Enteropathogenic *Escherichia coli* inhibits ileal sodium-dependent bile acid transporter ASBT

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Annaba F, Sarwar Z, Gill RK, Ghosh A, Saksena S, Borthakur A, Hecht GA, Dudeja PK, Alrefai WA. Enteropathogenic *Escherichia coli* inhibits ileal sodium-dependent bile acid transporter ASBT. *Am J Physiol Gastrointest Liver Physiol* 302: G1216–G1222, 2012. First published March 5, 2012; doi:10.1152/ajpgi.00017.2012.—Apical sodium-dependent bile acid transporter (ASBT) is responsible for the absorption of bile acids from the intestine. A decrease in ASBT function and expression has been implicated in diarrhea associated with intestinal inflammation. Whether infection with pathogenic microorganisms such as the enteropathogenic *Escherichia coli* (EPEC) affect ASBT activity is not known. EPEC is a food-borne enteric pathogen that translocates bacterial effector molecules via type three secretion system (TTSS) into host cells and is a major cause of infantile diarrhea. We investigated the effects of EPEC infection on ileal ASBT function utilizing human intestinal Caco2 cells and HEK-293 cells stably transfected with ASBT-V5 fusion protein (2BT cells). ASBT activity was significantly inhibited following 60 min infection with EPEC but not with nonpathogenic *E. coli*. Mutations in bacterial *escN*, *espA*, *espB*, and *espD*, the genes encoding for the elements of bacterial TTSS, ablated EPEC inhibitory effect on ASBT function. Furthermore, mutation in the bacterial *BFP* gene encoding for bundle-forming pili abrogated the inhibition of ASBT by EPEC, indicating the essential role for bacterial aggregation and the early attachment. The inhibition by EPEC was associated with a significant decrease in the V_{max} of the transporter and a reduction in the level of ASBT on the plasma membrane. The inhibition of ASBT by EPEC was blocked in the presence of protein tyrosine phosphatase inhibitors. Our studies provide novel evidence for the alterations in the activity of ASBT by EPEC infection and suggest a possible effect for EPEC in influencing intestinal bile acid homeostasis.

tyrosine phosphatases; infection with enteric pathogen

BILE ACIDS ARE PRODUCED BY the liver, conjugated to glycine or taurine, and then secreted into the intestine via the bile to emulsify dietary fat and cholesterol to facilitate their absorption (1). Ileal apical sodium-dependent bile acid transporter (ASBT) is responsible for the intestinal absorption of bile acids (1, 7). ASBT is a membrane-spanning protein predominantly expressed in the distal ileum and is localized to the apical membrane of epithelial cells (1, 7). A reduction in ASBT activity results in bile acid malabsorption that leads to an increase in the levels of bile acids in the colon (1, 23). High colonic concentrations of bile acids cause a variety of injurious effects on the colonic epithelia, including disturbances in mucosal permeability and induction of diarrhea (23). In this regard, ASBT function and expression were shown to be reduced in cases of intestinal inflammation such as Crohn's disease and radiation ileitis contributing to the pathophysiology of the associated diarrhea (22, 28). Whether enteric bacteria cause alterations in ASBT function contributing to the pathogenesis of the infection and the infectious diarrhea remains elusive.

Bile acids and intestinal microbes have been shown to closely interact and affect bacterial growth in the gut and bile acid homeostasis (21). For example, gut microbes generate the lipophilic unconjugated and secondary bile acids that enhance lipid and fat absorption from the intestine and may lead to hyperlipidemia (20). Also, bile acids have been shown to confer antibacterial effects as evident by an increase in bacterial growth and invasion in response to a decrease in the level of bile acids (8, 19). The antibacterial effects of bile acids were recently attributed at least in part to the activation of the farnesoid X receptor (FXR) transcription factor (18). As bile acids are the natural ligands for FXR, these recent observations suggest that bile acids should be first transported into the cells to directly bind and activate FXR nuclear receptor and subsequently elicit the antibacterial effects of bile acids. This notion indicates that the transport of bile acids into the cells may represent a crucial step to induce the protective effects of bile acid against enteric bacteria. It is likely, therefore, that ASBT-mediated bile acid transport may be modulated by pathogenic enteric bacteria such as enteropathogenic *Escherichia coli* (EPEC) to prevent FXR-induced antibacterial effects of bile acids. However, the effects of EPEC on ASBT are not known.

EPEC is a food-borne pathogen and a major cause of infantile diarrhea worldwide (16). EPEC is nontoxigenic and less invasive compared with other enteric bacteria but attaches to host cell membrane, inducing the formation of a unique attaching and effacing (A/E) lesion (27). EPEC manipulates numerous cellular processes in the host cells by its attachment and/or the translocation of a number of effector molecules into the host cells via the bacterial type three secretion system (TTSS) (27). The major phenotype of EPEC infection is protracted diarrhea, and recent studies suggested that the mechanism(s) of EPEC-induced diarrhea are multifactorial (16, 27). The interaction of EPEC with intestinal epithelial cells has been shown to modulate the function of a number of intestinal transporters via distinct mechanisms activated by EPEC-secreted effector molecules (5, 9, 11, 14).

Our findings showed that ASBT activity was decreased in Caco2 cells or HEK-293 cells stably expressing ASBT-V5 fusion protein (2BT cells) by infection with EPEC along with a reduction in ASBT level on the plasma membrane. EPEC-induced decrease in ASBT function appeared to be dependent on intact bacterial TTSS and was mediated by the activation of

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tyrosine phosphatases. Our results implicate ASBT inhibition in the pathophysiology of infection with EPEC and provide the first evidence for the modulation of the ileal bile acid transporter ASBT by an enteric pathogen.

MATERIALS AND METHODS

Materials. All chemicals were at least of reagent grade and were obtained from either Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Affinity-purified anti-rabbit or anti-mouse secondary antibodies conjugated to horseradish peroxidase and protein A/G agarose were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phenylarsine oxide (PAO) was purchased from Sigma and protein phosphatase inhibitor PTP III was obtained from Santa Cruz Biotechnology.

Cell culture. Human intestinal epithelial Caco-2 and human embryonic kidney HEK-293 cells were obtained from American Type Culture Collection. Human embryonic kidney HEK-293 cells stably transfected with human ASBT-V5 fusion protein (designated as 2BT cells) were previously described by us (3, 4). Cells were cultured in MEM supplemented with FBS (10% for 2BT and 20% for Caco-2 cells) and were plated at a density of 2×10^4 /well or 2×10^5/well in 24-well Falcon plates for Caco2 and 2BT cells, respectively. 2BT cells reached 90–100% confluence after 2–3 days in culture and were utilized for the uptake experiment. Caco-2 cells were cultured for 14 days on 24-well culture plates and then utilized for uptake studies as previously described by us (2).

Bacterial culture and infection of cells. On the day of experiment, 400 µl of overnight EPEC culture were inoculated to 10 ml of serum- and antibiotic-free DMEM cell culture medium supplemented with 0.5% mannose. Bacteria were grown ~3 h to an OD_{600} of 0.4. Cell monolayers were infected at a multiplicity of infection of 100. Nonadherent bacteria were removed by washing in PBS after 30–90 min. The following EPEC strains were used: wild-type EPEC strain E2348/69, CVD452 (E2348/69 *escN*:Km), UMD864 (E2348/69 Δ48−759 *espB1*), UMD870 [E2348/69 *espD1*:aph-3(Km)], *bfp mutant*, *espG*, *espF* (UMD874), *espH* (SE874), *map* (SE882), and the nonpathogenic isolate HS4. The EHEC strain used was 85–170 (O157:H7).

[³H]-taurocholic acid uptake. Sodium-dependent taurocholic acid (TC) transport in 2BT or Caco-2 cells was assessed as previously described by us (2). Briefly, medium was removed, and cells were incubated for 5 min at 37°C with buffer containing (in mM) 110 NaCl (with sodium) or choline chloride (without sodium), 4 KCl, 1 MgSO4, 1 CaCl$_2$, 50 mannitol, and 10 HEPES (pH 7.4). Cells were then incubated with the same buffer containing 10 µM of [³H]-TC (Perkin Elmer, Boston, MA) for the designated period of time. The transport process was terminated by rapidly washing the cells with ice-cold PBS. Cells were then solubilized with 0.5 N NaOH for at least 4 h. The protein concentration was measured by the method of Bradford (6) and the radioactivity was counted by liquid scintillation analyzer (model Tri-CARB 1600-TR; Packard Instrument, Downers Grove, IL). Sodium-dependent TC uptake was expressed as pmol·mg protein^{-1}·5 min^{-1}. For the kinetic parameters of TC uptake, values were analyzed for simple Michaelis-Menten kinetics utilizing a nonlinear regression data analysis from a computer model (GraphPad PRISM, GraphPad, San Diego, CA).

Cell surface biotinylation. Cell surface biotinylation was performed as previously described (3, 4) with labeling for 30 min at 4°C to stop endocytosis and internalization of antigens. Sulfo-NHS-SS-biotin (0.5 mg/ml; Pierce, Rockford, IL) was used in borate buffer containing (in mM) 154 NaCl, 7.2 KCl, 1.8 CaCl$_2$, and 10 H$_3$BO$_3$, pH adjusted to 9.0. Biotinylated proteins were immunoprecipitated with streptavidin agarose and then were released by incubation in 50 mM DTT reconstituted in Laemmli buffer. The immunoprecipitates were subjected to SDS-PAGE, and blots were then probed with anti-V5 antibodies.

Immunoprecipitation. 2BT cells grown to confluence were serum starved overnight and then infected with EPEC or left uninfected for 60 min. Cells were washed with ice-cold PBS three times and lysed in 20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA and 1× complete protease inhibitor cocktail. The lysate was further homogenized by passing 10 times through a 26-gauge needle. The lysate was centrifuged at 5,000 *g* for 5 min at 4°C, and protein concentration was determined by the method of Bradford (6). ASBT-V5 fusion protein in the lysate was immunoprecipitated using anti-V5 antibody (Invitrogen) by rotating overnight at 4°C. Protein A/G plus agarose beads were added [40 µl of a 50% (wt/vol) solution] and mixed for an additional 2 h at 4°C. Immunoprecipitates were washed three times with lysis buffer and solubilized in SDS-gel loading buffer. ASBT phosphorylation was then investigated using anti-phosphotyrosine (4G10) horseradish peroxidase-conjugated antibody (Upstate Biotechnology, Lake Placid, NY).

Western blot analysis. Proteins were solubilized in Laemmli sample buffer (2% SDS, 10% glycerol, 100 mM DTT, 60 mM Tris, pH 6.8, 0.01% bromophenol blue) and separated on 10% Tris-glycine SDS polyacrylamide gel. Separated proteins were then transferred onto nitrocellulose membranes, and Western blot analysis was performed by first blocking the blot overnight in buffer containing 5% nonfat dry milk in PBS. The blots were then incubated with the anti-V5 horseradish peroxidase-conjugated antibody diluted in the blocking solution for 3 h at room temperature. Blots were washed extensively with PBS containing 0.1% Tween-20, and then the bands were visualized by enhanced chemiluminescence ECL kit according to the manufacturer's instructions (Amersham, Arlington Heights, IL).

Statistical analysis. Results were expressed as means ± SE of three to four experiments performed on separate occasions. Student's *t*-test was utilized for statistical analysis. $P \leq 0.05$ was considered statistically significant.

RESULTS

ASBT function is inhibited by EPEC. Previous studies have shown that ASBT function is decreased by cytokines contributing to the pathophysiology of diarrhea associated with ileal inflammatory disorders such as Crohn's disease (22). Since cytokines are increased by infection with enteric microbes such as EPEC, whether these enteric microbes also have a direct effect on intestinal bile acids transport is not known. Therefore, we first investigated the effects of EPEC infection on ASBT activity. Serum-starved Caco-2 cells were infected with EPEC for different periods of time and Na$^+$-dependent ³H-TC uptake was then evaluated. As shown in Fig. 1*A*, Na$^+$-dependent ³H-TC uptake was significantly decreased in response to EPEC infection in a time-dependent manner with ~60–65% decrease after 60 min of infection and ~90% inhibition after 100 min of infection. To further determine whether the effects of EPEC were specific, Caco2 cells were also incubated with nonpathogenic *E. coli* or the EHEC (a strain that lacks shiga toxin) for 60 min, and ASBT function was measured. The results depicted in Fig. 1*B* demonstrate that ASBT activity was reduced by EPEC infection but not by nonpathogenic *E. coli* or by EHEC, indicating a specific effect of EPEC infection on ASBT function.

To examine whether the inhibition of ASBT by EPEC is cell line specific, we investigated the influence of EPEC infection on ASBT activity in HEK-293 cells stably transfected with ASBT-V5 fusion protein (2BT cells). ASBT activity was significantly reduced by 60 ± 3% in response to 60-min infection with EPEC compared with uninfected cells (Fig. 1*C*). Taken together, these data suggest that EPEC infection specif-
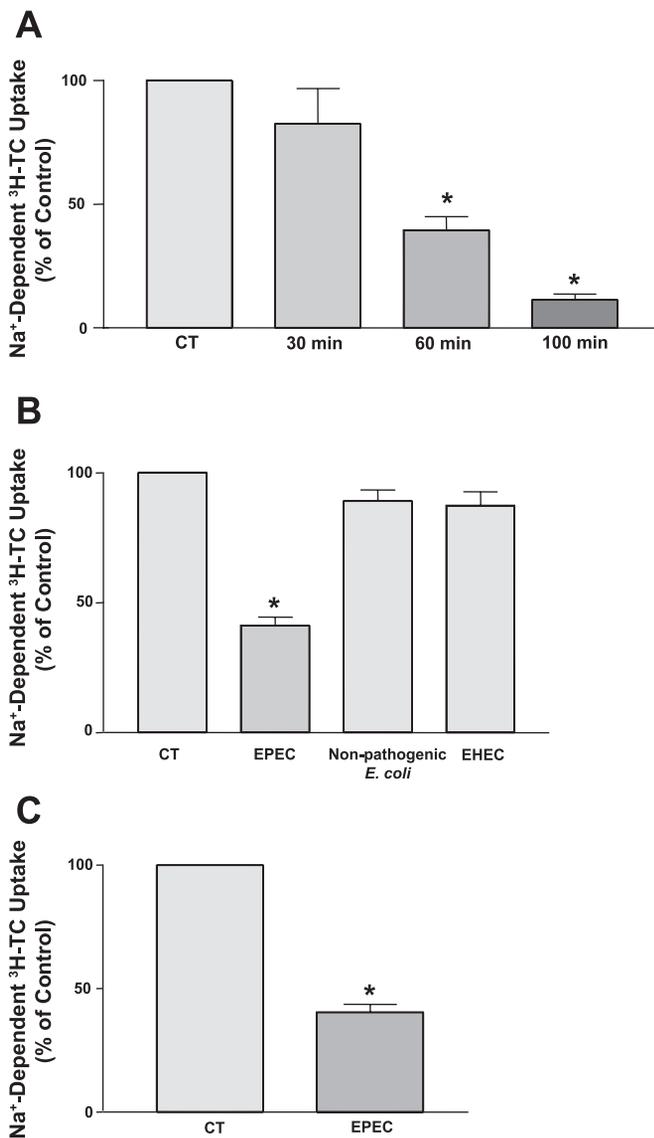
Fig. 1. Enteropathogenic *Escherichia coli* (EPEC) infection reduces apical sodium-dependent bile acid transporter (ASBT) activity. *A*: Caco-2 cells were infected with EPEC in the cell culture medium for different periods of time, and ASBT activity was then assessed by the measurement of Na-dependent ^{3}H-taurocholic acid (^{3}H-TC) uptake. *B*: Caco2 cells were infected with EPEC or nonpathogenic *E. coli* or EHEC strains (a strain that lacks shiga toxin) for 60 min, and ASBT activity was evaluated. *C*: 2BT cells were incubated with EPEC similar to Caco2 cells, and the ASBT activity was determined. Results are expressed as % control and represent means ± SE of 3 separate experiments performed in triplicates on separate occasions. *$P < 0.05$ compared with control.

ically inhibits ASBT function leading to a reduction in bile acid uptake.

EPEC-induced inhibition of ASBT activity is dependent on intact bacterial TTSS. EPEC contains a TTSS through which bacterial virulence proteins are translocated into host cells (27). The bacterial TTSS is composed of a needle-like structure that is made of the structural proteins, EspA, EspB, and EspD. The TTSS also contains the EscN protein that functions as the ATPase responsible for providing the energy for the translocation of the bacterial effector molecules. To examine the role of bacterial TTSS in the inhibition of ASBT, we infected

Caco2 cells with wild-type EPEC or EPEC strains harboring mutations in the *espA, espB, espD,* or *escN* genes, and Na$^+$-dependent ^{3}H-TC uptake was then evaluated. As shown in Fig. 2*A*, EPEC mutants failed to inhibit ASBT function, indicating that bacterial TTSS is essential to elicit the inhibitory effect of EPEC on ASBT activity.

To determine whether live bacteria are needed for the observed effect, Caco2 cells were incubated with live bacteria or bacterial culture supernatant, and ASBT activity was measured. Figure 2*B* shows that bacterial culture supernatant did not inhibit ASBT activity compared with incubating the cells with live bacteria. These findings indicate that translocation of the EPEC effector proteins into host cells during early infection is critical for the effects on ASBT activity, and that the bacterial factors secreted earlier into the culture media are not sufficient to inhibit ASBT function.

Attachment to host cells via bundle-forming pili is required. We next sought to investigate the role of EPEC attachment to host cells in the inhibition of ASBT function. The primary bacterial protein to mediate the early attachment of EPEC in vitro as well as during early infection is the bundle-forming pili (BFP) (25). To determine its role, cells were infected with either wild-type EPEC or *bfp* mutant strain. As shown in Fig. 3*A*, EPEC
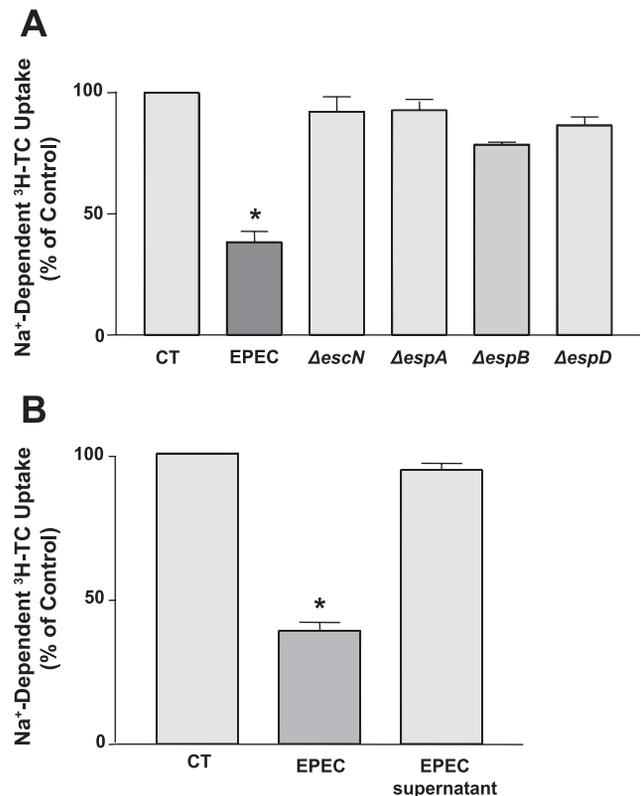

Fig. 2. Effect of EPEC on ASBT function is type 3 secretion system (TTSS) dependent. *A*: ASBT function in Caco2 cells was assessed after 60 min of infection with either wild-type EPEC or strains harboring mutations in bacterial *escN, espA, espB,* and *espD* genes encoding for the elements of EPEC TTSS. *B*: Caco2 cells were infected with wild-type EPEC or incubated with the bacterial culture supernatant for 60 min, and the ASBT activity was then evaluated. The bacterial culture supernatant was prepared by growing EPEC in cell culture media for mid-log phase and then spinning down the bacteria and filter sterilizing the supernatant. The results are % control and presented as means ± SE of values obtained from 3 separate occasions. *$P < 0.05$ compared with control (CT).
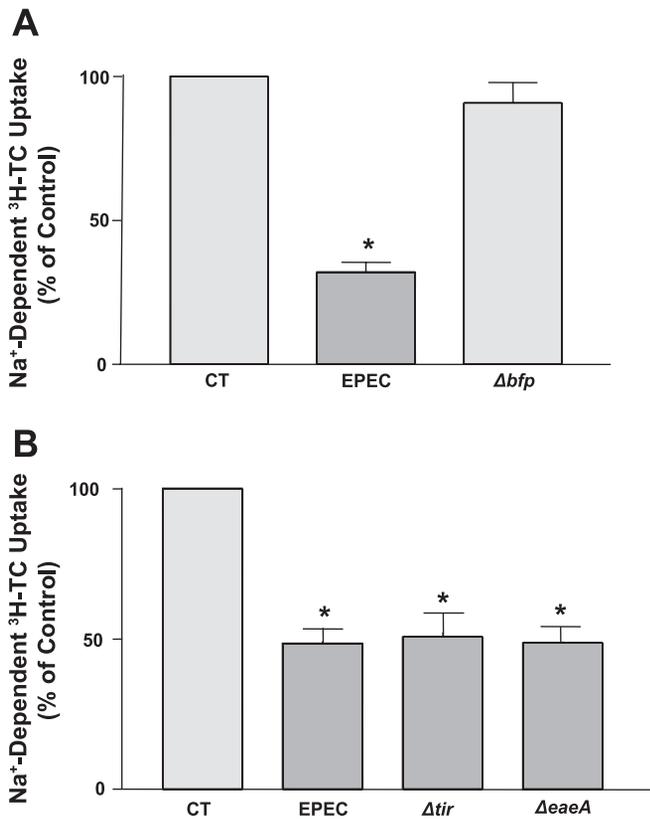
A

B

Fig. 3. Bacterial bundle-forming pili (BFP) but not intimin is essential for EPEC-induced ASBT inhibition. Caco2 cells were incubated for 60 min with wild-type EPEC or EPEC strain harboring mutation in the *bfp* gene (*A*) or strains harboring mutations in the intimin gene (Δ*eaeA*) or the translocated intimin receptor (*tir*) effector molecules (*B*). ASBT activity was then assessed after infection, and the data are expressed as % control and represent means ± SE of values from 3 separate occasions. *$P < 0.05$ compared with control.

had no effect on ASBT function in the absence of *bfp* gene, indicating the critical role of BFP-mediated early attachment in the inhibition of ASBT.

We next investigated the role of EPEC intimate adherence to host cells that is mediated by the binding of the bacterial protein intimin to the translocated intimin receptor protein (Tir). As depicted in Fig. 3*B*, ASBT activity was inhibited by EPEC strains harboring mutations in *intimin* (Δ*eaeA* mutant) and *tir* similar to the inhibition by wild-type EPEC. These data suggest that the adherence of EPEC mediated by intimin-Tir interaction is not essential for the inhibition of ASBT function.

EPEC infection decreases the V_{max} of the transporter. To determine whether changes in ASBT function were mediated by altering the number of active transporters or affecting the affinity of ASBT for the substrate, we next investigated the effects of EPEC infection of Caco2 cells on the kinetics of the Na-dependent ^{3}H-TC uptake. Caco2 cells were infected with EPEC, and ^{3}H-TC uptake was measured in the presence of increasing concentrations of the substrate TC. As shown in Fig. 4, EPEC infection resulted in a decrease in the uptake at each concentration of TC leading to a significant reduction in the V_{max} of the transporter (73 ± 20 in EPEC-treated cells vs. 135 ± 23 pmol·mg protein^{-1}·5 min^{-1} in control cells) with no significant change in the apparent K_m.

EPEC infection reduces ASBT protein levels on the plasma membrane. Changes in the V_{max} of the ASBT in response to EPEC suggest a decrease in the level of ASBT on plasma membrane. We next examined the alterations in ASBT expression on plasma membrane in response to EPEC infection by cell surface biotinylation studies. We performed these experiments in 2BT cells, taking advantage of the availability of antibodies (anti-V5 antibodies) suitable for the biochemical studies. Figure 5*A* shows that ASBT expression in the biotinylated fraction was significantly decreased by EPEC infection, whereas the total ASBT levels remained unaltered. We next sought to investigate whether changing the level of ASBT on the plasma membrane is involved in mediating the effect of EPEC on ASBT function. The effect of EPEC infection was examined in the presence or the absence of the endocytosis inhibitor PAO (26). Figure 5*B* shows that incubation of 2BT cells with increasing concentrations of PAO reduced the effect of EPEC on ASBT function and that the inhibition of ASBT was blocked at 5 μM concentration of PAO. These results strongly suggested that EPEC infection reduced ASBT function by decreasing its level on the plasma membrane.

Protein tyrosine phosphatases are involved in the inhibition of ASBT function by EPEC. In addition to inhibiting endocytosis, the PAO inactivates protein tyrosine phosphatases by cross-linking adjacent thiol groups in phosphatases processing motifs (10). To determine whether protein tyrosine phophatases (PTPases) are also involved in mediating the effects of EPEC on ASBT, we investigated the effect of increasing concentrations of PTP III, another inhibitor of PTPases, on the induced changes in ASBT function by EPEC infection in 2BT cells. As shown in Fig. 6*A*, PTP III abrogated the inhibition of ASBT by EPEC at 250 μM concentration. Interestingly, the data presented in the figure show that incubation with 500 μM of PTP III not only blocked the effect of EPEC but also increased the activity of ASBT alone or with EPEC infection. These results indicate that the inhibition of PTPases increases
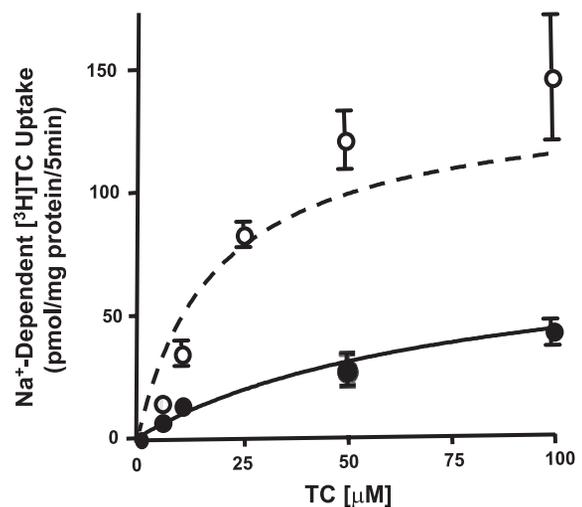

Fig. 4. EPEC infection decreases the V_{max} of ASBT transporter. ASBT activity was assessed in the presence of increasing concentrations (5–100 μM) of the substrate TC after infection with EPEC (●) or the nonpathogenic *E. coli* (○). Each point is a mean ± SE of 3 independent values, and the figure is a representative of 3 independent experiments with the same results. *$P < 0.05$ compared with control.
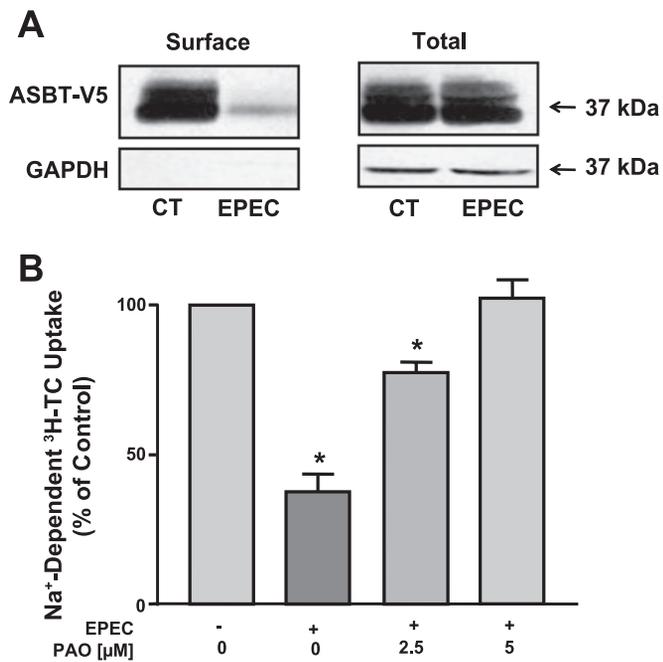
Fig. 5. ASBT level on the plasma membrane is decreased by EPEC infection. *A*: 2BT cells were infected with wild-type EPEC and then plasma membrane proteins in 2BT cells were labeled by cell surface biotinylation and then pulled down by streptavidin as described in MATERIALS AND METHODS. Surface protein (biotinylated) and total cellular proteins were separated by electrophoresis, and ASBT-V5 fusion protein was then detected by Western blot analysis using anti-V5 antibodies. *B*: 2BT cells were infected for 60 min with EPEC in the absence or the presence of different concentrations (2.5 and 5 μM) of the inhibitor of endocytosis, phenylarsine oxide (PAO). ASBT activity was then assessed, and the data are expressed as % control and represent means ± SE of 3 values from separate occasions. *P < 0.05 compared with control.

as a model for the human intestinal epithelium to study the regulation of ileal ASBT bile acid transporter (2). Recent studies from our laboratory have also utilized 2BT cells to investigate the molecular mechanisms of acute regulation of ASBT taking advantage of the availability of suitable antibodies (anti-V5 antibodies) to be utilized for various biochemical approaches, including Western blot analysis and immunoprecipitation (3, 4). Our data also showed that EPEC decreased ASBT activity in 2BT in the same manner as in Caco2 cells, indicating that the inhibition of ASBT activity by EPEC is not cell type specific. It is also important to note that the inhibition of ASBT occurred in the presence of EPEC but not in the presence of nonpathogenic *E. coli* bacteria or EHEC at the similar multiplicity of infection. These observations confirm that the effects on ASBT are specific to EPEC and are not due to increase in the osmolarity of the media due to the presence of microbes.

Live bacteria appear to be essential for eliciting the inhibitory effect on ASBT, as the bacterial culture medium alone failed to influence ASBT function. This observation suggested that the attachment of EPEC to epithelial cells is necessary to inhibit ASBT activity. EPEC is known to intimately adhere to host cells via the binding of intimin on EPEC surface to its translocated receptor Tir on the enterocytes (16). Mutations in either intimin or its receptor Tir did not obliterate the effects of EPEC on ASBT activity. While these results rule out the involvement of intimin and Tir receptor in ASBT inhibition,
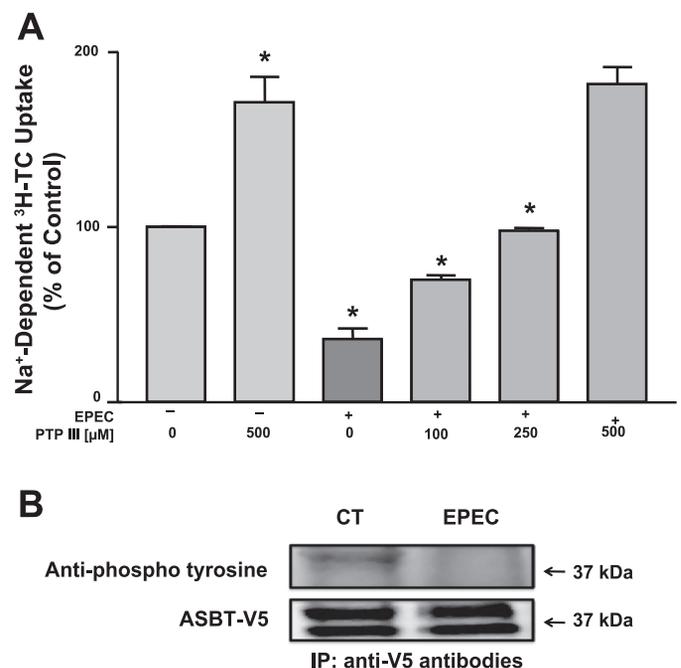
the ASBT basal activity and is sufficient to prevent its inhibition by EPEC infection.

To further assess changes in ASBT tyrosine phoshorylation by EPEC, control cells and EPEC-infected 2BT cells were harvested, and ASBT-V5 fusion protein was immunoprecipitated utilizing anti-V5 antibodies. The immunoprecipitates were then subjected to Western blot analysis utilizing anti-phosphotyrosine specific antibodies. As shown in Fig. 6*B*, ASBT-V5 fusion protein appeared to be tyrosine phosphorylated at the basal level and that EPEC infection caused a significant decrease in its level of tyrosine phosphorylation. These data indicate that EPEC infection may activate host PTPases that lead to ASBT tyrosine dephosphorylation and subsequent reduction in bile acid uptake.

DISCUSSION

In the present study, we show that ASBT function is directly affected by the pathogenic bacteria EPEC providing the first evidence for the modulation of ASBT function by enteric microbes. Our data show that EPEC infection acutely inhibited ASBT function via a decrease in its tyrosine phosphorylation and a reduction in its level on the plasma membrane. The effects of EPEC on ASBT in the present studies were investigated utilizing human intestinal Caco2 cells that were previously used as a suitable model to examine the EPEC-induced modulation of intestinal transport processes (9, 11, 14). Our previous studies have also shown the usefulness of Caco2 cells

Fig. 6. EPEC-induced effects on ASBT function are mediated by protein tyrosine phosphatases. *A*: 2BT cells were infected with EPEC for 60 min in the absence or presence of different concentrations of the protein phosphatases inhibitor PTP III, and ASBT function was evaluated. The results are % control (uninfected cells) and represent the means ± SE of 3 values from separate occasions. *P < 0.05 compared with control. *B*: 2BT cells were infected with EPEC for 60 min and harvested for immunoprecipitation with anti-V5 antibodies. The immunoprecipitates were separated by electrophoresis and investigated by Western blot analysis with anti-phosphotyrosine antibodies. The band with the same size as of ASBT was visualized and confirmed by probing the same blot with anti-V5 antibodies.

the data do not exclude the role of EPEC attachment, as intimin mutant did not decrease in vitro the rate of bacterial attachment to host cells (24). On the other hand, mutations in the BFP have been shown to severely impair the adherence of EPEC during the early infection (17, 29). BFP are bacterial surface appendages that are required for the full virulence of EPEC and are essential for localized EPEC adherence as well as the auto-aggregation into microcolonies (12). EPEC strains harboring BFP mutations showed no effect on ASBT activity, clearly indicating that initial attachment of EPEC to host cells and/or the bacterial aggregation are required for the inhibition of ASBT and occurs in cells expressing ASBT.

EPEC could also decrease ASBT function indirectly via the induction of proinflammatory cytokine secretion that are known to decrease ASBT expression. Our studies, however, were performed utilizing an in vitro cell culture model, and the inhibition of ASBT with EPEC infection occurred rapidly within 30–60 min of infection. Therefore, it is unlikely that cytokines secretion is involved in ASBT inhibition by EPEC observed in our cell culture models. One of the characteristic changes in the host cells induced by EPEC infection is the formation of a unique A/E lesion (16). A/E lesion is characterized by elevation of host cell membrane and accumulation of cytoskeletal proteins beneath adherent bacteria (27). However, many aspects of EPEC-induced pathology such as the induction of early diarrhea cannot be explained solely by the formation of A/E lesions (16). The fact that live bacteria and early attachment are needed for ASBT inhibition by EPEC suggests the involvement of molecules translocated from the bacteria into the host cells to influence ASBT activity. Interestingly, our data showed that the ASBT inhibition is dependent on intact bacterial TTSS, lending further support to the notion for the involvement of bacterial translocated effector molecules. EPEC may possibly interact with ASBT on the plasma membrane to influence its activity. However, the fact that the effects of EPEC infection on ASBT function are abrogated by mutation in the bacterial EscN, the protein that provides energy for translocation of effector molecules into host cells, suggests that a translocated effector molecule rather than EPEC itself inhibits ASBT. In this regard, previous studies have shown that *espF* and *espG* effector molecules mediate EPEC effects on the sodium hydrogen exchanger NHE3 and the Cl^-/OH^- exchanger, DRA in intestinal epithelial cells, respectively (11, 15). However, none of the key effector molecules examined including *espG, espF, espH,* and *map* appear to be involved in mediating the effects of EPEC on ASBT function (data not shown). The effect of EPEC on ASBT activity could possibly be mediated via other translocated bacterial proteins that are essential for EPEC virulence and pathogenesis, such as EspZ, NleH1, NleH2, NleD, and NleB (16). The possible roles of these effector molecules will be investigated in future studies. Since ASBT inhibition is independent of espG and espF, the present data, as well as our previous findings, collectively indicate that EPEC triggers multiple cellular pathways, each of which specifically affects different intestinal transport processes. Future studies will focus on identifying the bacterial translocated effector molecules involved in EPEC-induced inhibition of ASBT function.

EPEC has been shown to trigger host signal transduction pathways, including the activation of PTPases (13). Clearly, the activation of PTPases mediates the inhibition of ASBT function by EPEC since two PTPase inhibitors, PAO and PTP III, blocked EPEC effects on ASBT function. In this regard, we have recently shown that the activation of PTPases are involved in the reduction of the intestinal serotonin transporter (SERT) by infection with EPEC (9). It should be noted, however, that SERT inhibition by EPEC was not associated with changes in its level on the plasma membrane. In contrast, the ASBT level on plasma membrane was decreased along with a reduction in the V_{max} of the transporter, suggesting that EPEC infection induces internalization of ASBT from the plasma membrane (9). It appears, therefore, that EPEC infection may induce PTPases activation to reduce the activity of two intestinal transport processes, albeit via different molecular mechanisms involving the internalization of ASBT but not SERT transporter. Noticeably, ASBT appears to be a tyrosine phosphorylated protein at the basal level and that a reduction in its tyrosine phosphorylation may cause a decrease in its level on the plasma membrane and a reduction in its function. Future studies are warranted to explore the tyrosine residues that are phosphorylated in ASBT and their roles in maintaining its level on the plasma membrane.

In summary, our studies showed for the first time that the activity of ileal bile acid transporter ASBT is acutely reduced by infection with the enteric pathogenic microbe EPEC via a mechanism involving the activation of protein tyrosine dephosphorylation and a reduction in the level of the transporter on the plasma membrane. Further studies will be essential to determine whether the inhibition of ASBT and bile acid uptake into the intestinal epithelial cells may subvert the immune responses activated by the bile acid receptor FXR. The relationship between EPEC and ASBT represents a prototypic model that is important not only to unravel enteric pathogen-host interactions but also to investigate the effects of enteric bacteria on bile acid homeostasis.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

F.A., R.K.G., A.B., G.A.H., P.K.D., and W.A.A. conception and design of research; F.A., Z.S., R.K.G., A.G., and W.A.A. performed experiments; F.A., Z.S., R.K.G., A.G., S.S., A.B., G.A.H., P.K.D., and W.A.A. analyzed data; F.A., Z.S., R.K.G., S.S., A.B., G.A.H., P.K.D., and W.A.A. interpreted results of experiments; F.A., R.K.G., and W.A.A. prepared figures; F.A., R.K.G., and W.A.A. drafted manuscript; F.A., R.K.G., S.S., A.B., G.A.H., P.K.D., and W.A.A. edited and revised manuscript; F.A., R.K.G., P.K.D., and W.A.A. approved final version of manuscript.

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