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Inhibition of water absorption and selective damage to human colonic mucosa induced by Shiga toxin-2 are enhanced by *Escherichia coli* O157:H7 infection

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) strains are responsible for a variety of clinical syndromes including bloody and non-bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). Although multiple serotypes of STEC have been isolated from hemorrhagic colitis cases, *E. coli* O157:H7 is by far the most prevalent serotype associated with HUS. Shiga toxin is the major virulence factor of *E. coli* O157:H7 and is responsible for the more severe symptoms of the infection. However, the mechanisms involved in the pathogenesis of diarrhea mediated by Stx2 are not well known. In this study, we have determined the effects of *E. coli* O157:H7 strain 125/99 wild type (wt) on the human colonic mucosa mounted in an Ussing chamber. In response to 125/99wt, an inhibition of water absorption across human colonic mucosa was observed. Histological sections showed severe necrosis with detachment of the surface epithelium, mononuclear inflammatory infiltrate and loss of goblet cells after 1 h of incubation with 125/99wt. These alterations were not observed with the isogenic mutant strain lacking *stx2* or with the filter-sterilized culture supernatant from the 125/99wt strain. These results indicate that the cell damages in human colon are induced by Stx2, and that Stx2 production is increased by the interaction with bacterial cells. Identification of host cell-derived factors responsible for increasing Stx2 can lead to new strategies for modulating STEC infections.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) strains are responsible for a variety of clinical syndromes including bloody and non-bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (Karmali et al., 1985). HUS develops in 5–10% of children several days after bloody diarrhea and is a systemic disease characterized by thrombotic microangiopathy, hemolytic anemia, thrombocytopenia, and acute renal failure (Gianantonio et al., 1964; Boyce et al., 1995). Although multiple serotypes of STEC have been isolated from hemorrhagic colitis cases, *E. coli* O157:H7 is by far the most prevalent serotype associated with HUS. Shiga toxin (Stx), an AB_5 toxin, is the major virulence factor of *E. coli* O157:H7 and is responsible for the more severe symptoms of the infection. The A subunit (StxA) possesses N-glycosidase activity against 28S rRNA of 60S ribosomes in the cytosol, resulting in inhibition of protein synthesis in eukaryotic cells and activation of proinflammatory signaling cascade referred to as the ribotoxic stress response (Smith et al., 2003). In fact, it has been demonstrated that Stx induces both primary response genes c-jun and c-fos and activates the stress activated protein kinases, JNK/SAPK and p38, in intestinal epithelial cells. Stx enzymatic activity is also required for the referred kinase activation. The five B subunits (StxB) form a pentamer that binds to globotriaosyl ceramide (Gb3) receptors on the cell membrane (Thompson et al., 2000). *E. coli* O157:H7 can produce two antigenically distinct forms of Stx proteins (Stx1 and Stx2) and their variants, being Stx2 more virulent and epidemiologically more relevant than Stx1 (Palermo et al., 2009).

The genes *stxAB* are located in the genome of prophages that resemble the coliphage lambda (Neely and Friedman, 1998). Phage

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induction is critical to Stx gene expression and to the ability of STEC to cause disease (Tyler et al., 2013). The toxin genes are late-stage genes that are transcribed only during the lytic stage of the phage. The production of Stx is linked to the replication cycle of Stx phages, and the release of Stx is dependent on the lytic phase (Herold et al., 2004), which is induced under stress conditions (Zhang et al., 2000; Wagner et al., 2001). It has been proposed that differential Stx2 expression may account for differences in virulence. In this regards, STEC have been classified in pathotypes and clades according to Stx-phage type that determines Stx variant and level of Stx-production (Karmali, 2009). In particular, Stx2 overexpression is common to STEC strains from clade 8, which are highly associated with HUS (Neupane et al., 2011). While several groups have analyzed factors and conditions in the human gastrointestinal tract able to induce Stx2-phage in *E. coli* O157:H7 and the corresponding increase in Stx2 production (Bansal et al., 2012) others have recently shown the existence of putative eukaryotic promoter-like sequences located upstream of the genes encoding for the Stx2A and B subunits, and an eukaryotic machinery able to recognize it (Bentancor et al., 2013a) This property gives to the mammalian cells the capacity to transcribe and translate *stx2* genes e.g. after uptake of Stx2 encoding phages released after bacterial lysis, adding a putative alternative source of Stx production (Bentancor et al., 2013b).

In this study we examine the physiological and morphological effects of *E. coli* O157:H7 expressing Stx2 on human colonic mucosa. Our results show that the interaction of *E. coli* O157:H7 strain 125/99wt with the human colonic mucosa caused a significant inhibition of water absorption and histological damages on the surface epithelium. These alterations were not observed with the *stx2* mutant strain or with the filter-sterilized culture supernatant from the 125/99wt strain. These results indicate that the cell damages in human colon are induced by Stx2, and that Stx2 production is increased by the interaction with bacterial cells.

Materials and methods

Bacterial strains and growth conditions

Properties of *E. coli* strains used in this study are listed in Table 1. *E. coli* O157:H7 strain 125/99 wild type (125/99wt) isolated from a patient with HUS has been previously described (Rivas et al., 2006b). The strain 125/99wt carries the *stx2* EDL *sx2* variant and the Stx encoding phage is inserted in the yehV gene (Amigo et al. unpublished results). A mutant lacking *stx2* gene from the parenteral *E. coli* O157:H7 strain 125/99wt (125/99*Δstx2*) was made for the present study. A commensal non-pathogenic wild type *E. coli* was isolated from the feces of a healthy human (Gerhardt et al., 2013). This strain was used as a negative control.

Bacterial strains were grown in Luria Broth medium for 18 h at 37 °C with shaking at 150 rpm and then diluted 1/50 in DMEM/F12 medium and grown to exponential phase (OD_{600} = 0.3–0.4) at 37 °C with shaking at 50 rpm. In all the cases bacterial supernatants were collected after centrifugation at 10,000 × *g* for 5 min, followed by filtration through a 0.22-μm filter (Millipore, Billerica, MA, USA). Stx2 cytotoxicity in the filter-sterilized bacterial supernatants (SN) was determined in Vero cells assays.

*Construction of the mutant 125/99Δ*stx2

Primers for allelic replacement include in the 5′ end 50 nucleotides of gene sequence to replace, while the 3′ end is complementary to the kanamycin resistance gene (KmR) from plasmid pKD4 (Datsenko and Wanner, 2000). Primers mutStx2 were designed based on sequences flanking *stx2AB* (GenBank AF125520) plus 20 nucleotides corresponding to kanamycin cassette in pKD4 (GenBank AY048743.1). Primers used and generated in this study, the cycling conditions and size of the PCR products are listed in Table 2. PCR reactions for generating mutagenic PCR products were performed according to the conditions described in Table 2, with template pKD4 and primers mutStx2 F and mutStx2 R.

The 125/99wt was transformed with the plasmid pKD46 that expresses the λ Red system (Datsenko and Wanner, 2000) and electroporated with mutagenic PCR products. Recombination events in 125/99 stx2::KmR were confirmed by PCR. The absence of *stx2* gene as well as the presence of KmR cassette in *E. coli* genome was confirmed by DNA sequencing (data not shown).

Human colonic mucosa collection and preparation

The colon fragments used in this study were obtained from surgeries performed on 21 adult patients with cancer (informed consent was obtained) at the "Servicio de Cirugía Gastroenterológica, Hospital Churruca-Vizca", Buenos Aires, Argentina. The Ethics Committee of the Universidad de Buenos Aires approved the use of human tissues for research purposes. Colon fragments were removed immediately after ablation from macroscopically unaffected regions inside the "security zone" adjacent to the pathologically affected zone. Tissues were transported to the laboratory in oxygenated ice-cold high-potassium Ringer solution (in mM): 120 KCl, 10 $NaHCO_3$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 1.2 K_2HPO_4, 0.2 KH_2PO_4, 25 glucose, to preserve the transport functions. The mucosa and submucosa layers were then dissected from the underlying tissue (kept at 4 °C) and cut into 3–5 fragments depending on the size. Each mucosa fragment was mounted as a diaphragm on a modified Ussing chamber (1.76 cm^2) and used either for one of the treatments or for control. Both sides of the tissue were washed and bathed with a standard Ringer solution (in mM): 113 NaCl, 4.5 KCl, 25 $NaHCO_3$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 1.2 K_2HPO_4, 0.2 KH_2PO_4, 25 glucose, and bubbled with 95% O_2–5% CO_2. The bathing solution was maintained at 37 °C with water-jacketed reservoirs connected to a constant temperature circulation pump. This model of the Ussing chamber has a mucosal and serosal compartments bathed with 2 ml of Ringer solution each one, separated by the mounted tissue.

Net water flux and electrical measurements

Transepithelial net water flux (Jw) was recorded automatically across an Ussing chamber connected to a special electro-optical device (Dorr et al., 1997; Fernandez-Miyakawa et al., 2007). Briefly, the tissue was held against a nylon mesh by a hydrostatic pressure of 13 cm of H_2O. Water movement across the tissue was measured by displacement of a photo-opaque solution inside a glass capillary tube connected to the mucosal side of the chamber via an intermediate chamber. The liquid meniscus movement in the glass capillary was detected using the electro-optical device connected to a computer. The sensitivity of this instrument is approximately 50 nl.

The spontaneous potential difference (PD), expressed in mV, was simultaneously recorded in the other chamber across the micro-reference electrodes (Harvard Apparatus Inc, USA) placed adjacent to the epithelium under open-circuit conditions. The short circuit current (Isc) expressed in μA/cm^2 was measured with an automatic voltage clamp system that kept the PD at 0 mV. The transepithelial resistance (Rt) expressed in Ω cm^2 across the colonic mucosa was calculated from the Isc and open-circuit PD values, according to Ohm's law.

When the parameters were stabilized, the following samples were added to the mucosal side of each colon tissue (time 0): 200 μl (approximately 10^8 CFU) of bacterial culture of 125/99wt, 125/99*Δstx2*, filter-sterilized culture supernatant from 125/99wt or commensal *E. coli*. Then, both Jw and Isc were simultaneously

Table 1
E. coli strains used in this study.

Strain	Serotype	Sample source[a]	Gene profile[b]				Reference of source
			eae	stx1	stx2	ehxA	
O157:H7 125/99wt	O157:H7	HUS	+	–	+	+	Rivas et al. (2006b)
O157:H7 125/99*Δstx2*	O157:H7	Lab	+	–	–	+	This study
Commensal *E. coli*	Not determined	Lab	–	–	–	–	Gerhardt et al. (2013)

[a] O157:H7 was originally isolated from feces of a patient with HUS.
[b] Presence or absence of a gene was determined by multiplex PCR. *eae* and *ehxA* are markers for the LEE pathogenicity island and the large STEC virulence plasmid, respectively.

Table 2
Primers generated in this study.

Primer	Sequence (5′ to 3′)[a]	Cycle conditions	Size (bp)
mutStx2 F	ATGAAGTGTATATTATTTAAATGGGTACTGTGCCTGTTACTGGGTTTTTCTGTAGGCTGGAGCTGCTTCG	94 °C 5 min (1 cycle), 94 °C 30 s, 55 °C 30 s, 72 °C	1500
mutStx2 R	TCAGTCATTATTAAACTGCACTTCAGCAAATCCGGAGCCTGATTCACAGGCATATGAATATCCTCCTTAG	1 min (35 cycles), 72 °C 5 min (1 cycle)	

[a] Underlined base pairs correspond to kanamycin cassette in pKD4.

recorded for 60 min. Because of tissue variability, data are analyzed as ΔJw where ΔJw = Jw (at a given time) – Jw (at time 0) and ΔIsc, where ΔIsc = Isc (at a given time) – Isc (at time 0). Each assay was carried out 3–6 times with colon fragments obtained of from different patients. At the end of the experiment, Ringer solutions from mucosal compartment was recovered and sterilized by filtration through a 0.22-μm filter (Millipore, Billerica, MA, USA). Stx2 cytotoxicity in the filter-sterilized conditioned Ringer solution (CRS) was determined in Vero cells assays.

Cytotoxicity assay

Purified Stx2 (Phoenix Laboratory, Tufts Medical Center, Boston, MA, USA), SN and CRS were assayed for cytotoxicity on Vero cells as previously described (Karmali et al., 1985). Briefly, Vero cell monolayers grown in 96-well plates were treated for 72 h under growth-arrested conditions (serum-free medium) with different concentrations of Stx2 or different dilutions of SN and CRS. At the end of the incubation, plates were washed twice with PBS (145 mM NaCl, 10 mM NaH_2PO_4, pH 7.2) and incubated for 2 h with freshly diluted neutral red in PBS to a final concentration of 50 μg/ml. Cells were then washed with 1% $CaCl_2$ and 4% formaldehyde twice and then were solubilized in 1% acetic acid and 50% ethanol. Absorbance at 546 nm was read in an automated plate spectrophotometer. Results were expressed as percent viability, with 100% represented by cells incubated under identical conditions but without treatment. The 50% cytotoxic dose (CD_{50}) corresponded to the dilution required to kill 50% of Vero cells. This assay is able to detect approximately 2 pg/ml of Stx2.

In neutralization studies, CRS were preincubated at 37 °C for 1 h with the mouse monoclonal antibody 2E11 against the A-subunit of Stx2 (Rocha et al., 2012) at dilution 1:10 (250 μg/ml) or 1:100 (25 μg/ml). The mixtures were then added to each well containing Vero cells and incubated for 72 h. Cell viability was analyzed by neutral red uptake, and 100% represents cells incubated under identical conditions but without treatment.

Histological studies

Human colonic mucosa were recovered after physiological experiments and fixed for at least 24 h in cold fixative (4 °C) containing 4% formaldehyde in PBS. After fixation, longitudinal 2–4 μm thick sections were cut, dehydrated, and carefully embedded in paraffin to provide sections perpendicular to the mucosa. Sections were then stained with hematoxylin–eosin (H&E) and observed by light microscopy (Nikon Eclipse 200, NY, USA). The slides were then blindly scored on 3 criteria that typically were observed in human colonic mucosa treated with Stx2: necrosis, inflammation and loss of goblet cells. A scale from 0 to 3 was used with 1 being the least severe or lowest, and 3 being the most severe or highest. Details of the points assigned to the different degrees of necrosis, inflammation and loss of goblet cells are explained in Table 3. Six photomicrographs fields at ×200 magnification were analyzed for each experimental condition.

Statistiscal analysis

Results are reported as means ± standard errors of the means (SEM). The statistical significance between two mean values obtained for two different experimental conditions was calculated by the Student's *t*-test. All data from the curves were analyzed using Mann–Whitney test. Histological scores were analyzed by Kruskal–Wallis test with Dunn's multiple comparisons as a *posteriori* test. Statistical analysis was performed using Graph Pad Prism Software (San Diego, CA, USA). *P* values <0.05 were considered significant.

Results

E. coli *O157:H7 strain 125/99 inhibits the normal water absorption across human colonic mucosa*

Under basal conditions, an absorptive Jw (0.16 ± 0.02 μl/min cm^2, $n = 21$) was observed in the human colonic mucosa placed between two identical Ringer solutions in the Ussing chamber. The electrical parameters tested simultaneously with the net water flux showed a PD of 2.3 ± 0.3 mV, Isc of 27.4 ± 2.9 μA/cm^2 and Rt of 91.7 ± 11.4 Ω cm^2.

To establish the relative contribution of bacterial cells to the cytotoxic effect of Stx2, matched colonic mucosa obtained from the same patient were incubated with 200 μl (approximately 10^8 CFU) of 125/99wt, 125/99*Δstx2* or commensal *E. coli* strain. The 125/99wt strain caused a significant Jw inhibition compared with the commensal *E. coli* (Fig. 1, $P < 0.05$, $n = 5$). However, the respective isogenic mutant strain lacking *stx2* did not alter the absorptive water transport. The Jw values obtained after incubation with 125/99*Δstx2* strain were similar to those recorded with commensal *E. coli* used as negative control (Fig. 1, $n = 5$). These results suggest that Stx2 could be responsible for the water absorption inhibition measured across the human colonic mucosa. Jw remained unchanged when colonic mucosa was incubated with filter-sterilized culture supernatant from 125/99wt (SN 125/99wt)

Table 3
Scoring system used for histological evaluation of human colonic mucosa.

Score	Necrosis	Inflammation	Loss of goblet cells
0	No necrosis	No mononuclear inflammatory infiltrate.	No loss of goblet cells.
1	Mild cell necrosis affecting <25% of the colonic mucosa.	Mild mononuclear inflammatory infiltrate affecting <25% of the colonic mucosa.	Mild loss affecting <25% of the colonic mucosa.
2	Moderate cell necrosis affecting 25–50% of the mucosa.	Moderate mononuclear inflammatory infiltrate affecting 25–50% of the mucosa.	Moderate loss affecting <50% of the colonic mucosa.
3	Severe cell necrosis affecting >50% of the mucosa. Detachment of the surface epithelium and debris in the lumen.	Severe mononuclear inflammatory infiltrate affecting >50% of the mucosa.	Severe loss affecting >50% of the colonic mucosa.

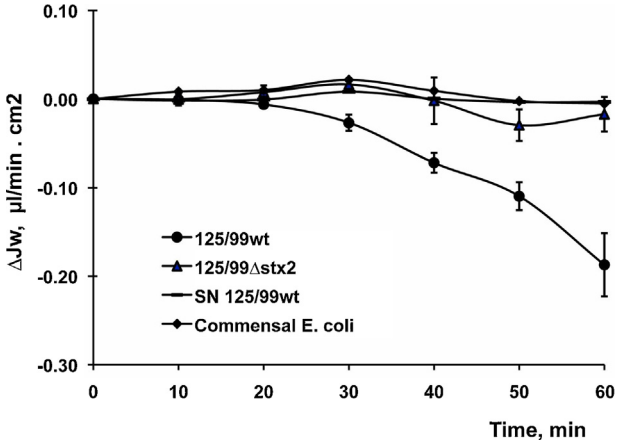

Fig. 1. Inhibition of water absorption in human colonic mucosa by *E. coli* O157:H7 expressing Stx2. Data represent the time course of the transepithelial net water flux (Jw) after addition of 125/99wt, 125/99Δstx2 or sterilized culture supernatants (SN) from 125/99wt (SN 125/99wt) on the mucosal side of colonic mucosa. A time-dependent Jw inhibition was observed in the case of tissues incubated with 125/99wt. A commensal *E. coli* was used as a negative control. Values are the means of 5 experiments for each time point. Error bars indicate ± 1 SEM.

(Fig. 1). Isc simultaneously measured in colonic mucosa incubated with 125/99wt, 125/99Δstx2, SN 125/99wt or commensal *E. coli* strain remained unchanged at least for 1 h (Fig. 2). At end of each experiment, the PD recorded under open-circuit conditions was similar to the initial values. In consequence, Rt also remained unchanged.

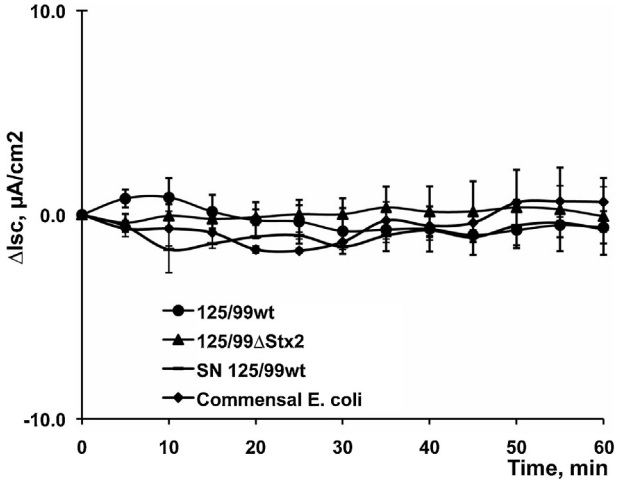

Fig. 2. Short-circuit current (Isc) in human colon treated with *E. coli* O157:H7 expressing Stx2. Data represent the time course of Isc after addition of 125/99wt, 125/99*Δstx2* or sterilized culture supernatants from 125/99wt (SN 125/99wt) on the mucosal side of colonic mucosa. A commensal *E. coli* was used as a negative control. Values are the means of five experiments for each time point. Error bars indicate ± 1 SEM.

E. coli *O157:H7 producing Stx2 causes significant morphological damages in human colonic mucosa*

The treatment of colonic mucosa with the 125/99wt strain for 1 h caused notable histological damage compared with tissues non-treated (control) or treated with commensal *E. coli* (Fig. 3). Severe cell necrosis with detachment of the surface epithelium, mononuclear inflammatory infiltrate and loss of goblet cells was observed (Fig. 3A). Treatment with 125/99*Δstx2* denoted isolated areas of epithelial necrosis (Fig. 3B). Human colonic mucosa incubated with commensal *E. coli* (Fig. 3C) presented a normal histoarchitecture compared with the controls (Fig. 3D). The histopathology score showed a statistically significant correlation ($P < 0.001$) between the treatment with 125/99wt and the epithelial damages (Fig. 3E). In contrast, the scores of 125/99*Δstx2* and commensal *E. coli* were not significant compared with the controls.

Cytotoxicity on Vero cells

A significant cytotoxic effect was observed when monolayers of Vero cells were exposed to different concentrations of purified Stx2 in growth-arrested conditions. The CD_{50} was maximal after 72 h of incubation with Stx2 and corresponded to 2.5×10^{-4} µg/ml (Fig. 4A). The CD_{50} of SN 125/99wt was reached at a dilution of approximately 1:60 whereas filter-sterilized culture supernatant (SN 125/99Δ*stx2* was non-toxic (Fig. 4A). Furthermore, a significant cytotoxicity was detected in the mucosal Ringer solution from the Ussing chamber conditioned by the presence of 125/99wt strain (125/99wt-CRS) in contact with the colonic mucosa for 1 h (Fig. 4B). In contrast, a lower cytotoxicity was detected in the mucosal Ringer solution conditioned by the presence of SN125/99wt (SN 125/99wt-CRS) (Fig. 4B). The average yield of Stx2 concentration in the 125/99wt-CRS (150 ng/ml), evaluated by comparing with the CD_{50} for the corresponding purified Stx2, was higher than in the SN-125/99wt-CRS (2 ng/ml) (Fig. 4B). The cytotoxicity of 125/99wt-CRS on Vero cells was attributable to Stx2, as it was neutralized with increasing concentrations the monoclonal anti-Stx2 antibody 2E11 in a dose-response manner (Fig. 5).

Discussion

It is generally accepted that water absorption in the human colon is associated with ionic active transport across colonic epithelium and that the resulting diarrhea is an imbalance of absorption and secretion of ions and solutes, followed by the movement of water in an attempt to restore the appropriate ion concentrations (Kunzelmann and Mall, 2002). Previous reports of our laboratory have shown that Stx2 alters the normal water absorption across human colon and causes irreversible histological damage without altering the short circuit current (Fiorito et al., 2000; Creydt et al., 2004; Gerhardt et al., 2013). These studies suggest that the inhibition of the water absorption may be consequence of the Stx2 cytotoxicity on the intestinal cells. Even though the presence of Gb3 receptors in the human intestine is still under discussion (Schüller

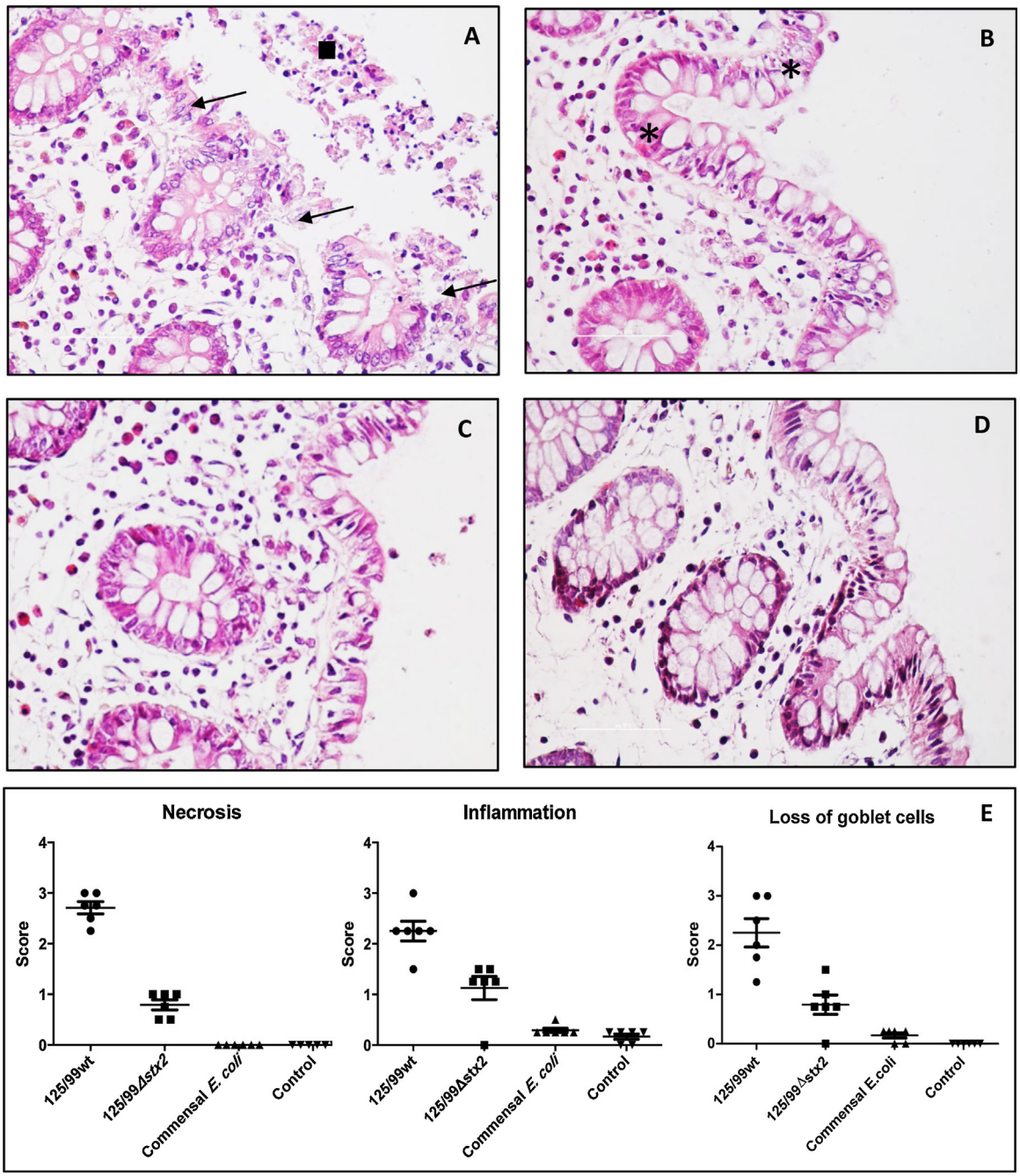

Fig. 3. Destruction of human colonic mucosa by *E. coli* O157:H7 expressing Stx2. Human colonic mucosa was treated with 125/99wt (A), 125/99*Δstx2* (B), commensal *E. coli* (C) or non-treated (D, control) for 1 h, and samples were stained with H&E. Bacteria from 125/99wt caused severe necrosis with detachment of the surface epithelium (black arrows), inflammatory cells and debris in the lumen (black square). Treatment with 125/99*Δstx2* denoted isolated necrosis on the surface epithelium (black asterisks). Human colonic mucosa incubated with commensal *E. coli* (C) presented normal histoarchitecture similar to the control (D). Blinded scoring (E) was performed for necrosis, inflammation and loss of goblet cells. Kruskal–Wallis test with Dunn's multiple comparisons revealed significances ($P < 0.001$, $n = 5$) between 125/99wt and the other experimental conditions. (A–D) Original magnification ×400.

et al., 2004, Zumbrun et al., 2011), it is possible that the Stx2-epithelium interaction induces cell death by inhibition of protein synthesis and induction of apoptosis (Ibarra et al., 2013).

In this study, we show that *E. coli* O157:H7 strain 125/99wt but not the 125/99*Δstx2* strain inhibited the water absorption across the human colonic mucosa and caused significant histological damages indicating the responsibility of Stx2 in the cytotoxic effects. Furthermore, there was no cytotoxicity when colonic mucosa was incubated with filtered culture supernatants containing 2 ng/ml of Stx2. Previously, we have reported a dose-dependent relationship between concentration above 10 ng/ml of Stx2 and water absorption inhibition (Gerhardt et al., 2013). In consequence, the amount of Stx2 may be insufficient to mediate these effects. However, the concentration of Stx2 increased to 150 ng/ml in the mucosal Ringer solution of the Ussing chamber after 1 h of colonic mucosa incubation with 125/99wt. This amount of Stx2 was 75-fold greater than in absence of bacterial cells (150 ng/ml compared with 2 ng/ml) and it was enough to cause the typical damages associated with Stx2. Previous report has shown that Stx phage genes are increased in the presence of molecules synthesized by human intestinal epithelial cells. Although titers of Stx2-converting phages does not necessary means an increase in the production of Stx2,

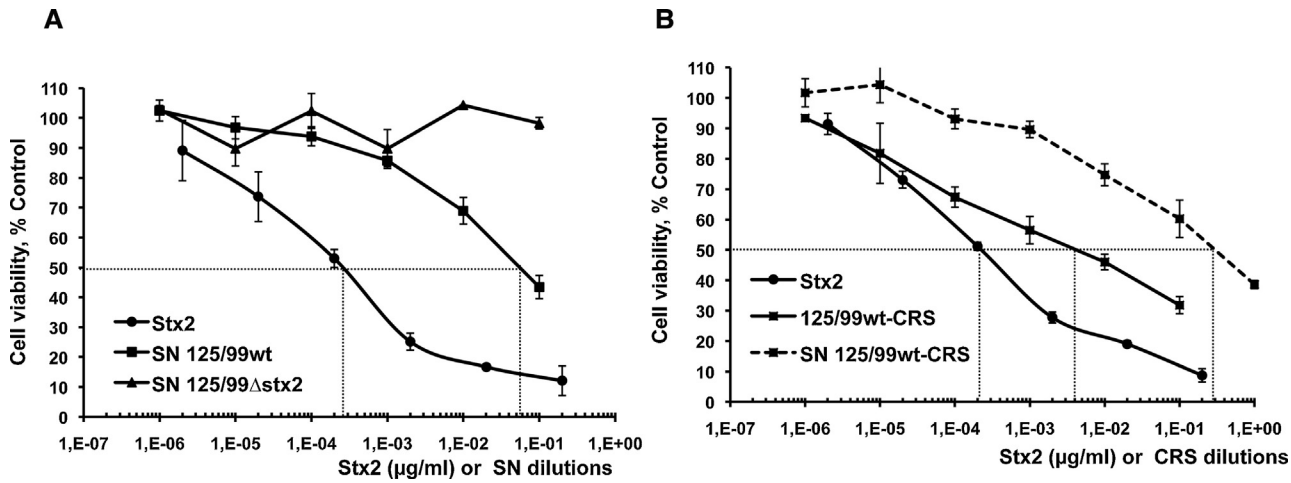

Fig. 4. Cytotoxicity of Stx2, SN of *E. coli* O157:H7 and CRS on Vero cells. Vero cell monolayers were plated in 96-well plates and grown to confluence in complete minimal essential medium. The cells were then exposed for 72 h to different dilutions of filter-sterilized culture supernatant (SN) from 125/99wt or 125/99 *Δstx2* (A) or filter-sterilized Ringer solution conditioned by the presence of 125/99wt (125/99wt-CRS) or conditioned by the presence of filter-sterilized culture supernatant (SN 125/99wt-CRS). Vero cells were also exposed to different concentrations of purified Stx2. Cell viability was analyzed by neutral red uptake, and 100% represents cells incubated under identical conditions but without treatment.

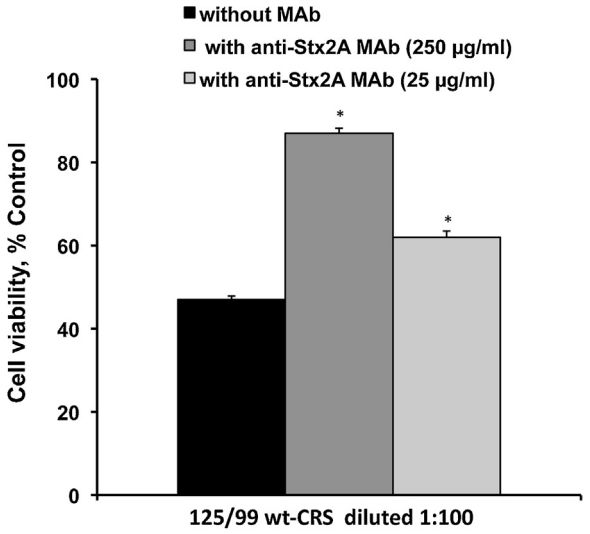

Fig. 5. Neutralization of 125/99wt-CRS with a monoclonal anti-Stx2 antibody. Preincubation of the 125/99wt-CRS (dilution 1:100) with the monoclonal anti-Stx2 antibody 2E11 (dilution 1:10 and 1:100) resulted in a significant increase in Vero cell viability in a dose-response manner. Data are reported as means ± 1 SEM of three triplicate experiments. $^{*}P < 0.001$.

a direct correlation with Stx2 levels has been reported in several STEC, including O157:H7 (Kohler et al., 2000). In addition, it was reported that STEC infection triggers the production of hydrogen peroxide, which in turn activated the SOS response in STEC. This response is a strong inducer of Stx and of Stx-encoding phage (Loś et al., 2010; Kimmitt et al., 2000). Another interesting phenomenon has been recently described (Bentancor et al., 2013b). The authors have shown that biologically active Stx2 may be expressed by the host and interacts with its target tissues and induces the typical pathological features associated with bacterial Stx2. These preliminary results allow to hypothesize that human intestine infected by *E. coli* O157:H7 could be an alternative source of Stx2 production (Lengeling et al., 2013).

As previously mentioned *E. coli* O157:H7 is by far the most prevalent serotype associated with bloody diarrhea and HUS mainly caused by the expression of Stx2, highly prevalent in Argentina (Rivas et al., 2006a). In addition, pathogenic *E. coli* lacking Stx may cause a non-bloody diarrhea (Kaper et al., 2004). Evidences of physiological alterations induced by the attaching and effacing (A/E) lesion have been reported in both enteropathogenic and enterohemorrhagic *E. coli* (Philpott et al., 1998; Tomson et al., 2004) suggesting that non-bloody diarrhea is the result of disruption of tight junction induced by A/E lesion. However, in our experiments, the mutant 125/99 *Δstx2* was unable to cause inhibition of water absorption. This result is expected because it is necessary more than 1 h of incubation to significant alters the barrier function resulting in diarrhea (Viswanathan et al., 2004; Guttman et al., 2007).

In summary, we have demonstrated that the incubation of *E. coli* O157:H7 with the human colonic mucosa caused a significant inhibition of the water absorption and selective histological damages. These effects were mediated by Stx2, which was enhanced at the mucosal side of intestinal tissue by the presence of bacterial cells. Identification of host cell-derived factors responsible for increasing Stx2 can lead to new strategies for preventing STEC infections.

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