Antiviral Activity of Shiga Toxin 1: Suppression of Bovine Leukemia Virus-Related Spontaneous Lymphocyte Proliferation†

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Human infections with Shiga toxin (Stx)-producing Escherichia coli (STEC) cause hemorrhagic colitis. The Stxs belong to a large family of ribosome-inactivating proteins (RIPs) that are found in a variety of higher plants and some bacteria. Many RIPs have potent antiviral activity for the plants that synthesize them. STEC strains, both virulent and nonvirulent to humans, are frequently isolated from healthy cattle. Interestingly, despite intensive investigations, it is not known why cattle carry STEC. We tested the hypothesis that Stx has antiviral properties for bovine viruses by assessing the impact of Stx type 1 (Stx1) on bovine peripheral blood mononuclear cells (PBMC) from cows infected with bovine leukemia virus (BLV). PBMC from BLV-positive animals invariably displayed spontaneous lymphocyte proliferation (SLP) in vitro. Stx1 or the toxin A subunit (StxA1) strongly inhibited SLP. Toxin only weakly reduced the pokeweed mitogen- or interleukin-2-induced proliferation of PBMC from normal (BLV-negative) cows and had no effect on concanavalin A-induced proliferation. The toxin activity in PBMC from BLV-positive cattle was selective for viral SLP and did not abrogate cell response to pokeweed mitogen- or interleukin-2-induced proliferation. Antibody to virus or StxA1 was most effective at inhibiting SLP if administered at the start of cell culture, indicating that both reagents likely interfere with BLV-dependent initiation of SLP. StxA1 inhibited expression of BLV p24 protein by PBMC. A well-defined mutant StxA1 (E167D) that has decreased catalytic activity was not effective at inhibiting SLP, suggesting the inhibition of protein synthesis is likely the mechanism of toxin antiviral activity. Our data suggest that Stx has potent antiviral activity and may serve an important role in BLV-infected cattle by inhibiting BLV replication and thus slowing the progression of infection to its malignant end stage.
A hallmark of PBMC from BLV-infected cattle is that they proliferate spontaneously in vitro (53, 54). This spontaneous lymphocyte proliferation (SLP) is particularly vigorous in PBMC cultures from cattle in the PL stage of infection. Since derepression of viral gene transcription and the synthesis of viral proteins (4, 22, 33) precede and are required for SLP to occur, we tested our hypothesis that Stx1 has antiviral activity by assessing the impact of toxin on SLP. Specifically, our goals were (i) to assess suppression of SLP by Stx1, (ii) to determine whether Stx1 acts selectively on BLV-positive PBMC, and (iii) to test the ability of Stx1 to inhibit expression of BLV proteins.

Our results indicate that Stx1 has a potent antiviral activity against BLV-positive bovine lymphocytes.

MATERIALS AND METHODS

Animals. Friesian-Holstein cows from the University of Idaho dairy were used as blood donors. Cows were identified as BLV-positive by the standard method of determining high titers of anti-BLV antibody. Five PL cows were identified by elevated numbers and percentages of B cells (3 standard deviations above normal levels) in peripheral circulation and used as BLV-positive donors. Cows with no detectable anti-BLV antibodies were used as BLV-negative donors.

Toxin. Recombinant Stx subunit A (Stx1A), Stx1A with the E-to-D amino acid substitution at position 167 (referred to throughout as E167D), and StxB were purified as previously described (3, 27, 58). Stx1A was purified from E. coli SY327(pSC25). Concentrated periplasmic proteins were adsorbed to Matrex Gel Green A agarose (Amicon) equilibrated with 10 mM phosphate-buffered saline (PBS) and Stx1A eluted as a single protein peak with approximately 0.3 M NaCl in a 0.15 to 1.0 M NaCl gradient. The E167D mutant was purified from E. coli SY327(pSC25.1) using the same protocol as for the wild-type StxA. StxB was purified from E. coli JM105(pSC32). Periplasmic proteins were fractionated by ammonium sulfate precipitation, and StxB was separated by isoelectric focusing and native polyacrylamide gel electrophoresis. Holotoxin was reconstituted in vitro by combining Stx1A and StxB at a 1:10 molar ratio in 10 mM Tris-HCl (pH 7.0) and dialyzed against 10 mM Tris-HCl (pH 7.0). The association of A and B subunits was confirmed by immunoblotting of proteins separated by analytical discontinuous native polyacrylamide gel electrophoresis. Before use in cultures, toxins were dialyzed exhaustively against 10 mM PBS, and concentrations were measured using a Bio-Rad assay with bovine serum albumin as a standard.

Lymphocyte culture and proliferation assay. Blood was collected by jugular venipuncture into acid-citrate-dextrose (ACD) (one part to four parts whole blood). PBMC were purified by density gradient centrifugation using Accupaque (1.068 g/ml; Accurate Chemical and Scientific Corp., Westbury, N.Y.) as previously described (20). Erythrocytes were lysed by incubation in warm ammonium chloride, and the PBMC preparation was washed several times in PBS-ACD mix (4:1) to remove platelets. PBMC were cultured in 96-well culture plates (Corning) at the initial density of 2.5 × 10⁶ cells/ml (0.5 × 10⁶ cells/well) in RPMI 1640 with 20% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 μg of streptomycin per ml, and 100 μg of penicillin per ml. To assess cell proliferation, [3H]thymidine was added to the wells (1.0 μCi/well) 18 to 24 h before harvest. The cells were harvested at 12, 18, 24, 48, and 72 h, centrifuged, and resuspended in 0.5 ml of 0.1 M Tris buffer (pH 7.5) with 0.1 M EDTA and 0.1 M phenylmethylsulfonyl fluoride. Samples were subjected to repeated freeze-thaw cycles until cells were lysed, as determined microscopically. Supernatants were transferred to nitrocellulose using a 96-well blotter, and cell lysates were probed with the murine monoclonal antibody BLV-24-kDa protein (referred to throughout as anti-p24) and anti-mouse antibody conjugated to alkaline phosphatase (Sigma, St. Louis, Mo.). Immunoblots were developed using 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Sigma) as substrate, according to manufacturer's instruction, and scanned with a Hewlett-Packard densitometer; the results were quantitated with the Molecular Analyzer analytical program. The cultures of BLV-negative PBMC served as negative controls.

Reagents. Concanavalin A (ConA) and pokeweed mitogen (PWM) were purchased from Sigma. Human recombinant interleukin-2 (IL-2) was purchased from Gibco BRL (Grand Island, N.Y.). Polyclonal antibody to Stx1A was generated by standard technique in New Zealand White rabbits. Lipopolysaccharide (LPS) of Salmonella enterica serovar Typhimurium was purchased from Difco Laboratories (Detroit, Mich.). Murine monoclonal antibodies BLV-1 against the 51-kDa glycoprotein of BLV (referred to throughout as anti-gp51) and control antibody COLIS69A of the same isotype (immunoglobulin G1) were purchased from WSU Monoclonal Antibody Center (Pullman, Wash.).

RESULTS

Stx1 suppresses SLP in cultures of PBMC from BLV-infected cows. PBMC from five BLV-positive cows in the PL stage of infection invariably proliferated in vitro, and this SLP was consistently suppressed by Stx1 (Fig. 1). Holotoxin and Stx1A were potent suppressors of SLP, acting in a dose-dependent manner over the range of concentrations tested. The effects of Stx1A or holotoxin were significantly different at 0.1 and 0.5 μg/ml because the 95% confidence intervals of the percent proliferation values did not overlap. Stx1B was far less potent than Stx1A in suppressing SLP even at molar concentrations more than fourfold higher than those of Stx1A. Moreover, in contrast to Stx1A, StxB did not act in a dose-dependent fashion. The 95% confidence intervals of the percent proliferation values were overlapping for all concentrations of StxB.

Cellular proliferation in spontaneously proliferating cultures of BLV-positive PBMC almost exclusively involves B lymphocytes (19, 30, 41). To evaluate Stx1 activity on normal B cells,
we measured Stx1 inhibition of PWM-induced proliferation of normal BLV-free PBMC. Normal B cells do not proliferate in culture unless stimulated to do so. Since a more appropriate control was not obvious, the PWM lectin, which is a B-cell mitogen, was used as the cell division stimulant. In contrast to SLP, PWM-induced proliferation of BLV-free PBMC was only weakly sensitive to Stx1 (Fig. 1). Low doses of Stx1A or Stx1 (0.1 \( \mu \)g/ml), sufficient to reduce SLP by 45 and 60%, respectively, caused <10% inhibition of proliferation induced by PWM. Stx1A at the highest concentration tested inhibited the PWM-induced proliferation by only 30%, whereas Stx1B and holotoxin either were marginally inhibitory or had a weak stimulatory effect in cultures from some donors.

To determine whether bovine T lymphocytes constitute targets for Stx1, we tested the impact of Stx1 on PBMC proliferation induced by ConA, a lectin that induces T-cell proliferation by specific interaction with the T-cell receptor complex. T-cell proliferation induced by ConA was not affected by Stx1 holotoxin or toxin subunits (data not shown).

These results indicated that SLP of BLV-positive PBMC is susceptible to Stx1-mediated inhibition and that the inhibitory effect is mediated by the A subunit of holotoxin. Subsequent experiments to further characterize toxin activity were performed with purified Stx1A or -B.

**Anti-Stx1A serum prevents inhibition of SLP by Stx1.** To determine if a spurious inhibitor was present in our toxin preparations, we tested the ability of anti-Stx1A immune serum to neutralize Stx1 or Stx1A suppression of SLP. Antitoxin neutralized Stx1 or Stx1A activity in a dose-dependent manner (Fig. 2) and did not affect cellular proliferation in cultures without toxin (data not shown). The antitoxin was effective within a range of titers from 1:1000 to 1:50 but did not have a neutralizing ability outside this range (data not shown). Within this range, the ability of antitoxin to neutralize increasingly greater doses of Stx1A was directly proportional to concentration. For example, antitoxin restored about 80 or >50% of the thymidine incorporation in BLV-positive cultures treated with up to 1.0 \( \mu \)g of Stx1A or Stx1, respectively, per ml (Fig. 2). A two-way ANOVA indicated statistically significant differences among the effects of various concentrations of toxin and antitoxin as well as a significant interaction of these two factors.

**BLV-positive PBMC treated with Stx1A retain responsiveness to immunostimulation.** To determine the mechanism of SLP suppression by Stx1, it was important to assess whether the impact of the toxin on SLP was mediated by selective targeting or by indiscriminate suppression of the ability of BLV-positive PBMC to respond to immunostimulation. We tested the impact of Stx1A on cellular proliferation in cultures of BLV-positive PBMC supplemented with PWM or IL-2, a potent B-cell activator. The addition of IL-2 (1.0 ng/ml) to PWM-negative cultures strongly augmented proliferation, evidenced by a gain of about 6.0 \( \times \) 10^4 cpm per well (Table 1). This IL-2-induced proliferation was preserved even in the presence of Stx1A at 1.0 \( \mu \)g/ml, a toxin concentration sufficient to cause almost complete suppression of SLP. Moreover, proliferation in these cultures exceeded proliferation in cultures of BLV-negative PBMC treated with combination of Stx1A and IL-2 (Table 1). BLV-positive cultures treated with Stx1A also retained the ability to respond to stimulation with PWM (Table 1). These results suggest that inhibition of SLP by Stx1 involves selective action on a subpopulation of PBMC and does not alter the ability of B cells not targeted by the toxin to respond to immunostimulation.

IL-2 and PWM induce substantial T-cell blastogenesis. In BLV-negative PBMC cultures up to 40 to 60% of blast-size cells are classified as T cells (defined as CD5-positive cells lacking B-cell markers). Since Stx1 did not inhibit T-cell proliferation, T-cell division may have masked Stx1-mediated inhibition of B-cell blastogenesis. The percentage of B cells enlarged to blast size in PWM-stimulated cultures ranged from 20% (BLV positive) to 40% (BLV negative) and was not affected by the presence of Stx1A at 1.0 \( \mu \)g/ml. However, in IL-2-stimulated cultures, the percentage of blasts was reduced by toxin (from 60% to 30%) in BLV-negative PBMC, although no such reduction occurred in BLV-positive cultures. This Stx1A activity correlated with the lack of CD5 expression on B cells. Among BLV-negative PBMC, in which CD5-negative cells predominate, toxin inhibited B-cell enlargement whereas among BLV-positive PBMC, in which CD5-positive B-cells predominate, toxin did not inhibit B-cell enlargement.

We also examined the impact of Stx1A on PBMC cultures stimulated with LPS. This gram-negative bacterial cell wall component can significantly influence immune responses and was shown to stimulate BLV expression in cultures of BLV-positive PBMC (34). LPS used at a concentration of 0.1 \( \mu \)g/ml increased proliferation of BLV-positive PBMC twofold but did not induce normal PBMC cultures to proliferate, indicating
that only BLV-positive cultures were susceptible to mitogenic stimulation by low concentration of LPS. The increased proliferation resulting from LPS application was completely abrogated by treatment with Stx1A (data not shown), further indicating that cells involved in SLP constitute the cellular targets of Stx1A.

Stx1A suppression of SLP is not accompanied by increased cytotoxicity. Additional support for the premise that Stx1 targets a selected and probably minor subpopulation of B cells comes from the finding that cell death, detected by trypan blue exclusion or cell shrinkage measured by flow cytometry, was not greater in cultures treated with Stx1A than in cultures without toxin. We analyzed PBMC from five PL cows incubated with and without toxin over a 3-day culture period and observed no differences in the number of dead cells. Results from a representative experiment are shown in Fig. 3. After 3 days in culture, 36% of the cells incubated without toxin were nonviable B cells (Fig. 3B); likewise, 26 and 34% of the cells incubated with Stx1A at 0.1 and 0.5 μg/ml, respectively, were nonviable B cells (Fig. 3C and D). The finding that treatment with Stx1A did not increase B-cell death is consistent with the fact that although the majority of B cells from cows in PL stage contain provirus, very few PBMC from BLV-positive cattle express viral proteins (4, 11, 24, 39).

Inhibition of SLP by antiviral antibody or Stx1A is time dependent. SLP in cultures of BLV-positive PBMC is preceded within 24 h of culture by de novo synthesis of viral proteins and dissemination of viral particles (4). It is known that anti-BLV serum can block SLP (55). To assess whether viral proteins accessible to antibody were required to sustain SLP, we examined the ability of antiviral antibody to interfere with SLP over a 2-day period. Monoclonal anti-gp51 was able to reduce thymidine incorporation in spontaneously proliferating cultures by 60% (Fig. 4). However, this inhibition required application of anti-gp51 at the beginning of cell culture (Fig. 4). Inhibition of SLP by anti-gp51 was due to a specific interaction with viral proteins, since this antibody did not affect IL-2-induced proliferation of BLV-negative PBMC, and control monoclonal antibody of the same isotype had no effect on SLP (data not shown). These results are in agreement with the findings that dissemination of BLV proteins is involved in initiation of SLP, but they also suggest that BLV proteins are not required for continuation of an established SLP event.

To determine if toxin also acts on SLP in a time-dependent fashion, we administered Stx1A or Stx1B to cultures of BLV-positive PBMC at various times after the start of cell culture. Similar to treatment with anti-gp51, the ability of Stx1A to inhibit SLP was reduced if cells were precultured in medium for 24 h before toxin application (Fig. 4). Stx1A applied on day 2 of culture at concentrations of up to 1.0 μg/ml had only minimal impact on SLP (Fig. 4), and even 5.0 μg of Stx1A per ml applied on day 2 of culture reduced thymidine incorpora-
Immunoblot analysis of cell lysates of PBMC cultured for 12 h showed a lesser amount of p24 protein in cells treated with Stx1A. The fact that susceptibility of SLP to inhibition by either Stx1A or anti-gp51 lessens within 24 h of culture argues that the cells involved in dissemination of viral proteins and the initiation of SLP constitute targets for Stx1.

In contrast to Stx1A, the relatively minor effect of Stx1B on SLP did not change when Stx1B was applied after a preculture without toxin (data not shown). This difference suggests that Stx1B and Stx1A have different modes of action and likely affect different subpopulations of PBMC.

**Stx1A reduces expression of BLV core protein.** To directly test antiviral activity of Stx1, we assayed the expression of BLV p24 core protein in PBMC cultured with or without Stx1A. Immunoblot analysis of cell lysates of PBMC cultured for 12 h showed a lesser amount of p24 protein in cells treated with toxin (1.0 μg/ml) than in cells in the control cultures without toxin (Fig. 5). The optical density of the immunoreaction in the sample treated with toxin was 442-fold less than the immunoreaction in the sample without toxin, suggesting that toxin suppressed viral protein synthesis.

**Stx1A enzymatic activity is required for antiviral effect.** We used a well-characterized site-specific mutant of the Stx1A chain to determine if the protein synthesis-inhibitory enzymatic activity of the toxin was required for its antiviral effect. The E167D catalytic center mutant maintains structural integrity but has enzymatic activity several orders of magnitude less than that of wild-type toxin (27). In contrast to wild-type Stx1A, the E167D mutant toxin had no inhibitory activity, and PBMC from BLV-positive cows treated with mutant toxin proliferated as if they were not treated with toxin (Fig. 6). This observation suggests that toxin-mediated protein synthesis inhibition is the mechanism by which Stx1A suppressed viral protein expression in cultures of BLV-positive PBMC and without viral proteins, the hallmark SLP does not occur.

**DISCUSSION**

The aim of this study was to test the hypothesis that Stx1 has antiviral activity in cattle, the animal reservoir for STEC. We accomplished this aim by examining the ability of Stx1 to inhibit BLV-dependent SLP of PBMC from cows in the PL stage of BLV infection. Our results provide the first demonstration of antiviral activity of Stx and are consistent with a copious body of research showing antiviral activity of the RIP family of toxins in the plants that express them (reviewed in reference 51). We showed that Stx1 specifically suppresses BLV-induced SLP (the hallmark of this viral infection) and that toxin does not suppress cytokine- or mitogen-induced cell proliferation in either BLV-infected or normal bovine cells. In addition, we show that toxin does not induce increased or indiscriminant cell death. The most likely explanation for these results is that Stx1 has a specific adverse impact on the cells that express the virus.

A high proportion (up to 70% and possibly more) of the B cells from BLV-infected cattle carry provirus, but due to repression of the BLV genome, only a small proportion (<1%) of these cells express viral proteins initially in culture (22). It is well established that SLP is preceded and accompanied by synthesis of viral proteins (32, 53). BLV-specific antibody inhibition of SLP is also well established (54, 55) and may result from interference of the release of BLV particles from cultured cells (16). The premise that Stx1 has antiviral activity is supported by our findings that maximal SLP sensitivity to Stx1 was exhibited within the first 24 h of culture. We also found a similar time-dependent loss of sensitivity of SLP to anti-gp51-mediated inhibition. Both of these findings are consistent with the fact that the expression of BLV particles in culture reaches maximum after 12 to 24 h of cell culture (57). Finally, we demonstrated that cell cultures treated with Stx1 express less BLV p24 protein. This could be due either to the nonlethal suppression of viral protein synthesis or Stx1-mediated death of the cells expressing viral proteins. Our assays did not allow distinction between these possibilities, since our determination
of the p24 protein level was limited to the protein present within cells harvested from the cell cultures at a given time.

Similar to ricin, the archetype of the A:B RIPs, Stx1 holo-toxin is composed of an enzymatically active A chain and a cell receptor-binding B-chain pentamer. The A subunit alone was able to abrogate SLP and was similarly efficacious as holotoxin. Thus, sensitivity of target cells in BLV-positive culture to Stx1 occurs via a mechanism that does not require the B subunit. This is in sharp contrast to the receptor-based mechanism by which Stx1 gains entry to Vero cells and other cellular targets described thus far (5, 29). For example, others have shown that human B lymphocytes are sensitive to Stx and this sensitivity parallels expression of CD77 that can bind the B subunit. However, normal bovine lymphocytes are not sensitive to toxin, and bovine cells have not been shown to express the CD77 ligand. Also, a precedence of antiviral activity without the B subunit has been set by many plant RIPs. Type 1 RIP hemitoxins composed solely of an enzymatic A chain are po-tent antiviral agents; examples include inhibition of HIV replication by pokeweed antiviral protein (45), bydronin (56), and trichosanthin (9). Similar anti-HIV activity is exhibited by an isolated A chain of ricin (45). Typically, inhibition of HIV-1 replication by plant RIPs occurs at the concentrations nontoxic to uninfected cells (37, 45).

The mechanism of Stx anti-BLV activity was not investi-gated; however, our finding that the E167D mutant lacks an-tiviral activity, along with the fact that this catalytic center has been highly conserved among all RIPs, strongly suggests that directed protein synthesis inhibition is the likely mechanism of antiviral activity. It should be noted, however, that inhibition of protein synthesis may not be the only mechanism of antiviral activity. Plant RIPs were shown to inhibit HIV-1 integrase via topological activity on long terminal repeats of viral DNA (37), and these proteins show structural similarity to retrovirus reverse transcriptases (49). Inhibition of HIV infection by plant RIPs involves regions of these proteins which are not required for ribosome inactivation, suggesting that the anti-HIV activity of ribosome-inactivating proteins may not be the result of N-glycosidase activity alone (38). Interestingly, some antiviral activity of RIPs has been associated with the B sub-unit. For instance, ricin can agglutinate hog cholera virus (a small RNA virus) due to a galactose-binding ability of B sub-unit (43). Ricin was also able to agglutinate cells of a variety of leukemic cell lines, including NIH 3T3 cells infected with Moloney leukemia virus (35).

Elucidation of the mechanism by which the A subunit enters cells was beyond the scope of this investigation. However, it is possible that Stx1A uptake by target cells in our experiments was facilitated by the cell membrane perforation. A variety of mammalian cells infected by virus display this type of increased membrane permeability (21), and it may occur in cells with replicating BLV. Another possibility is that internalization of Stx1A by target cells involved nonspecific endocytosis. This mechanism could explain the fact that proliferation of PWM-induced normal PBMC was somewhat reduced by high concentration of Stx1A. Since SLP is preceded by de novo synthesis of BLV proteins, the elevated metabolism of cells expressing BLV could increase the susceptibility of these cells to Stx1A. However, internalization of Stx1A by nonspecific endocytosis does not explain all of our findings. Specifically, endocytosis does not account for the fact that the spontaneously proliferating cultures became less sensitive to Stx1A within 24 h of preculture without the toxin, and it does not explain the inhibition of SLP by Stx1A used at concentrations which only marginally affected the proliferation of normal PBMC. These findings imply that BLV-expressing cells are exceedingly sen-sitive to Stx1 and that the toxin acts via a selective mechanism.

Very little information exists regarding the action of Stx1 on bovine cells. A recent publication (40) describes the impact of Stx1 on the metabolic rate of the bovine leukemic cell line BL-3 and on normal bovine PBMC. In both cell types, the metabolism was reduced by Stx1A but only if basal metabolism was first increased by a mitogen such as PWM, ConA, or phytohemagglutinin or by LPS. If cell metabolism was not stimulated to increase, Stx1 had no effect on basal metabolic rate. In agreement with our results, Meng et al. did not detect a cytotoxic impact of Stx1 on PBMC, even when Stx1 caused a 50% reduction of the metabolic rate (40). Since these authors did not clarify the BLV status of their PBMC donors, it is possible that these effects were due to antiviral activity of Stx1. Interestingly, the authors state that the BL-3 cell line was secondarily infected with BLV and with bovine diarrhea virus; however, the BLV activation in these BL-3 cells was not char-acterized.

BLV infections in cattle are chronic, and in most animals the disease does not progress to the malignant stage. Although antibodies to BLV are clearly important in viral repression (8, 47), they do not always prevent progression of BLV infection to the PL and malignant stages. Consequently, other factors interfering with BLV replication may play a role in a suppres-sion of this virus. Our results indicate that Stx1 may serve a protective role in BLV-infected cows. Gastrointestinal STEC release toxin systemically, because cattle have anti-Stx antibodies in serum and colostrum (46). More evidence to support the movement of the toxin out of the gastrointestinal tract comes from tissue culture experiments. Biologically active Stx1 is capa-ble of moving across a monolayer of intact polarized human intestinal epithelial cells (2), which suggests that Stx1 may also be capable of crossing the intestine in cattle harboring STEC. Stx1 is not cytotoxic to normal bovine PBMC (40), and consequ-ently the presence of Stx in tissues or body fluids of cattle harboring BLV could benefit these animals by causing deletion of the BLV-expressing cells and/or inhibiting viral protein expression and propagation.

These findings have implications for the pathogenesis and epidemiology of STEC as well as maintenance of its bovine reservoir. Ultimately, analyses to correlate gastrointestinal STEC and/or systemic Stx with a delayed progression of BLV disease are needed to demonstrate a selective advantage for cattle to harbor STEC. Future work is planned to assess the antiviral activity of other members of the Shiga toxin family and to more thoroughly study the role of previously identified enzymatic and translocation domains of the Stx1A subunit in antiviral activity (15, 27, 52).

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