

Importance of Genotypic and Phenotypic Tolerance in the Treatment of Experimental Endocarditis Due to *Streptococcus gordonii*

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Genotypic and phenotypic tolerance was studied in penicillin treatment of experimental endocarditis due to nontolerant and tolerant *Streptococcus gordonii* and to their backcross transformants. The organisms were matched for in vitro and in vivo growth rates. Rats with aortic endocarditis were treated for 3 or 5 days, starting 12, 24, or 48 h after inoculation. When started at 12 h, during fast intravegetation growth, 3 days of treatment cured 80% of the nontolerant parent compared with <30% of the tolerant derivative ($P < .005$). When started at 24 or 48 h and if intravegetation growth had reached a plateau, 3 days of treatment failed against both bacteria. However, a significant difference between the 2 organisms was restored when treatment was extended to 5 days. Thus, genotypic tolerance conferred a survival advantage in both fast- and slow-growing bacteria, demonstrating that the in vitro-defined tolerant phenotype also carried the risk of treatment failure in vivo.

Bacteria have developed at least two mechanisms that enable them to survive the lethal effect of β -lactam antibiotics and other bactericidal drugs: antibiotic resistance and antibiotic tolerance. Resistant bacteria can grow in the presence of increased concentrations of antibiotics, resulting in increased MICs. However, beyond the elevated MICs, resistant bacteria remain sensitive to the bactericidal effects of the drugs. In contrast, tolerant bacteria are sensitive to the bacteriostatic effect of antibiotics (i.e., their MIC is unchanged) but have a drastically decreased susceptibility to drug-induced killing [1, 2]. In other words, bactericidal agents behave like bacteriostatic drugs against tolerant bacteria.

Two types of tolerance have been described: genotypic and phenotypic. Genotypic tolerance is conferred by so-called survival mutations, is expressed during the whole bacterial growth cycle, and can be transmitted both vertically to the bacterial progeny and horizontally by DNA transformation to other cells, as shown in *Streptococcus pneumoniae* [3] and *Streptococcus gordonii* (this work). Of interest, tolerance mutations are not genetically linked to resistance mutations in these 2 organisms [4] (unpublished observation). Phenotypic tolerance is characterized by the well-known fact that slow-growing bacteria have increased resistance to the bactericidal effect of cell-wall inhibitors [5]. Phenotypic tolerance is only expressed during slow bacterial division, but it is a property of any bacterial strain.

Antibiotic resistance is clinically important because it can lead to treatment failure. Tolerance also has caused treatment

failure in several animal studies, but its clinical impact is more difficult to assess. First, although tolerance is an in vitro measurement of the ability of bacteria to survive antibiotic-induced killing, it still lacks a clear-cut definition as well as standardized procedures for its determination in the laboratory [6, 7]. Second, studies that have investigated the clinical implication of tolerance are retrospective, often lack adequate control groups, and sometimes provide controversial results [2, 8]. Third, most animal studies to date suffer from potential bias as they have compared unrelated pairs of tolerant and nontolerant bacteria that may have been different in parameters other than tolerance per se [9–13]. Only Voorn and colleagues [14, 15] and Meeson et al. [16] studied related pairs of nontolerant and tolerant staphylococci and streptococci. They showed that tolerance impaired therapy with β -lactam drugs [14, 16] but not with peptide antibiotics [15]. Finally, none of these studies investigated the impact of phenotypic tolerance (i.e., slow-growing bacteria in the experimental setting), the role of which was recently highlighted in an experimental model of endocarditis caused by *Staphylococcus epidermidis* [17]. Therefore, it is difficult to draw definitive conclusions from currently available data.

In the present study, we systematically investigated the role of genotypic and phenotypic tolerance in penicillin treatment of experimental endocarditis due to *S. gordonii*. Rats with catheter-induced aortic vegetations were infected either with the penicillin-nontolerant parent strain or with a penicillin-tolerant derivative produced by cyclic exposure of the parent cells to penicillin in vitro. Antibiotic therapy was started at various times after inoculation, either when bacteria were actively growing in the cardiac vegetations or when they had reached a plateau and displayed phenotypic tolerance. Eventually, the reliability of the system was confirmed by use of an isogenic pair of nontolerant-tolerant *S. gordonii* transformants that were constructed by backcrossing chromosomal DNA of either a nontolerant or a tolerant derivative into the parent cells.

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Materials and Methods

Microorganisms, growth conditions, and antibacterial agents. We used a spontaneous streptomycin-resistant mutant (St^R) of *S. gordonii* Challis (formerly *Streptococcus sanguis*) [18], which is susceptible to penicillin-induced killing (Tol^- phenotype), and a penicillin-tolerant (Tol^+ phenotype) derivative of this bacterium (see below) in the experiments. Unless otherwise stated, bacteria were grown at 37°C in brain-heart infusion broth (BHI; Difco Laboratories, Detroit) without aeration or on Columbia agar (Becton Dickinson Microbiology Systems, Cockeysville, MD) supplemented with 3% blood. Culture growth was assessed by spectrophotometer (Sequoia-Turner, Mountainville, CA) for changes in optical densities (wavelength of 620 nm; OD_{620}) and by viable cell counts. Bacterial stocks were frozen in BHI containing 10% (vol/vol) glycerol and stored at -70°C. We purchased penicillin G and procaine penicillin from Hoechst-Pharma (Zurich) and streptomycin from Sigma (St. Louis).

Antibiotic susceptibility and time-kill curves. The MICs of the test antibiotics were determined by a standard macrodilution method [19] in BHI using a 10^5 – 10^6 cfu/mL overnight culture of bacteria as inoculum. The MICs were defined as the lowest antibiotic concentrations inhibiting visible bacterial growth after 24 h of incubation. The MBCs of the drugs were measured by subculturing 0.01-mL samples from the MIC tubes showing no turbidity onto blood agar supplemented with 2000 U/mL penicillinase (Bacto-Penase concentrate; Difco) to minimize the effect of antibiotic carry-over on the plates [6]. MBCs were determined after incubation for 48 h at 37°C and defined as the lowest antibiotic concentrations resulting in $\geq 99.9\%$ killing of the original inoculum.

Time-kill curves were determined by adding 20 \times the MIC of penicillin (final concentration) to bacterial cultures at various times during the logarithmic and the early stationary phase of growth. In brief, tubes containing fresh prewarmed (at 37°C) medium were inoculated with 0.1 mL of an overnight bacterial culture and further incubated at 37°C without aeration. Samples of the cultures were removed at various times before and after the addition of penicillin, serially diluted, and plated on penicillinase-containing agar plates for colony counts.

Selection of penicillin-tolerant derivatives of *S. gordonii* and DNA-backcross experiments. Derivatives of *S. gordonii* that were tolerant to the bactericidal effect of penicillin were obtained by cyclic exposure of parent cultures to high concentrations of penicillin as previously described [20]. Nonmutagenized cultures of a spontaneous St^R mutant of *S. gordonii* were grown to the midlogarithmic phase ($OD_{620} \sim 0.2$) in 10 mL of BHI supplemented with 100 mg/L streptomycin and then treated with 2 mg/L penicillin (i.e., 500 \times the MIC, final concentration) for 18–20 h at 37°C. This concentration was chosen because it is easily reached in human serum during standard penicillin therapy (see Results). Streptomycin was constantly kept in the medium to avoid contamination during the enrichment cycles. The cells were then washed three times by centrifugation (6000 g for 15 min) and resuspended in fresh medium, and the survivors were allowed to regrow overnight in penicillin-free BHI. After eight such enrichment cycles, single colonies were further characterized for in vitro properties of antibiotic susceptibility, tolerance, and stability of the tolerant phenotype. Tolerance stability was tested by serial passages of the derivatives (up to 35 generation times) in antibiotic-free liquid cultures.

In certain experiments, competent cells of the original streptomycin-susceptible *S. gordonii* strain were transformed with either chromosomal DNA of the St^R parent strain described above or with DNA of one of its penicillin-tolerant derivatives [18]. Selection was for the acquisition of the St^R marker (on agar plates containing 200 mg/L streptomycin) either alone, to determine the transformation frequency of this known marker, or for the St^R marker plus the tolerant phenotype as described [3]. The behavior of these backcross transformants was then tested both in vitro and in vivo in the experimental endocarditis model.

Rat model of experimental endocarditis. Sterile aortic vegetations were produced in female Wistar rats (180–200 g) as previously described [21]. Bacterial endocarditis was induced 48 h later by intravenous (iv) challenge with 0.5 mL of saline containing various sizes of bacterial inocula. Inoculum sizes were controlled in parallel by plating serial dilutions of the bacterial suspensions injected into animals on agar plates. Infection was assessed at various times after bacterial challenge. The rats were killed in a 100% CO_2 atmosphere, and blood was immediately drawn for semiquantitative cultures. The cardiac vegetation and spleen were dissected, weighed, homogenized in 1 mL of saline, serially diluted, and plated for viable colony counts. Colonies growing on the plates were enumerated after 48 h of incubation at 37°C. The dilution technique enabled detection of $\geq 2 \log_{10}$ cfu/g of tissue. Vegetations with negative cultures were given a value of 2 \log_{10} cfu/g in subsequent calculations for statistical analysis.

Assessing the bacterial infectivity in vivo. To determine whether penicillin-tolerant derivatives of *S. gordonii* and the parent bacteria could provoke experimental endocarditis, we determined both the minimum size of inoculum producing endocarditis in 90% of the rats (ID_{90}) and the rate of bacterial growth in situ in the vegetation. The ID_{90} was determined by challenging groups of 8–10 rats with various inoculum sizes (10^3 – 10^6 cfu) of each test organism and measuring the rates and intensity of valvular infections 24 h later. To establish the kinetics of bacterial growth in the vegetation, a large group of animals was challenged with 100 \times the ID_{90} of the test organisms, and the progressive increase in bacterial densities in the vegetations was followed by killing subgroups of rats at various times after inoculation. Vegetations and spleens were processed as described above.

Penicillin treatment of experimental endocarditis. Penicillin treatment was started 12, 24, or 48 h after inoculation of the rats with 100 \times the ID_{90} . Groups of animals infected with either test organism were treated for 3 or 5 days with procaine penicillin (300,000 U/kg) given subcutaneously twice a day. This regimen produced peak and trough antibiotic levels in the rat sera that approximated drug concentrations in humans during iv penicillin therapy (see Results). Untreated control rats were sacrificed at the start of therapy to determine both the rate and the severity of infection. Treated animals were killed 24 h after the last antibiotic dose, when no residual antibiotic activity was detected in sera. Rats that died during therapy were taken into account for vegetation bacterial densities only if they had received treatment for ≥ 2 days. In addition, bacteria recovered from infected valves were retested in vitro to establish the stability of the nontolerant and tolerant phenotypes of the test strains.

Determination of serum concentrations of penicillin, serum inhibitory titers (SITs), and serum bactericidal titers (SBTs) in rats. Penicillin concentrations in sera were determined in groups of 3–

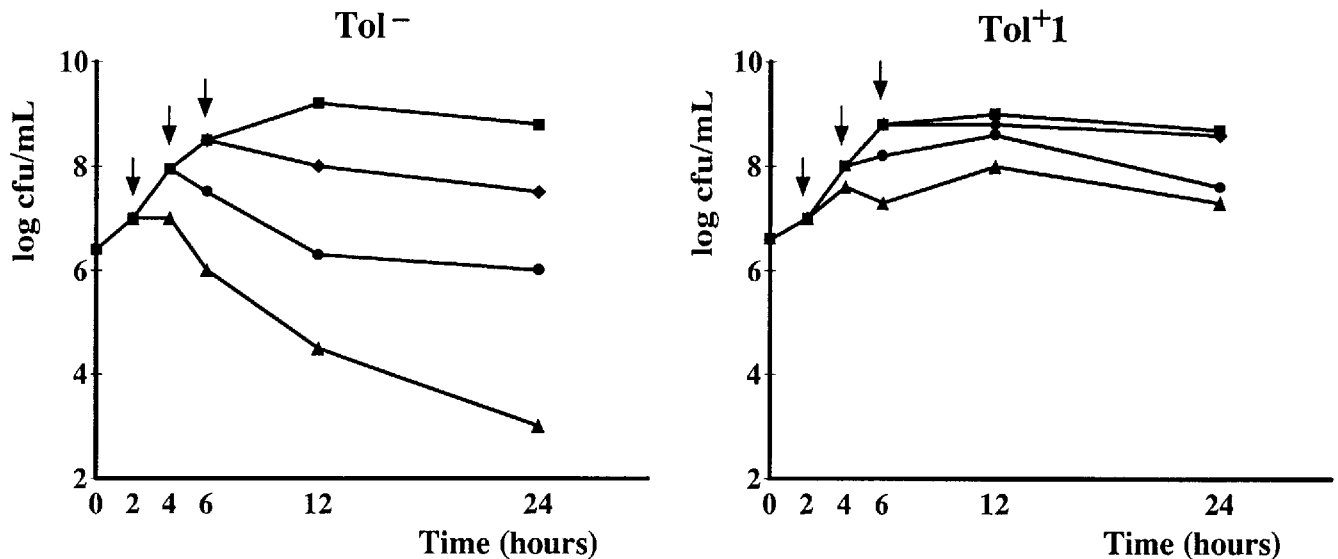


Figure 1. Penicillin-induced killing of nontolerant (Tol^-) *S. gordonii* parent strain (left) and its penicillin-tolerant Tol^{+1} derivative (right). Broth cultures of organisms were treated with $20\times$ MIC of penicillin (final concentration) during logarithmic or early stationary phase of growth (arrows), and viable cells in cultures were followed over time.

6 infected rats at various times before and after antibiotic administration. Blood samples were drawn from the retroorbital sinus, and antibiotic concentrations were measured by bioassay using *Bacillus subtilis* ATCC 6633 as an indicator organism. Antibiotic standards were dissolved in pooled rat serum. The assay limit of detection was 0.04 mg/L. The linearity of the standard curve was assessed by a regression coefficient of $r = .99$, and the intra- and interplate coefficient of variation of the assay was consistently $<10\%$.

The SITs and the SBTs were determined at peak (1 h) and trough (12 h) antibiotic concentrations by a standard method [22], using an inoculum of 10^5 – 10^6 cfu/mL and pooled rat serum containing 50% BHI as a diluent. The SIT was defined as the largest dilution of serum inhibiting visible bacterial growth, and the SBT was defined as the largest dilution killing $\geq 99.9\%$ of the original inoculum after 24 h of incubation at $37^\circ C$.

Statistical analyses. We used Fisher's exact test to compare the incidence of valvular infection. Differences between bacterial densities of various treatment groups were compared by the non-parametric Mann-Whitney Wilcoxon unpaired test or one-way analysis of variance when adequate. All significant ($P \leq .05$) differences were determined by the two-tailed test.

Results

In vitro characterization of the nontolerant parent *S. gordonii* and the tolerant derivative Tol^{+1} . The MIC of penicillin for the Tol^- parent *S. gordonii* was 0.004 mg/L. Penicillin treatment (with $20\times$ the MIC) of logarithmically growing cultures of this strain resulted in a loss of viable counts of 3–4 \log_{10} cfu/mL in 24 h (figure 1). This loss of viability was reduced to 1–2 \log_{10} cfu/mL after eight enrichment cycles of the parent culture with penicillin [23]. The bacteria were then plated, and single colonies were selected and regrown in sepa-

rate cultures for further characterization. One of these, Tol^{+1} , was used in the present experiments. Tol^{+1} had an unchanged MIC of penicillin (0.004 mg/L) compared with the wild type parent and grew at the same rate in broth cultures (figure 1). However, Tol^{+1} had a greatly increased MBC of penicillin (≥ 128 mg/L vs. 0.008 mg/L for the parent) and complied with the definition of penicillin-tolerance assessed by an MBC-to-MIC ratio ≥ 32 [24] (ratio ≥ 3200 and 2 for Tol^{+1} and the parent strain, respectively). The Tol^{+1} derivative was also stable. It did not lose the tolerance phenotype for up to 35 generation times in penicillin-free medium.

Figure 1 shows the time-kill curves of both the Tol^- parent and the Tol^{+1} derivative exposed to $20\times$ the MIC of penicillin. When added to cultures in the logarithmic phase of growth, penicillin killed the Tol^- parent cells but did not kill the Tol^{+1} derivative. In contrast, when added to the cultures in the early stationary phase of growth, penicillin failed to kill either organism because of phenotypic tolerance. These results clearly dissociated the genotypic and phenotypic tolerance traits of the 2 organisms in vitro and established a basis for further testing the 2 bacteria in vivo.

In vivo infectivity and bacterial growth in vegetations of the Tol^- parent and the Tol^{+1} derivative. The 2 organisms had similar ID_{90} (i.e., 10^5 cfu) in the rat model of experimental endocarditis. This indicated that the Tol^{+1} derivative was not affected in its ability to colonize damaged valves and induce infectious endocarditis. Moreover, in experiments using inocula of $100\times$ the ID_{90} , both the Tol^- parent and the Tol^{+1} derivative grew at similar rates in the vegetations after establishing infection (figure 2, upper). Blood cultures were positive in $>90\%$ of the animals, regardless of the infecting organism. Median

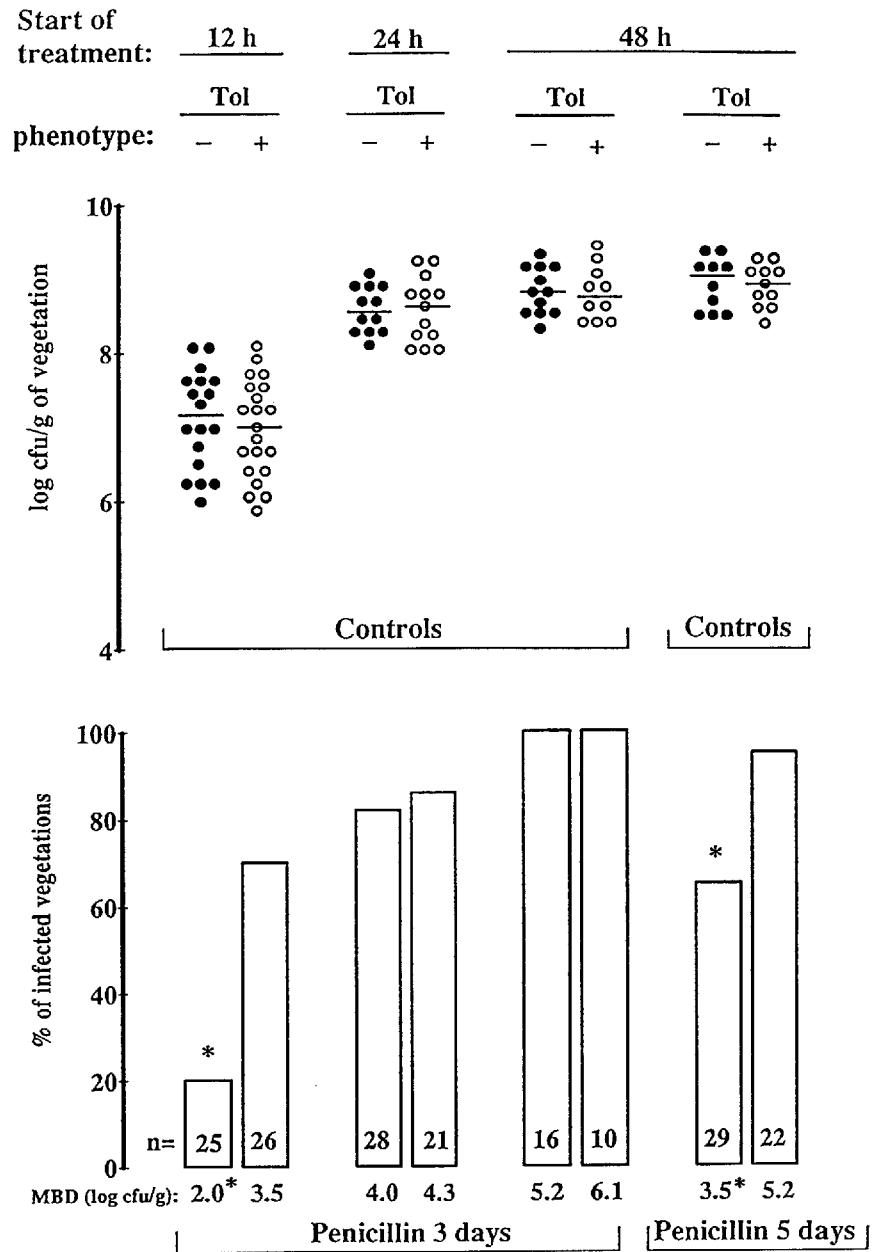


Figure 2. Intravegetation growth (upper) and outcome of 3 and 5 days penicillin treatment of experimental endocarditis caused by nontolerant (Tol⁻) *S. gordonii* parent strain or by tolerant derivative Tol⁺¹ (lower). Each dot in upper panel represents bacterial density in vegetation of separate control rats sacrificed at onset of therapy. Columns (lower panel) show % of infected vegetations after 3 or 5 days of penicillin therapy. Numbers of animals in each treatment group are noted at column bottoms (n=). MBD is median bacterial density of vegetations. * *P* < .005 vs. Tol⁺¹ derivative.

levels of bacteremia increased from 16 cfu/mL (range, 0–54) and 13 cfu/mL (range, 0–43) in 7–12 animals infected with the Tol⁻ parent or the Tol⁺¹ derivative, respectively, 12 h after inoculation to 1000 cfu/mL (range, 0–1000) for both organisms after 48 h (*P* > .05). Bacterial densities in the spleens also increased in parallel over time from 3.82 ± 0.56 and 3.84 ± 0.44 (mean log₁₀ cfu/g of tissue in 7–12 animals infected with the Tol⁻ parent or the Tol⁺¹ derivative, respectively) 12 h after inoculation to 4.37 ± 0.35 and 4.06 ± 0.64, respectively, after 48 h (*P* > .05). Therefore, both the Tol⁻ parent and its Tol⁺¹ derivative behaved alike in several important aspects of experimental endocarditis: the establishment of valvular infection, the in situ growth rates in the vegetation, the levels of bacter-

emia, and the colonization of target organs such as the spleen. These similarities allowed the further comparison of these 2 organisms in therapeutic experiments with penicillin.

Penicillin concentrations in the serum of rats: SITs and SBTs. Peak and trough concentrations of penicillin in rat sera during antibiotic therapy were (mean ± SD of 10–13 rats) 12.27 ± 4.16 mg/L at 1 h and 5.83 ± 3.32 mg/L at 12 h. These concentrations were within the range of penicillin levels obtained in humans: Peak serum concentrations can reach ~60 mg/L during iv therapy [25]. At peak serum concentrations, the SITs/SBTs of the rat sera were ≥1:256/1:128 for the Tol⁻ parent and ≥1:256/none for the Tol⁺¹ derivative. At trough levels, these values were 1:256/1:32 for the Tol⁻ and 1:128/

none for the Tol⁺1 strain. Thus, the 2 organisms were similarly susceptible to growth inhibition by sera, but only the Tol⁻ parent was sensitive to serum-induced killing (the Tol⁺1 derivative survived this treatment). This shows that the tolerant phenotype of Tol⁺1 was preserved when tested in the presence of serum.

Penicillin treatment of experimental endocarditis. Figure 2 (lower) shows results of two series of experiments during which therapy was given for 3 and 5 days (left and right panels, respectively). When treatment was started 12 h after inoculation during fast intravegetation growth (figure 2, upper), 3 days of penicillin successfully treated 80% of rats infected with the Tol⁻ parent strain. On the contrary, only 30% of rats inoculated with the Tol⁺1 derivative had sterile vegetation cultures ($P < .005$), indicating that genotypic tolerance had a strong impact on therapeutic success in this particular experimental setting. Yet, when the 3-day therapy regimen was started 24 or 48 h after inoculation, the difference in outcome progressively shrank, as bacterial growth in the vegetation came to a halt and infections due to the Tol⁻ parent became more difficult to eradicate. In this case, the effect of genotypic tolerance was masked by the effect of phenotypic tolerance in a manner reminiscent of that observed in *in vitro* time-kill curves (see figure 1). This observation raised the question as to whether genotypic tolerance might still confer a survival advantage during penicillin treatment of slow-growing bacteria or whether its beneficial effect was limited to logarithmically growing bacteria.

To address this question, we did a series of experiments in which rats with late onset of therapy (at 48 h) were treated with penicillin for 5 days instead of 3 days. Figure 2 (lower right panel) shows the results of these studies. The prolonged treatment (5 days) restored a statistically significant difference between the 2 treatment groups. Indeed, endocarditis due to the Tol⁺1 derivative was again more difficult to treat than infections due to the Tol⁻ parent strain ($P < .05$). Thus, genotypic tolerance could adversely affect the success of therapy in both early and late treatment onset, provided that prolonged antibiotic treatment enabled detection of the difference.

It is noteworthy that in all of the experiments, bacteria recovered from infected valves retained their original phenotype regarding penicillin tolerance. Moreover, mortality during therapy was equally distributed in both Tol⁻ and Tol⁺1-infected rats. Overall, ~30% of animals (27 in the Tol⁻ and 30 in the Tol⁺1 group) died in each group. Of these, 12% (11 and 12 in each group, respectively) died after 2 days of therapy. These animals were taken into account for vegetation bacterial titers and did not alter the results of the experiments.

Infection due to Tol⁻ and Tol⁺ transformants of the parent *S. gordonii* strain. It was important to establish whether the *in vivo* difference observed between the Tol⁻ parent and the Tol⁺1 derivative was indeed due to tolerance mutations rather than to unrelated mutations that might have arisen during the eight penicillin cycles of the original enrichment procedures. For this purpose, backcross experiments were

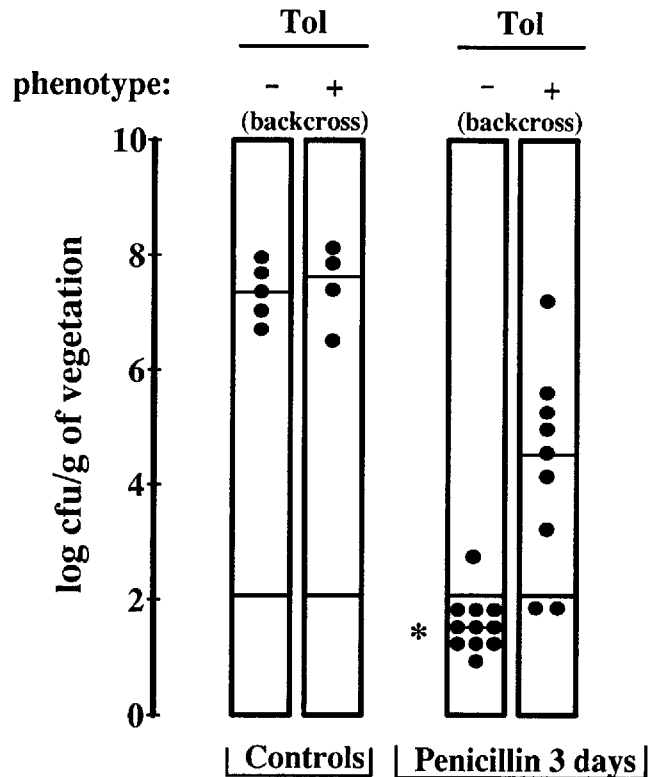


Figure 3. Results of 3 days penicillin treatment of experimental endocarditis caused by nontolerant (Tol⁻) parent and tolerant derivative (Tol⁺) backcross transformants of *S. gordonii*. Treatment was started 12 h after bacterial challenge. Each dot represents bacterial density in vegetation of separated rats sacrificed at onset (controls) or after 3 days of therapy. Bars in columns indicate median values of vegetation bacterial densities. * $P < .005$ vs. Tol⁺1 derivative.

done in which the original streptomycin-susceptible *S. gordonii* strain was transformed either with chromosomal DNA of the Tol⁻ but streptomycin-resistant parent strain described above or with DNA of its Tol⁺1 derivative, which also carried the St^R marker.

The frequency of transformation to St^R was 10^{-2} – 10^{-3} . Cultures of competent cells treated with DNA of the Tol⁺1 derivative and subsequently cycled with penicillin consistently converted to the tolerant phenotype after 2 or 3 passages. In contrast, cultures treated with the control DNA remained sensitive to penicillin-induced killing for more than four penicillin-enrichment cycles. When tested *in vitro*, both types of transformants had characteristics identical to their DNA donor for growth rates and penicillin susceptibility (data not shown). Moreover, the infectivity of these transformants in rats with aortic vegetations were similar to their parent strains, and they produced the same response patterns to penicillin treatment as the parent organisms in experimental endocarditis (figure 3). Penicillin therapy was started 12 h after inoculation in this set of experiments. These results support the reliability of the experimental system described.

Discussion

Our results clearly show that the *in vitro*-defined phenomenon of antibiotic tolerance is also relevant *in vivo*, as it may result in treatment failure of experimental endocarditis. The observation is important because it may have clinical relevance. Indeed, tolerant derivatives obtained by cyclic exposure of bacteria to antibiotics reflect a kind of antibiotic pressure that exists in the clinical environment [20]. In addition, the tolerant derivatives used in the present experiments and in previous studies that used other types of organisms [14–16] arose from nonmutagenized bacterial cultures, suggesting that such tolerant organisms might also be selected during antibiotic treatment in humans or animals.

The model of experimental endocarditis is particularly well suited for the investigation of antibiotic tolerance, because it tests the bactericidal effect of antimicrobial drugs in the absence of helping host defenses [26], except perhaps for platelet-derived microbicidal proteins [27]. On the other hand, the therapeutic success of a given drug also depends on such antibiotic-independent factors as the age of the vegetation [28], the rate of bacterial growth *in situ* at the time of treatment initiation [17], and pathogenic determinants, such as the production of exopolysaccharides by the bacteria [29]. Since these parameters can interfere with the outcome of therapy, they must be carefully matched before definitive conclusions can be drawn from therapeutic experiments that compare nontolerant with tolerant microorganisms.

In the present experiments, the *in vitro* and *in vivo* characteristics of the pair of related strains were carefully matched before their behavior was tested in the rats. The results showed that genotypic tolerance greatly decreased the success of penicillin in early treatment onset. The observation was made both with the original pair of nontolerant parent and Tol⁺1 strains and with the backcross-transformants of these organisms. This provided support for a specific role of tolerance mutations in treatment failure of early valvular infection. At a later stage, however, the effect of genotypic tolerance became masked by phenotypic tolerance leading to questions of the true importance of genotypic tolerance in the outcome of clinical infections. Indeed, slow-growing bacteria (i.e., phenotypically tolerant cells) are likely to be highly predominant at the treatment onset of human infections [30] and hence in “slow” endocarditis due to viridans streptococci. In this situation, the predominant effect of phenotypic tolerance might outweigh genotypic tolerance, leveling the latter to a mere laboratory curiosity. In the present experiments, however, when treatment was extended to 5 days, the genotypic tolerance could still confer a survival advantage to slow-growing bacteria and contribute to treatment failure when therapy initiation was delayed. These results confirm previous experiments that showed the adverse effect of tolerance on therapy, using related tolerant and nontolerant bacteria [14, 16]. In addition, they further demonstrate that the detrimental effect on antibiotic treatment conferred by

tolerance mutation(s) is maintained after DNA backcross of these mutations in wild type strains and when slow-growing bacteria express so-called phenotypic tolerance.

The question arises then as to whether existing antibiotic regimens against infectious endocarditis might overcome β -lactam tolerance *in vitro* and *in vivo*. In time-kill experiments, the combination of penicillin with gentamicin successfully killed both the Tol⁻ parent and the Tol⁺1 mutant to a similar extent *in vitro* (loss of viability >4 log₁₀ cfu/mL after 24 h of antibiotic treatment; data not shown), suggesting that this drug association might be effective *in vivo* as well. Indeed, Voorn et al. [14] showed that while treatment with cloxacillin alone failed against experimental endocarditis due to tolerant staphylococci, the combination of cloxacillin with gentamicin could restore therapeutic efficacy. Using the same experimental model, these authors also showed that other classes of antibiotics (i.e., glycopeptides and lipopeptides alone or combined with rifampin) were equally active against both tolerant and nontolerant staphylococci *in vivo* [15]. Thus, from an experimental point of view, therapeutic regimens effective against tolerant bacteria might exist in the current armamentarium of antibacterial agents.

Taken together, the present results underscore the relative importance of genotypic and phenotypic tolerance in penicillin treatment of experimental streptococcal endocarditis and provide a rationale for the interpretation of some controversial results reported in the literature. This revives the suggested, but incompletely solved, role of tolerant bacteria in treatment of human endocarditis and is a reminder that bactericidal antibiotics are more effective than nonbactericidal drugs in this disease. It is possible that patients infected with tolerant bacteria respond less well than those infected with nontolerant bacteria only during a limited window in the early course of therapy. On the other hand, patients infected with tolerant organisms might also experience relapse after short-course treatment. While these issues have yet to be solved, the present experimental data underline the potential adverse role of tolerance *in vivo*.

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