Intervention with Shiga Toxin (Stx) Antibody after Infection by Stx-Producing Escherichia coli

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Abstract
Shiga toxins (Stxs) produced by *Escherichia coli* (STEC) cause systemic vascular damage, manifested as hemolytic uremic syndrome in humans and as edema disease in pigs. Edema disease, a naturally occurring disease of pigs, was used to determine whether Stx antibodies, administered after infection and after the onset of Stx production, could prevent the systemic vascular damage and clinical disease caused by Stxs. A total of 119 STEC-infected pigs were treated with low, medium, or high doses of Stx antibody or with placebo. After inoculation with STEC, antibodies or placebo was injected intraperitoneally at 2 days postinoculation (DPI; low dose) or 4 DPI (medium and high doses). Edema disease was prevented with the low- and high-dose Stx antibody treatments administered at 2 and 4 DPI, respectively. High-dose antibody treatment also reduced the incidence and extent of vascular lesions. The degree of protection depended on the dose of antibody and the time of administration.

Disciplines
Veterinary Medicine | Veterinary Microbiology and Immunobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

Comments
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CONCISE COMMUNICATION

Intervention with Shiga Toxin (Stx) Antibody after Infection by Stx-Producing Escherichia coli

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and Harley W. Moon1

Shiga toxins (Stxs) produced by Escherichia coli (STEC) cause systemic vascular damage, manifested as hemolytic uremic syndrome in humans and as edema disease in pigs. Edema disease, a naturally occurring disease of pigs, was used to determine whether Stx antibodies, administered after infection and after the onset of Stx production, could prevent the systemic vascular damage and clinical disease caused by Stxs. A total of 119 STEC-infected pigs were treated with low, medium, or high doses of Stx antibody or with placebo. After inoculation with STEC, antibodies or placebo was injected intraperitoneally at 2 days postinoculation (DPI; low dose) or 4 DPI (medium and high doses). Edema disease was prevented with the low- and high-dose Stx antibody treatments administered at 2 and 4 DPI, respectively. High-dose antibody treatment also reduced the incidence and extent of vascular lesions. The degree of protection depended on the dose of antibody and the time of administration.

Clinical signs of edema disease develop several days after infection, thus providing a potential postinfection window for intervention, to prevent the vascular damage caused by Stxs. Microscopically, the hallmark of edema disease is vascular necrosis in the brain and gastrointestinal tract [5, 6]. In contrast to HUS, renal damage is not a feature of edema disease. The STEC strains associated with edema disease do not have the attaching and effacing attribute characteristic of some, but not all, STEC strains associated with HUS and HC [7].

Active or passive immunization against Stxs, before STEC inoculation, prevents the systemic complications of STEC infection in animal models [3, 8–14]. Passive transfer of neutralizing antibodies or active immunization with Stx2e toxoid protects pigs against the lethal effects of Stx2e given intravenously [9].

We have characterized elsewhere [4] the temporal relationships among colonization, Stx2e production, and the development of systemic manifestations in edema disease. Stx2e was detected in feces 1 day postinoculation (DPI). The mean time from inoculation to the onset of clinical disease was 6 days. The objectives of the study reported here were (1) to confirm that the systemic effects of STEC infection in swine can be prevented by postinfection treatment with Stx antibody and (2) to extend the concept to a naturally occurring disease induced by a host-adapted STEC strain and to treatment given several days after the onset of Stx production in the intestine.

Materials and Methods

Antibody and placebo. Hyperimmune serum was produced by vaccinating young adult pigs with genetically modified Stx2e (E167Q) toxoid, as described elsewhere [8, 13]. In brief, pigs were vaccinated

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Hyperimmune serum was produced by vaccinating young adult pigs with genetically modified Stx2e (E167Q) toxoid, as described elsewhere [8, 13]. In brief, pigs were vaccinated
intramuscularly 4–7 times, at weekly intervals, with 50–500 µg of toxoid in either Al₃(OH)₃, or Titer Max Gold (Sigma-Aldrich) adjuvant. Antibody was removed from serum by precipitation with 33% ammonium sulfate solution, as described elsewhere [9]. The titers of the resulting Stx2e antibody preparations had a range of 2000–100,000 log₂. Serum for the placebo (Stx2e antibody titer <2 log₂) was collected from nonimmunized pigs.

**Experimental design.** Three experiments, with a total of 6 replicates (table 1), were conducted, as described elsewhere [4]. Weaned pigs were orally inoculated with 10⁹ cfu of STEC strain S1191 (infected pigs) or nonpathogenic *E. coli* 123 (control pigs). *E. coli* S1191, which produces Stx2e, was isolated from a pig with edema disease [4]. *E. coli* 123, a nonpathogenic isolate, was obtained from a healthy pig.

Rectal swabs were collected from all pigs before and after inoculation. Rectal swabs were cultured on sheep blood agar, and fecal samples were assayed for Stx2e, as described elsewhere [4]. Serum samples for Stx2e antibody assays were collected from all pigs before inoculation, 1–2 days after antibody treatment, and at the end of experiments. Serum samples were processed and assayed as described elsewhere [4, 8]. The neutralizing titer was expressed as a reciprocal of the highest dilution that neutralized 3–5 Vero cytotoxins doses of Stx2e.

The objective of experiment 1 was to test whether a low-dose antibody treatment would prevent edema disease if Stx2e antibodies were given intraperitoneally at 2 DPI (table 1). The target antibody level to be attained was selected to approximate the protective antibody levels attained by active immunization [13].

There was evidence of protection in experiment 1. Therefore, the objective of experiment 2 was to test whether a medium dose of antibody treatment would prevent edema disease if antibody treatment was delayed to 4 DPI (table 1). The antibody dose given was higher than that given in experiment 1. There was no evidence of protection in experiment 2. Therefore, the objective of experiment 3 was to extend experiment 2 with administration of antibody at 4 DPI (table 1). Furthermore, the antibody dose was higher than that given in experiment 2.

Pigs were observed for signs of edema disease (i.e., subcutaneous edema or neurologic impairment). Infected pigs that remained free of clinical signs until the termination of experiments, 13–14 DPI, were designated subclinical pigs. At necropsy, all pigs were examined for gross lesions that are characteristic of edema disease and for lesions indicative of concurrent disease. Brain-stem samples and samples obtained from 2 sites on the ileum (1 and 2 m proximal from the ileocecal junction) were collected and preserved in formalin. Sections were examined microscopically for vascular necrosis. Slides were coded such that the pathologist was blinded to the treatment of the pigs. A tissue section was considered positive if it contained ≥2 necrotic arteriolar profiles. A pig was considered positive if ≥1 tissue sample was scored positive. The percentage of necrotic arteriolar profiles per section was measured, to determine whether the treatment had reduced the severity of disease in subclinical pigs.

**Statistics.** Fisher’s exact test (2 tailed), the χ² test, and the Wilcoxon signed-rank test were used for data analysis. Results were interpreted to be significantly different at *P* < .05. Whenever numerous tests of significance were done for a single data set, the significance level was normalized by use of the Bonferroni method.

### Table 1. Experimental design and incidence of clinical edema disease in pigs inoculated with Shiga toxin 2e (Stx2e)-producing *Escherichia coli* strain S1191 (infected pigs, *n* = 119) or nonpathogenic *E. coli* strain 123 (control pigs, *n* = 38).

<table>
<thead>
<tr>
<th>Experiment and group</th>
<th>Antibody (ab) treatment</th>
<th>No. of pigs</th>
<th>Total</th>
<th>Clinically ill</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>Placebo Low</td>
<td>2</td>
<td>30</td>
<td>9*</td>
</tr>
<tr>
<td>Infected</td>
<td>Stx2e ab Low</td>
<td>2</td>
<td>30</td>
<td>0*</td>
</tr>
<tr>
<td>Control</td>
<td>None NA NA</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Stx2e ab Low</td>
<td>2</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>Placebo Medium</td>
<td>4</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Infected</td>
<td>Stx2e ab Medium</td>
<td>4</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>None NA NA</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Stx2e ab Medium</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>Placebo High</td>
<td>4</td>
<td>20</td>
<td>7*</td>
</tr>
<tr>
<td>Infected</td>
<td>Stx2e ab High</td>
<td>4</td>
<td>20</td>
<td>0*</td>
</tr>
<tr>
<td>Control</td>
<td>None NA NA</td>
<td>4</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Stx2e ab High</td>
<td>4</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** Experiment 1 was performed with 3 replicates, experiment 2 with 1 replicate, and experiment 3 with 2 replicates. DPI, days postinoculation with *E. coli*; NA, not applicable.

*Significant difference in disease outcome between these 2 groups (*P* < .01).

*Significant difference in disease outcome between these 2 groups (*P* < .01).

### Results

STEC was not isolated from any pig before inoculation or from controls 2 or 5 DPI. All pigs inoculated with strain S1191 shed the organism in feces. Stx2e was detected at 2 DPI in feces from all of the 59 infected but none of the 29 control pigs so examined (data not shown). Stx2e antibodies were not detected in pigs before inoculation, and mean antibody titer remained <2 log₂ in the placebo-treated STEC-infected pigs and in nontreated control pigs until the end of the experiments (figure 1).

Intraperitoneal administration of Stx2e antibody to the control and STEC-infected pigs resulted in a significant increase in serum Stx2e antibody titers in infected pigs, in comparison with those of placebo-treated infected pigs and nontreated control pigs (*P* < .05, Wilcoxon signed-rank test; figure 1). Antibody titers attained and sustained in antibody-treated control and infected pigs were similar (*P* = .32–.83, Wilcoxon signed-rank test), decreasing slightly at the end of experiments in both groups.

None of the control pigs exhibited clinical signs or had gross lesions of edema disease. Clinical edema disease occurred in 21 (18%) of 119 STEC-infected pigs in experiments 1–3 (table 1). On histological examination, none of the control pigs was found to have vascular necrosis, whereas 89 (75%) of 119 infected pigs had vascular necrosis. Layers of bacteria adherent to ileal villi were seen in histological sections from 13 (11%) of 119 STEC-infected pigs but not in any from the control pigs.

**Experiment 1.** Clinical disease occurred only in placebo-treated STEC-infected pigs (table 1). The mean time of onset of clinical disease was 7 DPI. The difference in the incidence of clinical disease between antibody and placebo treatments
Figure 1. Geometric mean of Shiga toxin 2e (Stx2e) antibody (ab) titers (in log$_2$) of infected pigs (inoculated with Stx2e-producing *Escherichia coli* strain S1191) and control pigs (inoculated with nonpathogenic *E. coli* strain 123). Pigs were treated with low (experiment [Exp] 1), medium (Exp 2), or high (Exp 3) doses of Stx2e antibodies or placebo at 2 (Exp 1) or 4 (Exp 2 and 3) days after infection. Bars, SE.

was significant (*P* < .012, 2-tailed Fisher’s exact test). The incidence and extent of vascular necrosis in Stx2e antibody–treated and placebo-treated subclinical pigs were similar (76% of antibody-treated subclinical pigs had lesions, with an average of 25% of arterioles necrotic; 73% of placebo-treated subclinical pigs had lesions, with an average of 15% of arterioles necrotic).

**Experiment 2.** The mean antibody titers attained were ~10 times higher than those attained in experiment 1 (*P* < .05, Wilcoxon signed-rank test; figure 1). Clinical disease occurred in both antibody-treated and placebo-treated STEC-infected pigs (table 1). The mean time of onset of clinical disease was 5 DPI. All infected pigs that survived to the end of the study had vascular lesions. Vascular lesions were extensive in both groups (32% of arterioles necrotic in the antibody-treated group vs. 42% of arterioles necrotic in the placebo-treated group).

**Experiment 3.** The mean antibody titers attained in the STEC-infected pigs of experiment 3 were significantly higher than those attained in the STEC-infected pigs of experiment 2 (*P* < .05, Wilcoxon signed-rank test; figure 1). Clinical disease occurred only in placebo-treated STEC-infected pigs (table 1). The mean time of onset of clinical disease was 7 DPI. The difference in the incidence of clinical disease between antibody and placebo treatments was significant (*P* < .01, 2-tailed Fisher’s exact test). In contrast to the first 2 experiments, the incidence of vascular necrosis in antibody-treated subclinical pigs was significantly lower than that of placebo-treated infected pigs (30% and 85%, respectively; *P* < .01, *χ*$_2$ test). Additionally, the percentage of necrotic arterioles per section was reduced in the antibody-treated group (6% in the antibody-treated group vs. 32% in the placebo-treated group; *P* < .05, Wilcoxon signed-rank test).

**Discussion**

This study confirms the report that Stx-neutralizing antibodies, given parenterally after infection, can protect pigs against the systemic consequences of STEC infection [3]. This study extends that observation to disease caused by a host-adapted STEC strain in its natural host and extends the time of intervention to 4 DPI. We exploited the time period after infection and toxin production, but before the appearance of clinical signs, as a window for intervention against systemic
disease. In the edema disease model used here, the onset of Stx production occurred during the first DPI [4]. Protection appeared to depend on the dose of antibody and the time of intervention. Protection was induced with a low dose of antibody given 2 DPI (≥1 day after the onset of toxin production). Protection was also induced at 4 DPI, but only when the highest dose of antibody was given. This is consistent with evidence that protection of actively immunized pigs against intravenous Stx is antibody-titer dependent [9]. Our study supports the concept that it may be possible to prevent the systemic effects of Stx in humans with antibodies administered parenterally after the patient is infected with or exposed to STEC.

In humans, intravenous administration of large boluses of immunoglobulins poses the risk of inducing anaphylactoid reactions. These are attributed to immunoglobulin aggregates formed during precipitation and fractionation of serum. Although large amounts of immunoglobulins were given to the pigs in this study, neither anaphylactoid reactions nor vascular lesions indicative of immune complex–mediated vasculitis were observed. Antibody titers remained stable in treated pigs for the duration of the experiment, decreasing only slightly 10–12 days after the single injection. Although a reduction in the Stx2e antibody titers in the STEC-infected pigs, in comparison with control pigs, was expected (because of Stx2e antibody binding to the circulating Stx2e), we found no evidence for it.

Subclinical edema disease, characterized by microscopic vascular necrosis, is a consequence of STEC infection in pigs that remain free of clinical signs [4, 6, 13]. It has been proposed that, similarly, in humans infected with STEC, there may be microscopic vascular lesions in the absence of HUS [15]. Therefore, the effect of Stx2e antibody treatment on the incidence and extent of vascular lesions in subclinically infected pigs was assessed. We determined that protection against subclinical edema disease apparently depended on the antibody dose. Only the highest dose of Stx2e antibody reduced the incidence and extent of vascular lesions in subclinically infected pigs.

In conclusion, this study demonstrated protection of pigs against the systemic effects of STEC infection with passive antibody treatment given as late as 4 DPI. These results are consistent with the hypothesis that passive immune therapy may protect STEC-infected humans against systemic disease. Studies are needed to determine whether passive antibodies protect when administered at the time of onset of clinical edema disease.

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References


