitive ELISA detected IgG antibodies to solubilized peptidoglycan iso-

lated from S. aureus strain H in 170 individual sera from healthy donors and infected patients. Levels of antibodies to peptidoglycan varied with age, and transplacental transfer of peptidoglycanspecific IgG antibodies was demonstrated. Similar differences between age groups in the level of antibodies to streptococcal peptidoglycan have been reported [20]. Significantly elevated levels of antibodies to staphylococcal peptidoglycan were observed in 20 (80%) of 25 patients with serious S. aureus infection of internal organs or deep tissue sites, whereas only two (9%) of 22 patients with localized staphylococcal infection of the skin or mucosal surfaces showed mildly elevated levels of antibodies to peptidoglycan. IgM antibodies to S. aureus peptidoglycan were not found in sera from healthy donors and were present only in low titer in a diabetic patient with acute staphylococcal arthritis.

These results indicate that humoral immunity to S. aureus in humans may be due, at least in part, to antibodies to peptidoglycan, the biologically most active component of the staphylococcal cell wall. Increased amounts of IgG antibodies to peptidoglycan are produced by most patients with serious staphylococcal infections of deep tissue sites. The presence, albeit at a relatively low titer, of IgG antibodies to peptidoglycan in sera from normal individuals and the rare finding of IgM antibodies in our patients suggest that a secondary or booster response occurs to a bacterial antigen to which virtually everyone has been exposed. However, the sera were obtained from these patients at least seven to 14 days after the initial symptoms of disease had developed. An early IgM antibody response may, therefore, have gone undetected. The absence of increased levels of antibodies to peptidoglycan in patients with superficial S. aureus infections suggests that the antibody response is somehow related to the amount and accessibility of the peptidoglycan.

Five patients with deep tissue S. aureus infections did not have elevated levels of antibodies to peptidoglycan; none of these patients was receiving hemodialysis for chronic renal failure, a condition that may contribute to a poor antibody response in staphylococcal disease [24]. Also, serial determinations in one of these patients failed to reveal an increase in level of antibodies to peptidoglycan at a later phase of his disease.

Systemic and serious localized infections with

gram-negative Enterobacteriaceae did not induce elevated levels of antibodies to staphylococcal peptidoglycan in any of 34 patients tested. Although this finding indicates that the ELISA for antibody to S. aureus peptidoglycan is specific, cross-reactivity may occur during infection with other gram-positive bacteria. Of the 12 patients with serious gram-positive bacterial infections not due to S. aureus, one patient with enterococcal endocarditis did have a significantly elevated level of antibodies to peptidoglycan. Cross-reactions involving two of the three immunodominant sites of the peptidoglycan macromolecule can be observed between antisera raised in laboratory animals to staphylococcal and streptococcal peptidoglycans [1]. The present investigation, however, was aimed at the immune response of humans to the complex cell wall peptidoglycan of S. aureus. Future research should focus on the antigenic specificities of antibodies to peptidoglycan in the sera of healthy individuals and patients with staphylococcal infections.

Serial testing for levels of antibodies to peptidoglycan revealed that high levels may be present early during deep tissue S. aureus infection and that levels may drop to normal values within two to three months after initiation of adequate antimicrobial therapy. The antibody response to staphylolysine followed a more delayed pattern in the one patient tested; a very high titer of antibodies to staphylolysine was still present late in convalescence. Similarly delayed responses for antibodies to staphylolysine have been noted in other S. aureus infections [25]. Our two patients with chronic granulomatous disease had chronically elevated levels of circulating antibodies to peptidoglycan, which may be a reflection of slow and incomplete clearing of the S. aureus infections in these patients, even with vigorous antibiotic regimens.

Much is already known about humoral immunity of humans against teichoic acid, the other major cell wall component of *S. aureus*. When sensitive tests such as ELISA [26] or radioimmunoassay [27] were used, antibodies to staphylococcal teichoic acids were always detectable in normal human sera. Elevated levels of antibodies to teichoic acid are found in the majority of patients with serious staphylococcal infections, making them detectable by counterimmunoelectrophoresis or gel-diffusion techniques [9]. An-

tibodies to teichoic acid have usually been of the IgG class [26, 28]. Also, a correlation between the level of antibodies to teichoic acid and that of antibodies to staphylolysine has been reported in patients with *S. aureus* bacteremia [29].

When we compared the measurement of IgG antibodies to peptidoglycan with those of antibodies to teichoic acid in 51 selected human sera, a direct correlation between the levels of the two antibodies was observed. An increase in level of antibodies to peptidoglycan generally coincided with an increase in level of IgG antibodies to staphylococcal teichoic acid. Cross-reactivity between staphylococcal peptidoglycan and teichoic acid could be ruled out as a basis for the observed correlation between antibodies to peptidoglycan and antibodies to teichoic acid. An absence of cross-reactivity between peptidoglycan and teichoic acid has been reported [19].

The role of antibodies to peptidoglycan and the usefulness of their detection for the diagnosis and follow-up of patients with deep-seated staphylococcal infections remain to be further defined. Recent work has indicated that antibodies to peptidoglycan enhance the phagocytosis of invading staphylococci by human polymorphonuclear leukocytes and may protect the host from the toxic effects of this cell wall moiety [3, 6-8]. At present, no single serologic test exists that will reliably detect all patients with serious staphylococcal disease, nor is there a test that is totally specific [30]. The detection rate may be increased by serial testing for antibodies to staphylococcal antigens [31]. The ELISA for antibody to cell wall peptidoglycan may, either alone or in combination, further help in identifying patients with deep tissue staphylococcal infection.

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