Infection and Immunity

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Infect. Immun. **2012, 80(4):1418. DOI: 10.1128/IAI.06378-11. Published Ahead of Print 17 January 2012.**

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Identification of the Cellular Receptor of *Clostridium spiroforme* Toxin

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***Clostridium spiroforme* produces the binary actin-ADP-ribosylating toxin CST (*C. spiroforme* toxin), which has been proposed to be responsible for diarrhea, enterocolitis, and eventually death, especially in rabbits. Here we report on the recombinant production of the enzyme component (CSTa) and the binding component (CSTb) of *C. spiroforme* toxin in *Bacillus megaterium*. By using the recombinant toxin components, we show that CST enters target cells via the lipolysis-stimulated lipoprotein receptor (LSR), which has been recently identified as the host cell receptor of the binary toxins *Clostridium difficile* transferase (CDT) and *Clostridium perfringens* iota toxin. Microscopic studies revealed that CST, but not the related *Clostridium botulinum* C2 toxin, colocalized with LSR during toxin uptake and traffic to endosomal compartments. Our findings indicate that CST shares LSR with *C. difficile* CDT and *C. perfringens* iota toxin as a host cell surface receptor.**

Clostridium spiroforme is frequently found in the digestive tracts of rabbits, where it is proposed to be responsible for severe diarrhea, enterocolitis, and eventually death of the host (7). The virulence of *C. spiroforme* strongly correlates with the production of an enterotoxin termed CST (*C. spiroforme* toxin) (6) that belongs to the family of clostridial iota-like binary toxins, including *C. perfringens* iota toxin and *C. difficile* transferase (CDT). CST is an A-B-type toxin, with an enzyme component (CSTa) harboring mono(ADP-ribosyl)transferase activity and a separate binding component (CSTb) that forms heptamers, binds to target cells, and is responsible for the cell entry of the enzyme component. After endocytosis and traffic to acidic endosomes, the heptameric binding component forms pores in endosomal membranes, which allow the translocation of the enzyme component into the cytosol of target cells (1, 2). In the cytosol, CSTa ADP-ribosylates G-actin, thereby leading to actin depolymerization and disruption of microfilaments (18, 19, 21).

The binding components of binary actin-ADP-ribosylating toxins have significant similarity with the protective antigen (PA), which is the binding component of anthrax toxin (25). Structural studies of anthrax toxin were most instrumental for the understanding of the mechanisms underlying the cellular uptake of binary actin-ADP-ribosylating toxins. All these binding components are 80- to 100-kDa proteins and consist of 4 domains. Domain 1 is involved in proteolytic toxin activation. Cleavage of an ~20-kDa N-terminal peptide of domain 1 allows heptamerization, which is supported mainly by domain 3. Domain 2 is involved in membrane insertion, which occurs at the low pH of endosomes and depends on the formation of a beta-barrel structure based on the structural rearrangement of domain 2. Domain 4 appears to be involved in receptor binding. This domain of CST has similarity with the binding components of iota-like binary actin ADP-ribosylating toxins but is unrelated to the respective domains of C2 toxin or PA.

Recently, recombinant clostridial proteins (e.g., clostridial protein toxins) have been successfully expressed in *Bacillus megaterium*. This system was extremely instrumental for expression of members of the family of large glucosylating clostridial toxins, which possess a mass of 250 to 308 kDa and are notoriously poorly expressed in *Escherichia coli* (22). We previously employed this procedure for recombinant production of the clostridial toxins *C. sordellii* lethal toxin, *C. novyi* alpha-toxin, and *C. difficile* transferase (11, 17, 20). The current study describes the usefulness of the *B. megaterium* expression system for recombinant production of CSTa and CSTb. The availability of both toxin components enabled us to elucidate the target cell receptor of CST. We recently identified the lipolysis-stimulated lipoprotein receptor (LSR) as the target molecule for binding and internalization of the clostridial binary toxins *C. difficile* transferase and *C. perfringens* iota toxin (16). Here, we demonstrate that *C. spiroforme* toxin also uses the LSR for target cell entry.

MATERIALS AND METHODS

Cell cultivation. H1-HeLa and CaCo-2 cells were grown in Dulbecco modified Eagle medium (DMEM) (12 mM L-glutamine) supplemented with 10% fetal calf serum (FCS), penicillin (4 mM), streptomycin (4 mM), 1% nonessential amino acids (NEA), and for CaCo-2 cells only, 1% sodium pyruvate (1 mM). Cells were incubated at 37°C with 5% CO_2 under humidified conditions. Generation of H1-HeLa cells stably transduced with a retroviral element expressing an N-terminally FLAG-tagged version of LSR (FLAG-LSR) has been described elsewhere (16).

Cloning procedures. For cloning of CSTa and CSTb into the *B. megaterium* expression vector pHIS1522 (MoBiTec, Göttingen, Germany), the genes were amplified by PCR and by using genomic DNA from *C. spiroforme* strain CS246 as a template. PCR of both genes was performed with oligonucleotides introducing a 5′-BsrGI (CSTa) or a 5′-Acc65I (CSTb) restriction site and a 3′-KpnI restriction site (both genes) and by omitting the stop codons. Subsequent ligation of both amplification products into BsrGI/KpnI-digested pHIS1522 resulted in the generation of pHIS1522/CSTa-6His and pHIS1522/CSTb-6His, respectively.

Expression and purification of CSTa and CSTb. Briefly, *B. megaterium* protoplasts, transformed with either pHIS1522/CSTa-6His or pHIS1522/CSTb-6His (according to the manufacturer's instructions [MoBiTec, Göttingen, Germany]), were grown at 37°C in LB medium to an optical density at 600 nm (OD_{600}) of 0.8. Protein expression was induced by addition of 5% (wt/vol) xylose followed by incubation overnight

Received 22 December 2011 Accepted 5 January 2012

Published ahead of print 17 January 2012

Editor: S. R. Blanke

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doi:10.1128/IAI.06378-11

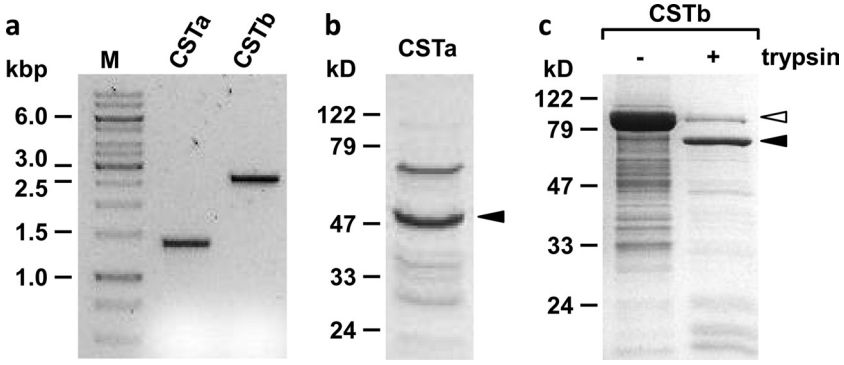

FIG 1 Cloning, recombinant expression, and purification of CSTa and CSTb. (a) The genes encoding CSTa and CSTb were amplified by PCR and by using genomic DNA from *C. spiroforme* strain CS246 as a template. PCR products were subjected to agarose gel electrophoresis and visualized by ethidium bromide staining. M, marker. (b and c) Representative SDS-PAGE analysis after Coomassie blue staining of CSTa (arrowhead) (b) and CSTb (before [white arrowhead] and after [black arrowhead] activation with trypsin) after recombinant expression in *B. megaterium* and nickel affinity purification. The amount of total protein added per lane was 5 μg for panel b and 10 μg for panel c. Protein bands corresponding to CSTa or CSTb were confirmed by MALDI-TOF analysis.

at 16°C (CSTa) or at 29°C (CSTb). Cells were then harvested by centrifugation, and the pellet was suspended in lysis buffer (20 mM Tris-HCl [pH 8.0], 300 mM NaCl, 20 mM imidazole, 10% glycerol, and 0.5 mM EDTA) supplemented with Complete protease inhibitor mix (Roche, Mannheim, Germany). After lysis of bacteria using a Microfluidizer (Microfluidics, Newton, MA) at 15,000 lb/in^2, cell debris was removed by centrifugation (18,000 × *g*, 30 min, 4°C). The supernatant was applied to preequilibrated Ni-iminodiacetic acid (Ni-IDA) beads (Macherey-Nagel, Düren, Germany) and incubated for 1 h at 4°C for binding of His-tagged proteins. The beads were then washed with buffer containing 20 mM Tris-HCl (pH 8.0), 300 mM NaCl, 20 mM imidazole, and 10% glycerol, and bound proteins were finally eluted with elution buffer containing 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 500 mM imidazole, and 10% glycerol. Eventually, imidazole was removed from eluates by gel filtration chromatography using a PD-10 desalting column (GE Healthcare, Freiburg, Germany) equilibrated with elution buffer without imidazole. Prior to use, CSTb was activated by treatment with 0.05 μg trypsin/μg of protein for 30 min on ice. Trypsin was inhibited by the addition of a 2-fold amount of trypsin inhibitor.

***In vitro* ADP-ribosylation assay.** After treatment with CST, cells were scraped into ice-cold ADP-ribosylation buffer (20 mM Tris, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol [DTT], and Complete protease inhibitor mixture, pH 7.5) and lysed by passaging the cell suspension through a 26-gauge needle with a syringe 10 times. The cell debris was then removed by centrifugation (21,000 × *g*, 15 min, 4°C). Cytosolic extracts (30 μg of total protein) were incubated with 1 μg C2I and [^{32}P]NAD for 60 min at 37°C, and the reaction was stopped by addition of Laemmli buffer. Radiolabeled actin was analyzed by SDS-PAGE and autoradiography.

Alexa dye labeling of purified proteins. Following the manufacturer's instructions, CSTb was labeled with Alexa Fluor 488 carboxylic acid succinimidyl ester (Invitrogen), and CDTb and C2I were labeled with Alexa Fluor 568 C5 maleimide (Invitrogen). Excess dye was removed with Micro Bio-Spin 6 columns (Bio-Rad Laboratories).

Microscopy. Cell morphology was analyzed in wells directly by using an inverted microscope (Axiovert 25; Carl Zeiss). Actin and nuclear stainings were performed with tetramethyl rhodamine isocyanate (TRITC)-conjugated phalloidin and ProLong Gold antifade reagent containing 4′,6′-diamidino-2-phenylindole (DAPI), respectively, and analysis was done by fluorescence microscopy (Axiophot; Carl Zeiss). Confocal fluorescence microscopy was performed with an inverted microscope (Axiovert 200 M; Carl Zeiss) equipped with a 40× Plan-Apochromat objective, a spinning-disk head (Yokogawa) with emission filters, and a CoolSNAP-HQ^2 digital camera (Roper Scientific). Alexa$_{488}$/fluorescein isothiocyanate (FITC) signals and Alexa$_{568}$/TRITC signals were detected by illuminating the specimen with 488-nm- and 561-nm-wavelength solid-state lasers, respectively. Images were processed with MetaMorph imaging software (Universal Imaging). For immunostaining of LSR, polyclonal rabbit anti-LSR antibody (sc-133765; Santa Cruz Biotechnology) and secondary Alexa 568- or Alexa 488-conjugated anti-rabbit antibodies (Invitrogen, Karlsruhe, Germany) were used.

Crystal violet staining. After CST treatment, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at 4°C, followed by staining of cells with 0.5% crystal violet solution (in 70% ethanol) for 30 min at 4°C. Excess staining solution was removed by washing the cells three times with H_2O. Stained cells were air dried at room temperature before imaging.

FACS analysis. Cells were detached from culture dishes by incubation with 10 mM EDTA in PBS and without trypsin to preserve cell surface proteins from degradation. The cell suspension was washed twice with PBS and kept on ice prior to addition of 10 μg activated, Alexa$_{488}$-labeled CSTb per 100,000 cells in 1 ml PBS. After incubation for 15 min on ice, cells were washed twice with PBS and subjected to fluorescence-activated cell sorter (FACS) analysis using the BD FACSCalibur platform. Cell surface-bound fluorescence was detected with an argon-ion laser (488 nm) and the 530-nm-band-pass filter (FITC). Competition experiments were performed by preincubating cells for 5 min on ice with 50 μg activated, unlabeled CSTb or 50 μg bovine serum albumin (BSA) (including trypsin and trypsin inhibitor, as used for the activation of CSTb) per 1 million cells in 0.5 ml PBS prior to addition of 1 μg activated, Alexa$_{488}$-labeled CSTb. Following two washings of cells with PBS, cell surface-bound fluorescence was detected by FACS analysis.

Other toxins used in this study. The components of *C. botulinum* C2 toxin (C2I and C2II) and *C. difficile* transferase (CDTa and CDTb) were purified from *Escherichia coli* and *Bacillus megaterium*, respectively, as described elsewhere, and binding components (C2II and CDTb) were activated by protease treatment (3, 5, 20).

RESULTS

Cloning, expression, and cytotoxic effect of recombinant *C. spiroforme* toxin produced in *B. megaterium*. The genes encoding either the enzyme component (CSTa) or the binding component (CSTb) of *C. spiroforme* toxin were amplified by PCR using genomic DNA from strain CS246 as a template (Fig. 1a). PCR products were then cloned into the *B. megaterium* expression vector pHIS1522 to generate C-terminally 6×His-tagged constructs, and plasmids were transformed separately into *B. megaterium* protoplasts. Tetracycline-resistant clones were grown in liquid LB medium, and protein expression was induced by the addition of xylose. The His-tagged proteins were then purified from crude

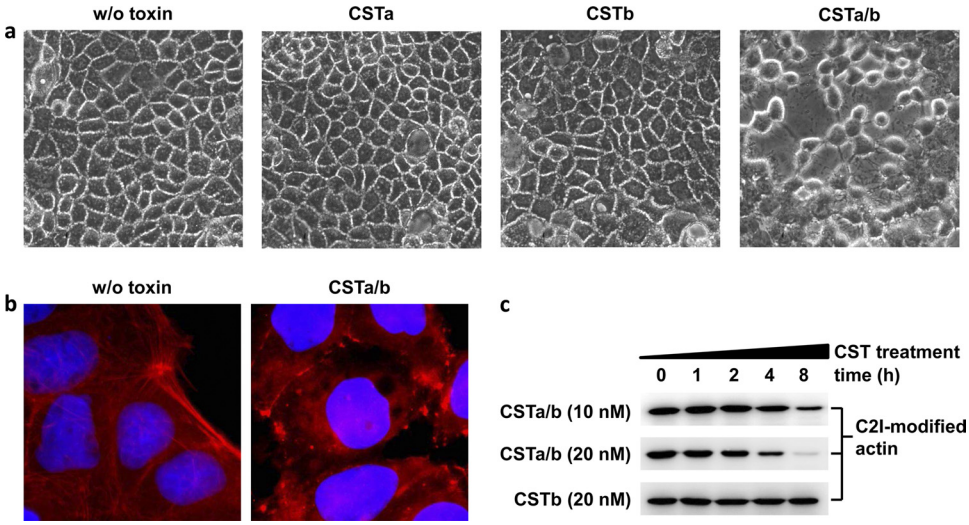

FIG 2 Cytotoxicity analysis of recombinant *C. spiroforme* toxin. (a) CST-induced cell rounding of CaCo-2 cells. Confluent CaCo-2 cell monolayers were incubated overnight with CSTa and/or CSTb (each at 10 nM) or were left untreated (w/o toxin) prior to microscopic analysis of cell morphology. (b) CST-induced collapse of the actin cytoskeleton. CaCo-2 cells were incubated with CSTa and CSTb (CSTa/b, each at 10 nM) for 8 h or were left untreated (w/o toxin) prior to fixation of cells and staining with TRITC-phalloidin (red, actin) and DAPI (blue, nuclei). Cells were then analyzed by fluorescence microscopy (using the 100× objective). (c) ADP-ribosylation of cellular actin with CST. CaCo-2 cells were treated for the indicated time intervals with CSTa and CSTb (each at 10 or 20 nM) or with CSTb only (20 nM). Subsequently, cells were lysed and cytosolic extracts subjected to *in vitro* ADP-ribosylation with C2I (modifies actin at the same amino acid residue as CST) and ^{32}P-NAD as a cosubstrate. C2I-modified actin was visualized by SDS-PAGE and autoradiography.

bacterial lysates by nickel affinity chromatography. The purity of a representative preparation of recombinant *C. spiroforme* toxin is illustrated in Fig. 1b (CSTa) and Fig. 1c (CSTb, left lane, −trypsin). CSTb was further activated by incubation with trypsin, which is required to achieve full toxicity of CST toxin (Fig. 1c, right lane, +trypsin) (18). Typically, approximately 0.05 mg CSTa and 2 mg CSTb were purified from 1 liter of bacterial culture.

In order to prove that recombinant CSTa and CSTb (trypsin activated) are correctly folded, we added both components in combination or separately (each at 10 nM) to cultured CaCo-2 cells. Only cells that were treated with both components of the recombinant toxin displayed severe morphological alterations (cell rounding) after overnight incubation (Fig. 2a), implying that a binary toxin with full biological activity was formed by both purified CST components. Our next aim was to prove that recombinant toxin-induced cell rounding of CaCo-2 cells is due to the collapse of the actin cytoskeleton. Therefore, we intoxicated CaCo-2 cells for 8 h with recombinant CST (10 nM), fixed the cells, and visualized the actin cytoskeleton by TRITC-phalloidin staining followed by fluorescence microscopy. Depolymerization of actin filaments was obvious only in cells that were treated with toxin (Fig. 2b). Finally, we aimed to substantiate that recombinant CST modifies cellular actin by ADP-ribosylation. This was confirmed by probing the ADP-ribosylation state of actin in lysates of CST-intoxicated cells using an *in vitro* actin-ADP-ribosylation assay. The amount of actin amenable to *in vitro* actin-ADP-ribosylation decreased over time only in CaCo-2 cells that were treated with both CST components (each at 10 nM) and not in cells that were treated only with the binding component of CST (Fig. 2c). The ADP-ribosylation state of cellular actin was increased when a higher concentration of recombinant CST (20 nM) was applied to the cells. Collectively, the recombinantly produced components of CST are able to constitute a fully functional binary toxin.

LSR is the cellular receptor of *C. spiroforme* toxin. Recently, a haploid genetic screen identified LSR as the functional cellular receptor of the CST-related binary toxins *C. difficile* transferase (CDT) and *C. perfringens* iota toxin (16). Here, we aimed to clarify whether *C. spiroforme* toxin also uses LSR for cell entry. We approached this question by the use of H1-HeLa cells, which do not express detectable amounts of LSR protein and are highly resistant to CDT and iota toxin, and FLAG-LSR-transduced H1-HeLa cells (16). Strikingly, after addition of CST to these cells and overnight incubation, clear signs of intoxication, including cell rounding (Fig. 3a) and detachment from culture plates (Fig. 3b), were observed exclusively in FLAG-LSR-transduced H1-HeLa cells [H1-HeLa(+LSR)]. Nontransduced H1-HeLa cells were resistant to toxin addition.

LSR-dependent intoxication was further confirmed by probing the ADP-ribosylation state of actin in lysates of CST-intoxicated H1-HeLa and H1-HeLa(+LSR) cells. The amount of actin in toxin-treated cells that is available for *in vitro* actin-ADP-ribosylation (carried out with the enzyme component of *C. botulinum* C2 toxin [C2I]) decreased over time only in FLAG-LSR-transduced H1-HeLa cells and not in nontransduced cells (Fig. 3c).

To further confirm that LSR acts as a cell surface receptor and mediates binding of CST to the cell membrane, Alexa-coupled CSTb was incubated with suspensions of nontransduced and LSR-transduced H1-HeLa cells on ice to prevent endocytosis, and cell surface-bound fluorescence was then detected by FACS analysis. H1-HeLa cells expressing LSR protein showed considerably increased binding of fluorescence-labeled CSTb compared with naïve H1-HeLa cells (Fig. 3d). To substantiate the specificity of CSTb binding to LSR-expressing H1-HeLa cells, we performed a competition assay, where cells were preincubated with a 50-fold excess of activated, unlabeled CSTb or with the same amount of BSA (including trypsin and trypsin inhibitor). Importantly, binding of

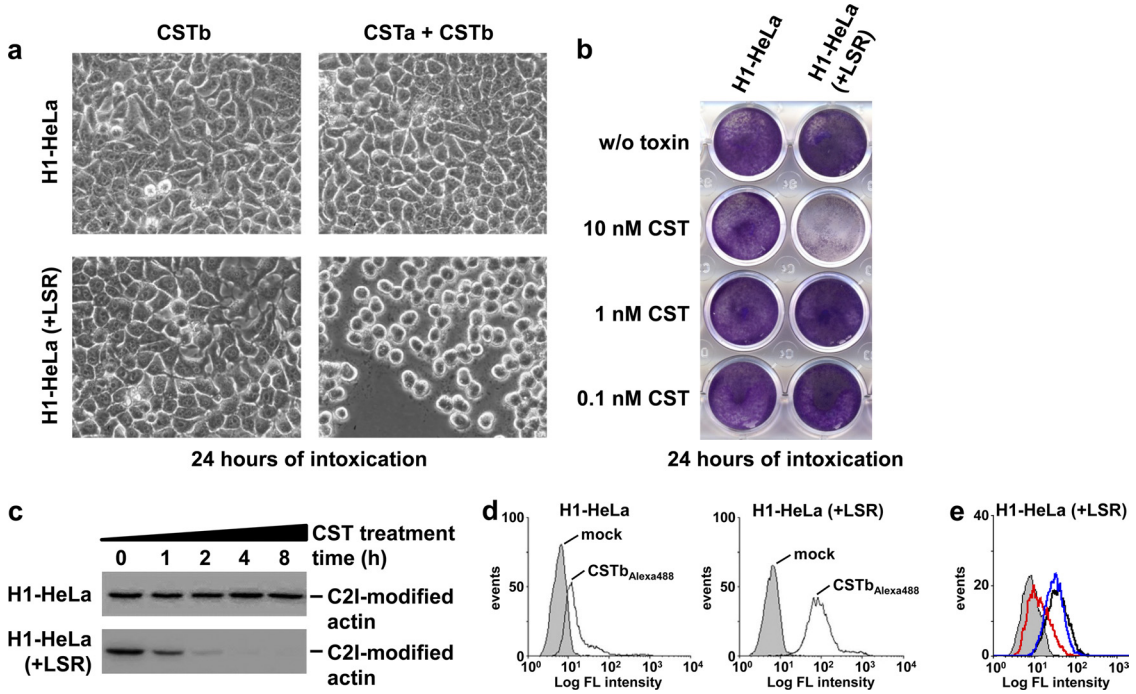

FIG 3 Ectopic expression of LSR increases sensitivity of H1-HeLa cells to CST. (a) Nontransduced (H1-HeLa) and FLAG-LSR-transduced [H1-HeLa(+LSR)] H1-HeLa cells were intoxicated with 10 nM CST (CSTa + CSTb) or with 10 nM CSTb only, and cell morphology was analyzed microscopically after overnight incubation. (b) Intoxication of nontransduced (H1-HeLa) and FLAG-LSR-transduced [H1-HeLa(+LSR)] H1-HeLa cells was performed with increasing concentrations of CST as indicated, and cell detachment was analyzed by crystal violet staining of nondetached cells after 24 h of intoxication. (c) H1-HeLa and H1-HeLa(+LSR) cells were treated for increasing time intervals (as indicated) with 10 nM CST before lysis of cells and *in vitro* ADP-ribosylation of cytosolic extracts with C2I and ^{32}P-NAD as a cosubstrate. Samples were subjected to SDS-PAGE, and C2I-modified actin was visualized by autoradiography. (d) Suspensions of H1-HeLa and H1-HeLa(+LSR) cells were mixed with 10 μg fluorescence-labeled CSTb ($CSTb_{Alexa\ 488}$) or without protein (mock) and incubated for 15 min on ice to prevent endocytosis. Cells were then washed twice with PBS and subjected to flow cytometric analysis. Single cell events were plotted against intensity of bound fluorescence (log FL intensity), where gray peaks represent cells without addition of protein (mock) and white peaks cells that were mixed with fluorescence-labeled CSTb ($CSTb_{Alexa\ 488}$). (e) Suspensions of H1-HeLa(+LSR) cells were preincubated with either 50 μg activated, unlabeled CSTb (peak with red line) or 50 μg BSA (peak with blue line) or were left without preincubation (peak with black line). Following incubation of the cells for 5 min on ice, fluorescence-labeled CSTb (1 μg) was added. Cells were then washed twice with PBS prior to FACS analysis. The gray peak represents cells that were left without preincubation of unlabeled CSTb and without addition of Alexa-labeled CSTb.

Alexa-labeled CSTb was specifically reduced on cells that were preincubated with unlabeled CSTb (Fig. 3e).

To validate that LSR binding mediates cell entry of CST, we performed colocalization studies with CaCo-2 cells. For this purpose, Alexa-coupled CSTb was added together with CSTa to CaCo-2 cells that were kept at 4°C to allow binding of the toxin to its receptor at the cell surface. Following an incubation period of 20 min at 37°C to induce endocytic uptake of the receptor/toxin complexes, cells were fixed, permeabilized, and subjected to immunostaining of endogenous LSR. Comparison of fluorescent signals of CST with signals of endogenous, immunolabeled LSR by confocal fluorescence microscopy confirmed colocalization of both proteins in endocytic vesicles (Fig. 4, CST). A similar colocalization pattern was observed when Alexa-labeled CDT was used as a positive control (Fig. 4, CDT). In contrast, endocytic vesicles containing Alexa-labeled *C. botulinum* C2 toxin, a more distantly CST-related actin-ADP-ribosylating toxin that enters cells via an asparagine-linked complex and hybrid carbohydrate structures (9), did not colocalize with LSR-containing endocytic compartments (Fig. 4, C2). Thus, our collective data demonstrate that CST is an additional member of the iota-like family of binary actin-ADP-ribosylating toxins that targets LSR for host cell entry.

DISCUSSION

Our study describes the recombinant production of the enzyme component (CSTa) and the binding component (CSTb) of the *C. spiroforme* toxin in the *Bacillus megaterium* expression system. The methodology was adapted from protocols that were previously established for the recombinant production of various other clostridial toxins (11, 17, 20, 22). The *B. megaterium* expression system often offers an advantage over conventional bacterial expression systems (e.g., *Escherichia coli*) in producing clostridial toxins, obviously due to a similar codon usage of the related genera *Bacillus* and *Clostridium*. However, we noticed that the efficiency of producing CSTa in *B. megaterium* was less than that for CSTb. A significant amount of CSTa protein was found in the insoluble fraction after lysis of bacteria and centrifugation of cell debris. Because we produced both CST components as C-terminally His-tagged proteins, we speculate that the His tag might negatively influence the stability of CSTa, resulting in increased aggregation and partially in the formation of inclusion bodies. As we show that the combined addition of both recombinantly produced CST components to cultured CaCo-2 cells led to the ADP-ribosylation of cellular actin, disruption of the actin cytoskeleton, and changes in cell morphology (cell rounding), our findings in-

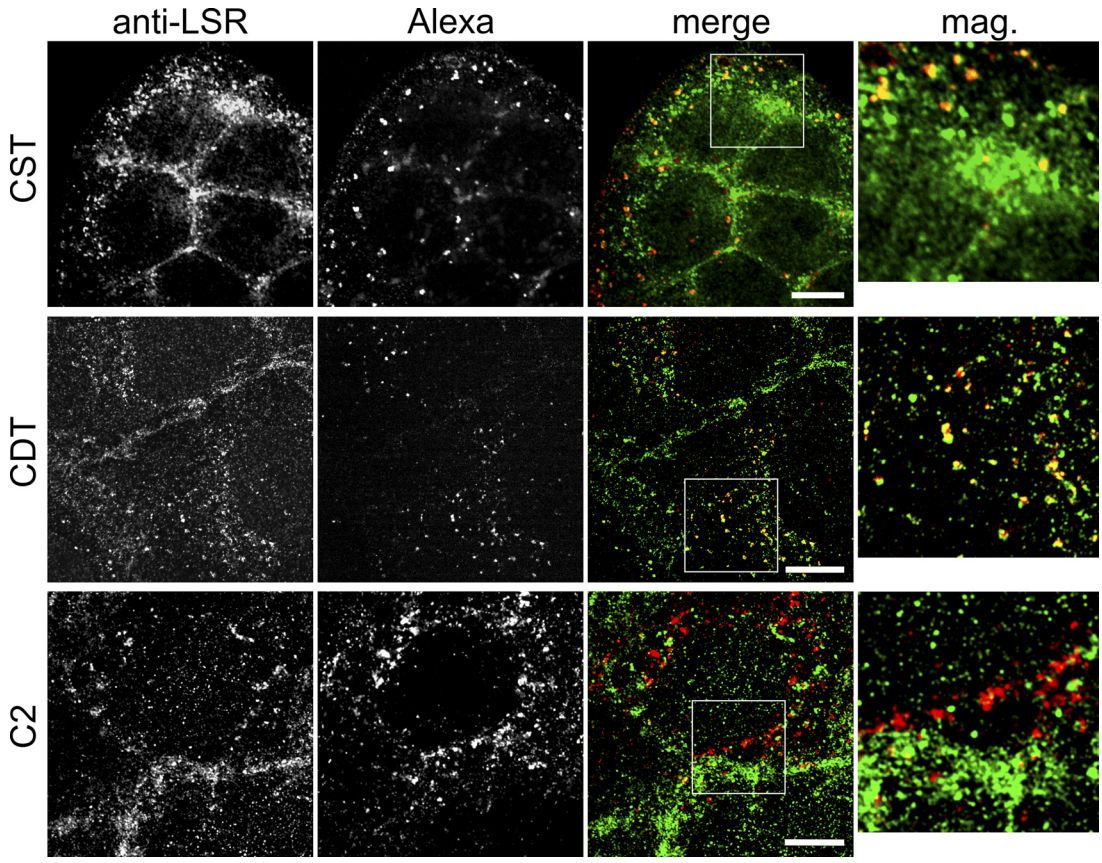

FIG 4 CST colocalizes with LSR in endocytic vesicles of CaCo-2 cells. CaCo-2 cells were incubated with either CSTa/CSTb$_{\text{Alexa 488}}$ (CST), CDTa/CDTb$_{\text{Alexa 568}}$ (CDT), or C2I$_{\text{Alexa 568}}$/C2II (C2) for 30 min on ice to allow binding of the toxins to the cell surface. Subsequently, cells were shifted to 37°C to induce endocytic uptake of the receptor/toxin complexes. Following an incubation period of 20 min, cells were fixed, permeabilized, and subjected to immunostaining of endogenous LSR. Localization of CST, CDT, or C2 (green signals in merged images, Alexa) and endogenous LSR (red signals in merged images, anti-LSR) was analyzed by confocal fluorescence microscopy (scale bars, 10 μm). Yellow signals in the merged images (merge) indicate colocalization of Alexa signals with immunostained LSR. mag., magnification of boxed area in the merged image.

dicate that recombinant CST produced in *B. megaterium* constitutes a fully functional actin-ADP-ribosylating toxin. Since the separate addition of the purified CST components did not lead to intoxication characteristics in CaCo-2 cells, possible toxic contaminants that might be copurified from the expression host can be excluded.

Clostridial actin-ADP-ribosylating toxins are subdivided into two families on the basis of their biological activities and immunological relatedness. One family corresponds to the C2 toxins from *C. botulinum* (C2 toxin family), and the other family (iota-like toxin family) includes the eponym *C. perfringens* iota toxin, as well as CST and *C. difficile* transferase (CDT) (8, 14, 15). Interestingly, binding and enzymatic components are mutually interchangeable among the members of the iota-like toxin family, to form fully active toxins, but not between the C2 toxin and the iota-like toxin group (8, 18). Further interfamily comparisons revealed that C2 toxin recognizes unique cell surface receptors for internalization (9, 10). These findings are in agreement with a recent study from our laboratory that identifies the cellular receptor of CDT and iota toxin, namely, the lipolysis-stimulated lipoprotein receptor (LSR), which does not mediate cell entry of C2 toxin (16). So far, it has not been investigated whether additional members of the iota toxin family, such as, for example, CST, also use the LSR for entry into host cells. The current study clearly provides evidence that the LSR also represents the target molecule for binding and internalization of CST. Our reasoning was based on H1-HeLa cells that do not produce LSR, thereby exhibiting increased resistance to the CST-related toxins CDT and iota toxin that can be circumvented by ectopic expression of LSR (16). In the current study, intoxication of H1-HeLa cells with CST and binding of the toxin to these cells was strictly dependent on the presence of LSR protein. The role of LSR in the cellular uptake of CST could be substantiated by using an additional cell line (CaCo-2) for colocalization studies, revealing overlapping fluorescence signals of Alexa-labeled toxin and immunolabeled LSR protein in endocytic vesicles.

LSR is a type I single-pass transmembrane protein of the cell surface, featuring an Ig-like V-type domain in the N-terminal, extracellular portion of the protein. The protein is expressed mainly in the liver but also in the intestine and in various other tissues (13, 24). Previous studies suggest that the LSR functions in the cellular uptake of triglyceride-rich, low-density lipoproteins for clearance of chylomicron remnants from blood circulation (4, 23). Recently, an additional role in the organization of tricellular junctions that are involved in epithelial barrier function has been attributed to the LSR protein (12). The finding that disruption of

the LSR gene causes lethality in mice also reveals an important role of this protein in early steps during development (13). Our study now highlights that pathogenic clostridia producing iota-like toxins have evolved by hijacking the LSR protein as a membrane target for smuggling their toxins into host cells.

ACKNOWLEDGMENTS

We thank Otilia Wunderlich, Sven Hornei, and Lars Ellenrieder for excellent technical assistance and Michel R. Popoff (Paris, France) for providing genomic DNA from *Clostridium spiroforme* strain CS246.

This work was supported by Deutsche Forschungsgemeinschaft grants AK6/16-3 and AK6/20-1 (to P.P. and K.A.).

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