Lack of Benefit of Intravenous Immune Globulin in a Murine Model of Group A Streptococcal Necrotizing Fasciitis

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Penicillin, clindamycin, and intravenous immune globulin (Venoglobulin-S; IVIG) alone and in combination were studied in a murine model of group A streptococcal necrotizing fasciitis. As assessed by bacterial clearance, treatment with IVIG was not significantly different from no treatment. All treatment regimens that contained penicillin or clindamycin were more effective (P < .05) than no treatment or treatment with IVIG alone. No significant differences were detected among results of treatment with penicillin, penicillin/clindamycin, penicillin/IVIG, clindamycin/IVIG, or all agents combined. Clindamycin alone was less effective than penicillin/IVIG (P = .02), penicillin/clindamycin (P = .009), clindamycin/IVIG (P = .04), or all agents combined (P = .02). No antagonism was observed with the addition of clindamycin or IVIG to penicillin.

Several outbreaks of invasive infections caused by group A streptococci have been reported in recent years, including one reported from our institution [1]. Invasive group A streptococcal infections are associated with a high mortality rate [1], despite universal in vitro susceptibility of Streptococcus pyogenes to penicillin. A reduction in the efficacy of penicillin in vitro when large numbers of organisms are present can be demonstrated [2]. Lack of expression of certain penicillin-binding proteins by streptococci during the stationary growth phase may be responsible for the inoculum effects observed in vitro as well as in vivo [3]. The addition of clindamycin to penicillin for the treatment of streptococcal toxic shock syndrome has been advocated because clindamycin, a protein-synthesis inhibitor, acts independently of the size of the inoculum or the stage of bacterial growth [4]. Furthermore, clindamycin suppresses synthesis of bacterial toxins and facilitates phagocytosis of S. pyogenes by inhibiting M protein synthesis [3]. Clindamycin may also modulate immune response by suppressing lipopolysaccharide-induced monocyte synthesis of tumor necrosis factor–α [5]. In one study, clindamycin alone was shown to have a greater efficacy (as assessed by mortality) than penicillin alone in a murine model of streptococcal myositis [2]. However, in that study, the efficacy in vivo of the combination of penicillin and clindamycin was not evaluated. Theoretically, negative interactions between these two antibiotics may exist (e.g., by clindamycin’s suppression of the synthesis of penicillin-binding proteins) [6]. This is potentially clinically important, because clindamycin-resistant S. pyogenes have been reported [7].

It has been suggested that intravenous immune globulin (IVIG) may exert a beneficial effect in the management of streptococcal toxic shock syndrome by neutralizing the activity of streptococcal superantigens. Supporting evidence for this concept includes the finding that patients with invasive group A streptococcal infections lack antibodies against streptococcal pyrogenic exotoxins (e.g., SpeB and SpeF) [8, 9]. Furthermore, IVIG contains neutralizing antibodies against a broad variety of streptococcal pyrogenic exotoxins and opsonic antibodies against surface M protein of S. pyogenes, both of which are transferable to the plasma of patients who receive IVIG [10, 11].

Seven published case reports describe patients with streptococcal toxic shock syndrome who improved after administration of IVIG [12–18]. In one series, 1 of 2 patients died while receiving IVIG preparations for group A streptococcal infection with septic shock [19]. In addition, in a recently published comparative observational study, IVIG adjunctive therapy for streptococcal toxic shock syndrome was associated with a reduction in mortality (from 66% to 33%), compared with that for controls [20]. Although IVIG has been recommended as adjunctive therapy for streptococcal toxic shock syndrome, no randomized clinical trial to evaluate its efficacy has been reported [21]. Potential negative aspects of IVIG therapy include cost, adverse effects (e.g., anaphylaxis), and the theoretical possibility of adverse interactions with other treatment modalities, including antagonism with antimicrobial agents. The purpose
of this study was to compare the efficacy of penicillin, clindamycin, and IVIG alone and in combination in the treatment of murine experimental necrotizing fasciitis.

Materials and Methods

Group A streptococcal isolate. A clinical isolate of S. pyogenes associated with an epidemic of invasive group A streptococcal disease in southeastern Minnesota [1] was used for all studies. This isolate expressed M-3 protein as well as streptococcal pyrogenic exotoxin A [22].

Antimicrobial susceptibility testing. Susceptibility of S. pyogenes to penicillin and clindamycin was determined by using a microbroth dilution assay [23]. A 2-dimensional broth dilution (checkerboard) study was done in Mueller-Hinton broth as follows. Penicillin in 0.5-mL volumes (Sigma, St. Louis) was added to 0.5-mL volumes of clindamycin (Sigma) to yield serial 2-fold dilutions from 0.25 to 0.015 μg/mL of each antimicrobial in a checkerboard matrix. Tubes containing dilutions of each antibiotic alone were also included. One milliliter of broth containing 5 × 10⁶ colony-forming units (cfu) of S. pyogenes was added to each tube. These tubes were mixed and were incubated for 18 h at 35°C in room air. After incubation, the tubes were mixed and checked for turbidity.

Murine necrotizing fasciitis model. Experimental necrotizing fasciitis was established in healthy 25-g C57Bl/6 mice (Jackson Laboratories, Bar Harbor, ME) by a modification of a previously described technique [24]. The left gluteal muscle was inoculated with a suspension of S. pyogenes (cfu amounts described below). Mice were killed (at various times after infection, as described below) with a lethal injection of 200 mg/kg of pentobarbital (Sleipnay, Fort Dodge Laboratories, Fort Dodge, IA). For quantitative cultures, an 8-mm biopsy sample of the left gluteal muscle was excised aseptically, weighed, and homogenized with 2 mL of Todd–Hewitt broth in a sterile tissue grinder (Stomacher 80; Tekmar Co., Cincinnati). The homogenate was quantitatively cultured for penicillin and clindamycin concentrations were determined by microbiologic assay [25]. Procaine penicillin and clindamycin dosages were selected to produce peak concentrations in mouse serum similar to peak concentrations in human serum after administration of therapeutic doses.

To determine whether our isolate of S. pyogenes would infect mice, 10 mice were inoculated with 10⁷ cfu of log phase S. pyogenes. Five of these mice were killed 24 h after infection, and 5 were killed 48 h after infection. Survival and log₁₀ cfu S. pyogenes/g of gluteal muscle or kidney were determined for the 10 mice, and for 5 mice results of blood cultures were determined.

To determine the minimum bacterial inoculum needed to establish fatal infection in our model, groups of 3 mice were inoculated with 10⁷ cfu, 10⁶ cfu, 10⁵ cfu, or 10⁴ cfu of log phase S. pyogenes, and the mice were observed for survival. The inoculum 6 × 10⁷ cfu of log phase S. pyogenes was selected for subsequent studies, on the basis of results from these preliminary studies.

To evaluate the effect of the incubation period on response to treatment, experiments were performed with treatment initiated at timed intervals (2, 4, 8, 16, and 24 h) after bacterial inoculation. In these preliminary studies, animals were treated with procaine penicillin, 200,000 U/kg intramuscularly every 6 h, in combination with IVIG (Venoglobulin-S; Alpha Therapeutic, Los Angeles) at 1 g/kg intraperitoneally once daily. Antimicrobial therapy was administered for 48 h. Six hours after completing the assigned treatment regimen, the mice were killed, and biopsy specimens were cultured quantitatively. The timing of the initiation of treatment in subsequent studies was determined from an intermediate (submaximal) effect observed in these preliminary studies.

After these preliminary studies, another group of mice was randomly assigned to receive 1 of 8 treatment regimens: no treatment; clindamycin, 50 mg/kg intraperitoneally every 6 h; procaine penicillin, doses as outlined earlier; IVIG, doses as outlined earlier; or combinations of the aforementioned doses of penicillin/clindamycin, penicillin/IVIG, clindamycin/IVIG, or penicillin/clindamycin/IVIG. The number of mice in each treatment group is shown in table 1. Two lots of IVIG were used evenly among IVIG-treated animals. Cumulative mortality records were kept for the duration of the experiment. Statistical analysis of quantitative culture results was done by rank-sum analysis.

Results

In vitro studies. The S. pyogenes isolate used in our study showed minimum inhibitory concentrations of ≤0.125 μg/mL for penicillin and 0.03 μg/mL for clindamycin and minimum bactericidal concentrations of ≤0.125 μg/mL for penicillin and 2 μg/mL for clindamycin. Two-dimensional broth dilution (checkerboard) studies of the bacteriostatic activity of penicillin and clindamycin against the study S. pyogenes isolate showed no visible turbidity in any of the tubes containing any com-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>No. of surviving mice</th>
<th>Median log₁₀ cfu/g gluteal muscle</th>
<th>Range, 25th–75th percentile log₁₀ cfu/g gluteal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>19</td>
<td>17</td>
<td>8.6a</td>
<td>7.7–9.4</td>
</tr>
<tr>
<td>IVIG</td>
<td>16</td>
<td>15</td>
<td>8.9a</td>
<td>8.5–9.3</td>
</tr>
<tr>
<td>Penicillin</td>
<td>16</td>
<td>16</td>
<td>5.3b,c</td>
<td>3.9–7.2</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>16</td>
<td>16</td>
<td>6.0b,c</td>
<td>5.7–6.8</td>
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<tr>
<td>Penicillin/ clindamycin</td>
<td>16</td>
<td>15</td>
<td>5.2b,c</td>
<td>3.4–6.3</td>
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<tr>
<td>Penicillin/IVIG</td>
<td>17</td>
<td>17</td>
<td>5.3b,c</td>
<td>4.4–5.7</td>
</tr>
<tr>
<td>Clindamycin/IVIG</td>
<td>16</td>
<td>16</td>
<td>5.1b,c</td>
<td>3.9–7.0</td>
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<tr>
<td>Penicillin/ clindamycin/IVIG</td>
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<td>16</td>
<td>5.6b,c</td>
<td>0.8–7.0</td>
</tr>
</tbody>
</table>

NOTE. cfu, colony-forming units; IVIG, intravenous immune globulin (Venoglobulin-S).

a P < .05 for no treatment or IVIG treatment vs. other therapies.
b P is not significant among any treatment comparisons.
c P < .02 for clindamycin compared with other treatment combinations.
bination of penicillin and clindamycin. No antagonistic interactions between penicillin and clindamycin in vitro were observed.

**In vivo studies.** The mean 30-, 60-, 90-, and 120-min concentrations in serum were 35, 32, 19, and 8 μg/mL, respectively, of penicillin after administration of 200,000 U/kg of procaine penicillin intramuscularly and 9.7, 4.3, 3.6, and 1.2 μg/mL, respectively, of clindamycin after administration of 50 mg/kg of clindamycin intraperitoneally.

In preliminary studies, 10 mice were inoculated with 10^7 cfu of *S. pyogenes*. Five mice were killed 24 h after infection, and all had positive blood cultures for *S. pyogenes*. The mean log_{10} cfu/g of gluteal muscle was 7.3, and the mean log_{10} cfu/g of kidney was 6.0 in these animals. Two of the remaining 5 mice died within 48 h of infection. For the remaining 3 animals, the mean log_{10} cfu/g of gluteal muscle was 7.7, and the mean log_{10} cfu/g of kidney was 5.9.

To determine the effect of bacterial inoculum in our model, we inoculated groups of 3 mice with 10^7 cfu, 10^6 cfu, or 10^5 cfu of *S. pyogenes* and observed them for survival over a 4-day period. All mice inoculated with 10^7 cfu and 10^6 cfu of *S. pyogenes* survived; 2 mice infected with 10^5 cfu of *S. pyogenes* died on the fourth day after infection; and all 3 mice infected with 10^4 cfu of *S. pyogenes* died on the third day after infection.

To determine the effect of the incubation period on response to treatment, we performed experiments using 5 groups of mice, beginning treatment with penicillin and IVIG at timed intervals after challenge with 10^7 cfu of bacteria. Table 2 shows that, when penicillin/IVIG therapy was delayed for 8 h after initial challenge, the median log_{10} cfu/g of tissue was 5.29. This was approximately half that of the untreated controls; therefore, all subsequent studies were done with treatment initiated at 8 h after infection with *S. pyogenes*.

Further results of treatment are shown in table 1. Two different lots of IVIG were studied, alone and in combination with clindamycin and penicillin. No statistically significant difference was found between the 2 lots of IVIG studied, either when used alone or when used in combination with penicillin or clindamycin; therefore the data from the 2 lots of IVIG were combined. Treatment with IVIG alone was not significantly different from no treatment. All treatment regimens that contained penicillin or clindamycin were more effective (*P < .05*) than no treatment or treatment with IVIG alone. No significant differences were detected among results of treatment with penicillin, penicillin/clindamycin, penicillin/IVIG, clindamycin/IVIG, or penicillin/clindamycin/IVIG. In particular, no antagonism was observed in vivo. Clindamycin alone was less effective than penicillin/IVIG (*P = .02*), penicillin/clindamycin (*P = .009*), clindamycin/IVIG (*P = .04*), or penicillin/clindamycin/IVIG (*P = .02*).

### Table 2. Results of no treatment versus penicillin and intravenous immune globulin (IVIG) combination treatment of murine necrotizing fasciitis with treatment initiated at varying times after bacterial challenge.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Median log_{10} cfu/g gluteal muscle (no. of animals) at time (in h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8.59 (3) 8.78 (2) 7.80 (4) 8.04 (3) 8.84 (3)</td>
</tr>
<tr>
<td>Penicillin/IVIG</td>
<td>4.61 (14) 5.22 (14) 5.29 (14) 6.96 (13) 7.53 (14)</td>
</tr>
</tbody>
</table>

**NOTE:** Time in hours is onset of therapy after infection. cfu, colony-forming units.

### Discussion

In a murine model of necrotizing fasciitis, the addition of IVIG to penicillin or to penicillin/clindamycin did not affect treatment efficacy, as assessed by quantitative bacterial clearance. Our study is the first to evaluate the value of IVIG adjunctive therapy for experimental invasive group A streptococcal infections, and our results are at variance with recent recommendations for the treatment of streptococcal toxic shock syndrome [21]. The recommendation to use IVIG as adjunctive treatment of streptococcal toxic shock syndrome is based on the premise that immune globulin will neutralize streptococcal superantigens. Clinical experience, however, is documented only by a small number of published anecdotal cases that describe clinical improvement in patients with streptococcal toxic shock syndrome after administration of IVIG [12–18] and a recently published comparative observational study that showed reduced mortality in patients with streptococcal toxic shock syndrome who received IVIG [20]. Although we were able to produce experimental murine *S. pyogenes* necrotizing fasciitis, it is possible that we were unable to demonstrate adjunctive benefit of IVIG in our model because the mice in our experiments had not developed toxic shock syndrome. Unpublished data suggest that the rabbit may be more susceptible than the mouse to streptococcal toxic shock syndrome after administration of IVIG [21]. Alternatively, measurement of cfu/g of tissue may not be an appropriate measure of response to the clinical manifestations of toxic shock syndrome. In future studies, measurement of mortality may be a more appropriate primary end point, although we were unable to demonstrate any beneficial effect of IVIG on mortality. The model was, however, purposefully designed to control mortality, because one cannot measure both survival and quantitative bacterial load in the same study without bias.

Recently, variation was shown in the titers of neutralizing antibodies of streptococcal superantigens in different preparations of IVIG. Venoglobulin-S, the preparation used in our study, reportedly contains higher neutralizing antibody titers against group A streptococcal superantigens than do other preparations [27]. Variation in neutralizing activity against group A streptococcal superantigens was demonstrated among...
different lots of Venoglobulin-S [27]. It is possible that the lots of Venoglobulin-S used in our study had relatively low levels of neutralizing antibodies against streptococcal superantigens. However, we studied 2 lots of IVIG and found no differences between them.

We did not demonstrate antagonism in vitro between clindamycin and penicillin in a checkerboard assay. Other investigators reported that, for *S. pyogenes* at 2 or 4 times the MIC, antagonism was demonstrated between penicillin and clindamycin [28]; in this same study, at one-half times the MIC, synergy was demonstrated between penicillin and clindamycin. The clinical significance of these concentration-dependent variable effects is unclear. The results of our study do agree with those of Stevens et al. [28], which showed no antagonism between penicillin and clindamycin when therapeutic concentrations of both drugs were used. The results of in vitro studies are method dependent; our failure to demonstrate antagonism in vitro between clindamycin and penicillin in a checkerboard assay agrees with our results in vivo.

Studies in vitro have shown that the combination of clindamycin and penicillin does not have a bactericidal advantage over either drug used alone against *S. pyogenes* [28]. Our results confirm and extend these findings to show a lack of difference in efficacy in vitro of the combination of clindamycin and penicillin compared with penicillin alone. In our study, the combination of penicillin and clindamycin in vivo was more effective than clindamycin alone. In another study, under certain circumstances, clindamycin was more effective than penicillin therapy in a murine model of experimental streptococcal myositis [2]. Compared with our study, this study used a higher inoculum of 3.5 × 10⁸ cfu *S. pyogenes*, compared with 6 × 10¹ cfu in our study, and higher dosages of antibiotics administered over shorter periods of time, and measured mortality as opposed to bacterial clearance as the end point [2]. In addition, the clindamycin minimum bactericidal concentration of our study isolate (2 µg/mL) was higher than that of the strain used in the previously published study (0.22 µg/mL) [2, 28], and our study isolate was tolerant to clindamycin, whereas the strain used in the previously published study was not tolerant to clindamycin [2, 28]. We used a clinical isolate of *S. pyogenes* associated with a recent epidemic of invasive group A streptococcal disease, whereas an archived type strain of *S. pyogenes* was used in the previously published study [2]. Any or all of these factors may account for the incongruity of results.

In conclusion, we demonstrated that, in a murine model of group A streptococcal necrotizing fasciitis, IVIG had no significant effect on the rate of bacterial clearance when combined with penicillin or penicillin/clindamycin. No evidence of antagonism was detected between clindamycin and penicillin in vitro or in our murine model of group A streptococcal necrotizing fasciitis.