Efficacy of Postinfection Treatment with Anti–Shiga Toxin (Stx) 2 Humanized Monoclonal Antibody TMA-15 in Mice Lethally Challenged with Stx-Producing Escherichia coli

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Infection with Shiga toxin (Stx)–producing Escherichia coli (STEC) causes hemorrhagic colitis and hemolytic uremic syndrome. TMA-15 is a humanized monoclonal antibody against Stx2, a major pathogenic factor. In a mouse infection model that used B2F1, a virulent STEC strain, the efficacy of TMA-15 was assessed when it was administered after bacterial and toxin exposure. In this model, a time-course analysis of the serum Stx2 level showed that the toxin was detectable from 24 h after infection. In an evaluation of the time-dependent efficacy, treatment with TMA-15 up to 24 h after infection ameliorated the lethal challenge, although treatment at 48 h showed no efficacy. To determine the effective dose, escalating doses were administered at 24 h after infection. The number of mice that survived after doses of 0, 0.25, 0.5, 1.0, and 2.0 mg/kg were 0/20, 11/20, 17/20, 20/20, and 20/20, respectively. These findings suggest that TMA-15 shows potential for prevention of severe complications associated with STEC infection.

Infection with Shiga toxin (Stx)–producing Escherichia coli (STEC) strains, including O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome (HUS) [1–3], is a serious problem—mostly in developed countries [4]. Two antigenically different types, Stx1 and Stx2, are the primary pathogenic factors [5]. Epidemiologic and experimental studies have suggested that Stx2 is more clinically significant than Stx1: Stx2-producing strains are more frequently associated with the development of HUS than are Stx1-producing strains [6, 7]. In mice, the lethal dose of purified Stx2 is 400 times lower than that of Stx1 [8]. Recently, in an experimental study of STEC-infected piglets, strains producing Stx2 alone caused more-severe neurologic symptoms than those producing both Stx1 and Stx2, whereas those producing Stx1 alone induced only diarrhea [9].

There is no effective treatment for HUS. Thus, it is important to prevent HUS and associated severe complications caused by STEC, especially Stx2-producing strains. Because the efficacy of antibiotic treatment for the development of HUS is controversial [10, 11], more-specific approaches against Stx are essential. One candidate approach is absorption of Stx in the gut. A toxin absorber, SYNSORB-Px (SYNSORB Biotech), is currently in clinical trials [12, 13]. Another technique being tested is use of a recombinant bacterium expressing glycolipid receptors for Stx, the efficacy of which was shown in a mouse model of STEC infection [14]. Systemic administration of neutralizing antibody against Stx is another promising approach. In experimental infection models, anti-Stx–neutralizing antibody protected the animals from death or neurologic complications [15–18]. However, the effective time window of these agents, relative to infection, has not been fully elucidated.

Because we believe that neutralizing antibody against Stx2 is the most promising candidate for prevention of HUS associated with STEC infection, we generated an anti-Stx2 murine monoclonal antibody (MAb), VTm1.1 [19], that was humanized by antibody engineering (so-called humanized antibodies have a longer half-life in human circulation and lower antigenicity to humans). For the present study, we used a streptomycin-treated mouse model of infection with a highly virulent STEC strain, B2F1 [16], in which the time course of the Stx2 level had been analyzed. We investigated whether the anti-Stx2 humanized MAb, TMA-15, exerts its efficacy even when administered after bacterial and/or toxin exposure.

Materials and Methods

Bacteria. STEC O91:H21 strain B2F1, which produces an Stx2 variant called “Stx2d” [16, 20], was obtained from the American
Type Culture Collection (ATCC 51435). A streptomycin-resistant clone of B2F1 was selected by passage culture of a Luria broth agar plate containing 30–100 μg/mL streptomycin.

Animals. We purchased 5-week-old male DBA/2J mice from CLEA Japan. The animals were quarantined and acclimated for 1 week before use in infection experiments.

Antibody. A humanized MAb against the Stx2 B subunit, designated “TMA-15,” was generated from the parental murine MAb VTm1.1 [19] by Protein Design Labs, by use of recombinant engineering antibody. A mouse anti-Stx2 A subunit MAb, 11E10 [21], was purified from the culture supernatant of a hybridoma cell line (ATCC CRL-1907).

Infection procedures. Infection experiments were done essentially as described for the streptomycin-treated mouse model [16, 22], with some modifications. DBA/2J mice were given drinking water containing 5 mg/mL streptomycin for 24 h and then were starved for food and water for 24 h. The animals were orally administered a bacterial suspension (0.2 mL) that contained 10^10 cfu starved for food and water for 24 h. The animals were orally administered a bacterial suspension (0.2 mL) that contained 10^10 cfu of B2F1 in 20% sucrose solution. After bacterial inoculation, feed and water containing streptomycin were provided ad libitum. The animals were monitored for death twice daily until 10 days after infection.

Measurement of toxin levels in blood. Serum samples were obtained from 10 animals per group. The collection time points were 1 h before and 12, 24, 48, 72, and 96 h after infection. We determined the serum Stx2 concentrations with a sandwich fluorimunoassay system constructed by using 11E10 MAb and the acetyl kinase–labeled Fab fragment of VTm1.1 as the capture and detection antibodies, respectively. The fluorescence signals after incubation with luciferin-luciferase reagent (Kikkoman) were measured by a plate reader (Luminous CT-9000D; DIA-IATRON). Recombinant Stx2 was used as a standard. The detection limit was 10 pg/mL.

TMA-15 treatment procedures. We performed 2 experiments on the efficacy of TMA-15 in the STEC-infection model. To investigate the time- and dose-dependent efficacy, TMA-15 at 0.25 and 2.5 mg/kg was intravenously administered to the mice via the tail vein 1 h before and 12, 24, and 48 h after infection. Control animals were infected but not administered TMA-15. Each group comprised 5 animals. For a dose-response study, 20 animals per group were given TMA-15 at 0.25, 0.5, 1.0, and 2.0 mg/kg 24 h after infection. Twenty control animals were infected and treated with saline containing the same content of the formulation vehicle (4% sucrose, 0.01% polysorbate 80, and 20 mM sodium citrate [pH 6]).

Results

Selection of mouse strain for STEC infection. To evaluate the efficacy of the agent in terms of the survival rate, we selected a streptomycin-treated mouse model by using a highly virulent STEC O91:H21 strain, B2F1 [16, 22]. A preliminary study revealed that DBA/2J mice, which are susceptible to O157:H7 strains [20], were even more susceptible to O157:H7 than the CD-1 outbred mice used in a previous study [16]. Accordingly, we decided to use a combination of STEC B2F1 and DBA/2J mice for further studies.

Quantitation of toxin levels in DBA/2J mice infected with B2F1. Figure 1 shows the serum Stx2 concentrations of infected mice at the indicated times. The serum Stx2 levels were below the detection limit until 12 h after infection. Stx2 was detectable (>10 pg/mL) in the serum of 6 of 10 mice at 24 h.

Time- and dose-dependent efficacy of TMA-15 in B2F1-infected mice. To examine the time- and dose-dependent efficacy, TMA-15 at 0.25 and 2.5 mg/kg was administered to streptomycin-treated DBA/2J mice at several time points before and after infection with 10^10 cfu of B2F1. All 5 control mice infected but untreated died within 5 days after infection (figure 2A). Pretreatment with TMA-15 at either dose completely protected the infected mice from death (figure 2B). The efficacy of the low dose was partial when administered at 12 and 24 h after infection, but the efficacy of the high dose was still complete (figure 2C, 2D). In contrast, even the high dose of TMA-15 at 48 h after infection had no effect on the survival rate (figure 2E), indicating that this timing was too late for TMA-15 to exert therapeutic efficacy.

Dose-dependent efficacy of TMA-15 at 24 h after STEC B2F1 infection. Another study was designed to determine the dose-dependent efficacy in detail at 24 h after infection, which was thought to be the time limit of the efficacy of TMA-15. DBA/2J mice (n = 20/group) that had been infected with B2F1 24 h earlier were administered the formulation vehicle or TMA-15 at 0.25–2.0 mg/kg. Figure 3 shows the survival curves for the...
mice treated with each dose. The 10-day survival rates at 0.25 and 0.5 mg/kg were 11 (55%) of 20 and 17 (85%) of 20, respectively. TMA-15 at 1.0 and 2.0 mg/kg prevented death from B2F1 infection in all 20 animals.

Discussion

In this study we evaluated the efficacy of a humanized MAb against the Stx2 B subunit, TMA-15, when administered after oral bacterial inoculation. It is important to determine whether treatment after infection is effective, since, in the clinic, treatment of patients with STEC infection is usually initiated after the onset of clinical symptoms. TMA-15 at ≥1.0 mg/kg completely protected the mice from lethal challenge even when administered 24 h after STEC infection. Thus, in this mouse model, there is a time window in which TMA-15 can be effective after infection. However, at 48 h after infection, TMA-15 treatment was totally ineffective, a time at which the serum Stx2 level of infected mice reached its maximum in the experiment measuring the serum Stx concentration. These data suggest that there is no chance for TMA-15 to be effective once large amounts of Stx2 enter the bloodstream and cause irreversible tissue damage.

It is difficult to extrapolate these results to postulate a clinical therapeutic time window, for 3 reasons. First, there are no reports of serum Stx level in humans with STEC infection, possibly because of interference by serum factors and/or blood cells [23]. Thus, it is not known when Stx enters the bloodstream and induces irreversible damage to target organs in humans. Second, STEC-infected mice likely do not develop gastrointestinal symptoms such as diarrhea [22, 24]; we did not observe such symptoms in our mouse model. Because disease days are usually counted from the onset of diarrhea in humans, the lack of diarrhea in the mouse model makes it difficult to compare the time courses of the disease between these species. Finally, disease severity varies greatly between mouse models and human patients: All of the untreated STEC-infected mice in our model died within 6 days after infection, whereas ∼10% of infected humans develop HUS more slowly and most HUS cases are not fatal.

Two possible therapeutic approaches against Stx are being tested. One is the use of a neutralizing antibody and the other is a toxin absorber in the gut (SYNSORB-Pk). The latter is
Figure 3. Dose-dependent efficacy of TMA-15 when administered 24 h after mice were infected with Shiga toxin–producing *Escherichia coli*. TMA-15 at 0.25, 0.5, 1.0, and 2.0 mg/kg was intravenously administered to mice that had been inoculated with B2F1 24 h earlier.

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References

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