Inactivation of Ras by Clostridium sordellii Lethal Toxin-catalyzed Glucosylation*  

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The lethal toxin (LT) from Clostridium sordellii belongs to the family of large clostridial cytotoxins causing morphological alterations in cultured cell lines accompanied by destruction of the actin cytoskeleton. C. sordellii LT exhibits 90% homology to Clostridium difficile toxin B, which has been recently identified as a monoglucosyltransferase (Just, I., Selzer, J., Wilm, M., von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) Nature 375, 500-503). We report here that LT too is a glucosyltransferase, which uses UDP-glucose as cosubstrate to modify low molecular mass GTPases. LT selectively modifies Rac and Ras, whereas the substrate specificity of toxin B is confined to the Rho subfamily proteins Rho, Rac, and Cdc42, which participate in the regulation of the actin cytoskeleton. In Rac, both toxin B and LT share the same acceptor amino acid, threonine 35. Glucosylation of Ras by LT results in inhibition of the epidermal growth factor-stimulated p42/p44 MAP-kinase signal pathway. LT is the first bacterial toxin to inactivate Ras in intact cells.

Clostridium sordellii produces two major virulence factors, the hemorrhagic toxin and the lethal toxin (LT), which are causally involved in diarrhea and enterotoxemia in domestic animals and in gas gangrene in man (1, 2). The hemorrhagic toxin exhibits hemorrhagic activity, whereas LT causes severe edema. In addition both toxins are lethal and cytotoxic. The cytotoxic effects on cell monolayers are characterized by redistribution of the actin cytoskeleton (3-5). In respect to physicochemical and immunological properties LT resembles toxin B from C. difficile (6, 7). ToxB-induced destruction of actin filaments results in accumulation of the breakdown products in small cell clusters (3, 5, 11). ToxB-induced cytotoxic effects are clearly distinct from those induced by ToxA. Whereas ToxA causes cell shrinkage and formation of neurite-like extensions, LT induces more pronounced rounding of cells, which become grouped in small cell clusters (3, 5, 11). ToxB-induced destruction of actin filaments results in accumulation of the breakdown products in the perinuclear space, whereas LT leads to a diffuse distribution (3, 11).

Recently, we reported that ToxA and ToxB from C. difficile are monoglucosyltransferases that selectively modify the low molecular mass GTP-binding proteins of the Rho subfamily (12, 13), whereas other members of the Ras superfamily are not substrates. The cosubstrate UDP-glucose is cleaved by ToxA/ToxB, and the glucose moiety is transferred to amino acid threonine 37 of Rho, which is located in the effector domain of the Rho proteins (12-14). The substrate proteins of ToxA and ToxB are Rho, Rac, and Cdc42, which are involved in the regulation of the actin cytoskeleton. Whereas Rho governs the formation of focal adhesions and stress fibers (15), Rac is involved in membrane ruffling (16), and Cdc42 is involved in the formation of filopodia (17, 18). Glucosylation renders Rho functionally inactive, eventually resulting in a redistribution of the microfilament system (12).

We report here the identification of C. sordellii lethal toxin as a glucosyltransferase that modifies the low molecular mass GTP-binding proteins Rac and Ras.

EXPERIMENTAL PROCEDURES

Materials—14C-labeled UDP-hexoses were obtained from DuPont NEN (Dreieich, Germany). All other reagents were of analytical grade and purchased from commercial sources. C. difficile ToxA and ToxB (19) and C. sordellii LT (20) were purified as described. The purity of lethal toxin was estimated to be 92% by densitometric analysis of Coomassie blue-stained SDS-PAGE of LT fractions.

Preparation of Recombinant GTP-Binding Proteins—Rac1, RhoA, Cdc42, RhOG, and Ha-Ras were prepared from their fusion proteins (e.g. RhoA-glutathione S-transferase) as described (21). GST fusion proteins from the Escherichia coli expression vector pGEX-2T were isolated by affinity purification with glutathione-Sepharose (Pharmacia, Germany) followed by cleavage of GTP-binding proteins from the GST fusion protein by thrombin treatment (100 μg/ml for 30 min at 22 °C). Thrombin was removed by binding to benzamidine-Sepharose, and the GTP-binding proteins were concentrated with Centricon (Amicon).

Cell Culture—Fibroblast NIH 3T3 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 4 mM glutamine/penicillin/streptomycin. After 24 h the medium was changed, and the cells were incubated with either C. sordellii LT (40 ng/ml) for 24 h or with C. difficile ToxB (0.3 ng/ml) for 9 h. Alternatively (for the MAP kinase assay) the cells were incubated with Dulbecco’s modified Eagle’s medium with 0.1% fetal calf serum for further 24 h. During this time the cells were incubated with C. sordellii LT and with C. difficile ToxB, respectively.

Before cell lysis, the cells were rinsed with ice-cold phosphate-buffered saline (pH 7.2) and were then disrupted mechanically by sonication (5 times on ice) in the presence of lysis buffer (2 mM MgCl2, 40 μM aprotinin, 0.1 mM phenylmethylsulfonyl fluoride, 20 μM leu-

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The abbreviations used are: LT, C. sordellii lethal toxin; ToxA, C. difficile toxin A; ToxB, C. difficile toxin B; EGF, epidermal growth factor; PAGE, polyacrylamide gel electrophoresis; MAP, mitogen-activated protein.
peptin, 80 μg/ml benzamidine, 50 mM HEPES, pH 7.4), followed by centrifugation for 10 min at 2,000 × g. The supernatant was used for the glucosylation reaction. For the MAP kinase assay, the cells were rinsed with ice-cold phosphate-buffered saline and were then disrupted mechanically by sonication (5 times on ice) in the presence of lysis buffer (150 mM NaCl, 2 mM EGTA, 2 mM diithiothreitol, 1 mM orthovanadate, 40 μg/ml aprotinin, 0.1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 10 mM HEPES, pH 7.4), followed by centrifugation for 15 min at 14,000 × g. The supernatant was used for the MAP kinase assay.

Glucosylation Reaction—Recombinant GTP-binding proteins (50 μg/ml) were incubated with LT (4 μg/ml) in a buffer containing 10 μM UDP-[14C]glucose, 1 mM MgCl2, 2 mM MgCl2, 100 μM bovine serum albumin, and 50 mM HEPES (pH 7.4) or with ToxB (1 μg/ml) in the same buffer but without MnCl2 for the indicated times at 37°C.

Cytosolic Subtraction (F500)—Rat brains were homogenized in 3 volumes of lysis buffer and centrifuged for 60 min at 100,000 × g. The supernatant (cytosolic fraction) was incubated for 15 min at 95°C, and denatured proteins were removed by centrifugation. The supernatant was passed through an ultrafiltration membrane (ultrafiltration membranes, Amicon Corp.) with a 500-Da cut-off. The flow-through termed F500 was used as cytosolic subtraction.

Toxins Effect in Cell-free System—Cell lysates from NIH 3T3 cells were incubated without or with LT (4 μg/ml) or ToxB (1 μg/ml) for 45 min at 37°C. Recombinant proteins (dissolved in 25 mM HEPES, pH 7.4, 2 mM MgCl2, 1 mM MnCl2, 100 μM bovine serum albumin) were incubated with ToxB (1 μg/ml) or LT (4 μg/ml) plus 1 mM MnCl2 for 45 min at 37°C. The reaction was terminated by addition of Laemmli sample buffer.

Gel Electrophoresis—Proteins were dissolved in sample buffer and subjected to 12.5% SDS-PAGE (22) followed by analysis using the PhosphorImager SF from Molecular Dynamics.

Immunoblot—Immunoblotting was performed according to Towbin et al. (23) with anti-v-Ha-Ras monoclonal antibody from Oncogene Science. Visualization was performed with the Amersham enhanced chemiluminescence system.

Sequential Glucosylation—Rac1 (50 μg/ml dissolved in 50 mM HEPES, pH 7.4, 2 mM MgCl2, 100 μM bovine serum albumin) was glucosylated with either LT (4 μg/ml plus 1 mM MnCl2) or ToxB (1 μg/ml) in the presence of unlabeled UDP-glucose (10 μM) for 45 min at 37°C. Thereafter, a second glucosylation in the presence of UDP-[14C]glucose (10 μM) and ToxB (1 μg/ml) or LT (4 μg/ml plus 1 mM MnCl2) was performed for 45 min at 37°C. RasK (50 μg/ml dissolved in 50 mM HEPES, pH 7.4, 2 mM MgCl2, 100 μM bovine serum albumin) was incubated with LT (4 μg/ml plus 1 mM MnCl2) or ToxB (1 μg/ml) in the presence of UDP-[14C]glucose (10 μM). For sequential glucosylation, Rac1 was first incubated with unlabeled UDP-glucose and LT in the presence of MnCl2 (1 mM) followed by a second [14C] glucosylation with ToxB.

Deglucosylation—The membrane fraction from lysates of NIH 3T3 cells (pellet of centrifugation 60 min at 100,000 × g) were resuspended in 50 mM HEPES, pH 7.4, 2 mM MgCl2, 1 mM MnCl2, and incubated with LT (4 μg/ml) in the presence of UDP-[14C]glucose (30 μM) for 90 min at 37°C. The membranes were washed twice with 50 mM HEPES, pH 7.4, 2 mM MgCl2 followed by resuspension in the same buffer. Deglucosylation was initiated by the addition of UDP (30 μM) and either LT (4 μg/ml plus 1 mM MnCl2) or ToxB (1 μg/ml). After 90 min at 37°C the reaction was analyzed by 12.5% SDS-PAGE followed by evaluation with the PhosphorImager.

Immunoprecipitation with anti-Ras Beads—Lysates from NIH 3T3 cells or from rat brain homogenate were [3H] glucosylated with LT and ToxB, respectively, as described above. A sample was taken for SDS-PAGE. To 300 μl of the lysate radiomimprintation precipitation buffer was added to form a final concentration of 10 mM MgCl2, 150 mM NaCl, 0.1% (w/w) Nonidet P-40, 0.05% (w/w) SDS, 0.5% (w/w) desoxycholate, 0.1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 40 μg/ml aprotinin, 50 mM HEPES, pH 7.4. Anti-Ras beads (15 μl of anti-v-Ha-Ras monoclonal antibody coupled to Sepharose beads, Oncogene Science) were added, and the samples were gently rotated head-over-head for 2 h at 4°C. The beads were collected by centrifugation for 20 s at 12,000 × g and then washed 4 times with radioimmunoprecipitation buffer. The proteins were extracted from the beads by Laemmli buffer (5 min at 95°C) and separated by 12.5% SDS-PAGE followed by blotting to nitrocellulose. Ras was detected by immunoblot analysis with anti-Ras. Nitrocellulose was then analyzed by PhosphorImager SF from Molecular Dynamics.

MAP Kinase Assay—The lysates from NIH 3T3 cells grown in medium depleted of fetal calf serum were used for the MAP kinase assay (Amersham, Braunschweig, Germany), which was performed according to the recommendations of the manufacturer. Before lysis the cells were stimulated with 2 ng/ml epidermal growth factor (EGF) (Sigma, Germany) for 10 min. Phosphorylation reactions were performed in the lysis after the addition of [γ-32P]ATP and of a peptide specific for extracellular regulated kinase 1/extracellular regulated kinase 2 kinase. A sample of the reaction mixture was placed on binding membranes followed by washing with phosphoric acid (75 mM) and distilled water. After drying, 32P-phosphorylated peptide was determined with a phosphorimage. Incorporation of [32P] into the peptide was determined in the linear phase (35 min) of the phosphorylation reaction.

RESULTS

Lysates from NIH 3T3 cells were incubated with C. sordellii LT in the presence of UDP-[14C]glucose, UDP-[14C]galactose, and UDP-[14C]-N-acetylglucosamine (Fig. 1A). Proteins in the molecular mass range of 20–22 kDa were only labeled in the presence of UDP-[14C]glucose, indicating incorporation of
[14C]glucose into these cellular proteins. When the concentration of LT was increased (up to 40 μg/ml) and the incubation time was prolonged (up to 180 min) no additional proteins were labeled (data not shown). To study the protein substrate specificity of LT in more detail, recombinant low molecular mass GTP-binding proteins of the Ras superfamily were tested. Under conditions that were sufficient to elicit full transferase activity of C. difficile ToxA and ToxB (12, 13), LT catalyzed only minimal incorporation of glucose into the recombinant GTP-binding proteins. Therefore, we tested whether the transferase activity of LT might require a factor that is present in cell lysates. Several cellular subfractions were analyzed to enhance glucosylation of recombinant Rho subfamily proteins. A cytosolic factor that was heat-stable and had a molecular mass below 500 Da (fraction F500) was preliminarily characterized to stimulate LT-catalyzed glucosylation (Fig. 1C).

As shown in Fig. 1B, Rac was the exclusive substrate of the Rho subfamily. Neither Rho nor Cdc42 was glucosylated. Surprisingly, Ras was modified by LT, whereas other members of the Ras superfamily such as Rab and Arf were not substrates. Denaturation of either Rac/Ras or LT completely inhibited glucosylation, indicating that the native structure of both substrate protein and transferase are essential for the glucosylation reaction (data not shown).

Since the activities of various eukaryotic glycosyltransferases are stimulated by Mn2+ (24, 25) several metal ions were tested. LT-catalyzed glucosylation was only stimulated in the presence of Mn2+ with maximal incorporation of glucose at 1 mM of Mn2+. Combination of Mn2+ with fraction F500 caused no additional increase in glucosylation, indicating that the stimulating factor in the fraction F500 is most likely Mn2+. In contrast, glucosyltransferase activity of the related ToxA/B was inhibited rather than stimulated by Mn2+ (data not shown). Chelation of Mn2+ by EDTA (2 mM) or the addition of EDTA (2 mM) to fraction F500 completely blocked glucosylation of Rac by LT but not by ToxB (Fig. 1C). Increasing concentrations of MgCl2 up to 10 mM caused a decrease in LT-catalyzed glucosylation but did not change ToxB-catalyzed modification of Rac. All these findings indicate that Mn2+ is required for LT transferase activity but does not interfere with the Mg2+ dependent GTP-binding to Rac protein.

To test whether the guanyl nucleotide-bound state of Rac affects the incorporation of glucose, Rac was incubated with either GDP or GTP prior to glucosylation. Rac was a better substrate for LT in the GDP-bound form than in the GTP-bound form (Fig. 1D). Identical effects of GDP/GTP were observed for Ras (Fig. 1D). The guanyl nucleotide dependence of glucosylation of the Rho subfamily proteins by ToxA/B is based on the location of the acceptor amino acid threonine 37 in the GTP-binding and hydrolysis domain (12, 26). To determine whether LT uses the same acceptor site as ToxA/B, sequential glucosylation by either transferase was performed. Rac was modified by LT (or ToxB) in the presence of unlabeled UDP-glucose, followed by a second glucosylation performed in the presence of UDP-[14C]glucose and ToxB (or LT) (Fig. 2A). Previous glucosylation completely inhibited the second glucosylation with either toxin, indicating that LT most likely catalyzes incorporation of glucose at the same acceptor amino acid of Rac as does ToxB. As expected, treatment of RhoA with LT did not inhibit subsequent 14C glucosylation by ToxB (Fig. 2A). To confirm these data by a different approach we made use of the deglucosylation reaction, which might be even more stereospecific than the sequential glucosylation. Instead of recombinant Rac we used membrane fractions of NIH 3T3 cells because they can be washed to remove UDP-glucose, which blocks deglucosylation. In the presence of a surplus of UDP (30 mM), LT was able to cleave the previously incorporated glucose moiety and to again form UDP-glucose (Fig. 2B). The reversal of LT-catalyzed glucosylation of Rac (lower band) was blocked after the removal of LT. However, when ToxB was added, deglucosylation of Rac was observed again. The upper band (Figs. 1 and 2B), which is not a substrate protein for ToxB, was not deglucosylated by ToxB. These experiments indicate that LT and ToxB share the same acceptor amino acid in the Rac protein.

LT catalyzed 14C glucosylation of three substrate proteins (Fig. 1). The middle band of the triplet was identified as Rac by immunoblot of two-dimensionally separated cell lysates. 14C glucosylation by LT (not shown). The faint lower band corresponds to Ras (see below). The identity of the upper one is still unclear, but glucosylation is GDP-dependent, indicating that this substrate is a low molecular mass GTP-binding protein. To study whether cellular Ras is actually a substrate of LT, lysates from rat brain homogenates were glucosylated by either LT or ToxB in the presence of UDP-[14C]glucose followed by immunoprecipitation of Ras by anti-Ras antibody immobilized to Sepharose beads. As illustrated in Fig. 3A, 14C-glucosylated Ras was precipitated only from lysates treated with LT but not from ToxB-treated lysates. The precipitated Ras comigrates in SDS-PAGE with the faint lower band. Immunoblot analysis of the precipitated Ras protein showed that both samples had the same amount of protein (data not shown). These results were reproduced with lysates from NIH 3T3 cells, but the amount of precipitated Ras was lower than from rat brain lysate (data not shown). These precipitation experiments indicate that cellular Ras is a substrate for LT. To test whether Ras is glucosylated in the intact cell, differential glucosylation was applied. If LT catalyzes glucosylation of Ras in the intact cell, subsequent 14C glucosylation of the lysate should show decreased incorporation of [14C]glucose. Because of the low concentration of Ras in NIH 3T3 cells, Ras was enriched by immunoprecipitation with anti-
Glucosylation of Ras and Rac

Ras after the 14C glucosylation. As shown in Fig. 3B, treatment of intact cells with LT completely blocked subsequent glucosylation of the lysate, indicating previous modification in the intact cell.

To test whether glucosylation induced inactivation of Ras resulting in decreased MAP kinase activity, EGF-stimulated p42/p44 MAP kinase activity was determined. Serum-starved NIH 3T3 cells were treated with LT and ToxB, respectively, until the cells showed the typical morphology. The Ras-regulated MAP kinase signal pathway was stimulated with EGF for 10 min. In cell lysates phosphorylation of specific substrates of p42/p44 MAP kinases were determined in the linear phase of the phosphorylation reaction. As depicted in Fig. 4, EGF stimulation increased basal p42/p44-induced phosphorylation by a factor of about 2.5. The same was true for lysates from ToxB-treated cells, which showed no difference in basal and stimulated phosphorylation compared with control. In contrast, LT treatment completely inhibited EGF-stimulated kinase activity without altering basal MAP kinase activity. Even after prolonged incubation times, stimulated phosphorylation was not different from basal values, indicating complete inactivation of Ras by LT. The same results were obtained with stimulation by 10% fetal calf serum (not shown).

DISCUSSION

C. sordellii lethal toxin belongs to the family of large clostridial cytotoxins, which is characterized by a single-chain structure and a molecular mass of 250–300 kDa (11). This family, which comprises C. difficile ToxA and ToxB, C. novyi α-toxin, C. sordellii lethal toxin (LT), and hemorrhagic toxin, exhibits cytotoxicity by affecting predominantly the microfilament cytoskeleton. ToxA and ToxB, which are coexpressed by pathogenic C. difficile strains, show an identity of 49% (63% homology) at the amino acid level (27). LT from C. sordellii has 76% identity (90% homology) with ToxB and 47% with ToxA. Thus, LT is more closely related to ToxB than ToxB is to ToxA (8). This high degree of identity prompted us to study whether ToxB and LT share the same molecular mode of action. Here we present evidence that LT is a glucosyltransferase that selectively uses UDP-glucose as a cosubstrate as was shown previously for ToxA and ToxB. UDP-galactose or UDP-N-acetylgalcosamine are not used. Whereas ToxA and ToxB glucosylate the Rho subfamily proteins Rho, Rac and Cdc42 (12, 13), LT modifies only one member of this subfamily, namely Rac. Moreover, Ras, which is the prototype of a separate subfamily of the large superfamily of low molecular mass GTPases, is a substrate for LT but not for the C. difficile toxins. The Rho subfamily has been shown to be involved in the regulation of the actin cytoskeleton. Rho participates in the formation of focal adhesions and stress fibers, whereas Rac regulates membrane ruffling and Cdc42 regulates the formation of microspikes and filopodia (15–18, 28). In addition, Rac exhibits a cell type-specific function in neutrophil granulocytes, where it regulates superoxide anion production (29). Recently, the Rho proteins have been identified as being involved in the activation of transcription factors via the Ras pathway (30) and a Ras-independent signal cascade (31–33). Thus, LT as well as ToxB could act in two ways. First, they inactivate the regulatory proteins of the actin cytoskeleton. Second, they may interfere with gene expression by inhibiting the signal cascade in which Rho and Rac lead to activation of transcription factors. The morphological alterations of cultured cells induced by LT are different from those caused by ToxB. Whereas ToxB causes cell shrinkage, resulting in cell adhesion points resembling neurite-like extensions, LT induces merely rounding of cells (3, 5, 11). However, despite the different morphology both toxins predominantly cause destruction of the actin filament system. The different features in morphology and the recent finding that overexpression of the isoforms RhoA, B, and C increases resistance to ToxA and ToxB but not to LT (34) are consistent with the differences in the target proteins for these toxins.

In cell lysates LT exhibits glucosyltransferase activity leading to the modification of cellular proteins. Glucosylation of the recombinant target proteins, however, depends on the presence of a cellular factor, which has been identified as the cation Mn2+. Combination of the cytosolic subfraction F500 with Mn2+ did not result in further stimulation, and the bivalent cation chelator EDTA blocked the stimulatory effect of the F500 fraction as well as that of Mn2+, indicating that Mn2+ is actually the cellular factor required for LT enzymic activity. Stimulation by Mn2+ is also observed with eukaryotic glycosyltransferases (24, 35). In contrast, Mn2+ does not enhance transferase activity of ToxA or ToxB. This finding suggests that Mn2+ does not compete with Mg2+ binding at Rac but most
likely acts directly on LT. Since EDTA has no effect on ToxA/B transferase activity, the possibility of binding of Mn2+ to ToxB can be excluded. Therefore, the Mn2+ dependence of LT activity may reflect structural differences between LT and ToxB, although both toxins show 90% homology. As observed with ToxA/B, LT glucosylates recombinant proteins, indicating that isoprenylation, the functionally relevant posttranslational modification of low molecular mass GTPases, is not essential for glucosylation.

The acceptor amino acid of both ToxB- and ToxA-glucosylated RhoA has been determined as Thr37 (12, 13). Thr37, which corresponds to Thr35 in Rac, is located in the effector domain and is crucial for GTP binding. As can be deduced from the crystal structure of Ras, the hydroxy group of threonine is ligand for Mg2+, which coordinates the β- and γ-phosphates of GTP (26, 36). In the GDP-bound form, the hydroxy group is exposed to surface of the molecule and is accessible for glucosylation. Consistent with this concept is the finding that GDP-bound Rac and Ras, respectively, are glucosylated to a greater extent than the GTP-bound form.

The sequential glucosylation of Rac by LT followed by ToxB, and vice versa indicates that both toxins share the same acceptor amino acid in Rac. This finding was verified by the technique of deglucosylation. The deglucosylation of LT-modified Rac by LT and ToxB, respectively, gives evidence that ToxB and LT glucosylate Rac at the same acceptor amino acid, namely Thr35.

Applying immunoprecipitation we showed that Ras from cell lysates is a substrate for LT. Furthermore, using the method of differential glucosylation we present evidence that Rac is actually a substrate in the intact cell. Inhibition of EGFr- and fetal calf serum-stimulated p42/p44 MAP kinases (extracellular signal-regulated kinase) in LT-treated cells indicates that glucosylation renders Ras inactive, resulting in a blocked signal pathway. Inhibition of the MAP kinase pathway is due to glucosylation of Ras but not of Rac, because ToxB that glucosylates Rac does not alter basal or EGF-stimulated Ras. This finding was verified by the technical novelty of indirect effects of the destroyed cytoskeleton on MAP kinases. Furthermore, the possibility of indirect effects of the destroyed cytoskeleton on MAP kinases and the ability of indirect effects of the destroyed cytoskeleton on MAP kinases. Furthermore, the possibility of indirect effects of the destroyed cytoskeleton on MAP kinases and the ability of indirect effects of the destroyed cytoskeleton on MAP kinases. Furthermore, the possibility of indirect effects of the destroyed cytoskeleton on MAP kinases.

It is likely that glucosylation of Rac, which is reported to be involved in the regulation of the cortical actin cytoskeleton and which acts upstream of Rho (16), 28, contributes to the cytoxic effects of LT. Recently, Hall and co-workers (16) presented evidence that oncogenic Ras acts upstream of the Rac protein, which itself is upstream of Rho subtype proteins. There is some evidence that oncogenic Rac mediates malignant phenotype by interfering with Rho subfamily proteins (37). However, at the moment it is not clear whether inactivation of Ras by glucosylation does contribute to the morphological effects of LT or whether inactivation of Ras results only in blockade of cell proliferation, which may be effective at conditions of long term intoxication.

Ras is also a substrate of Pseudomonas aeruginosa exoenzyme S, which catalyzes ADP-ribosylation. However, Ras-long is a group of heterogenous substrate proteins including Ral, Rap1A, Rap2, Rab3, Rab4, and even the intermediate filament protein vimentin (38, 39). Furthermore, the substrate specificity depends on exoenzyme S concentration (38). In contrast, the substrate specificity of LT does not vary with concentration of LT. The most important difference, however, is that LT modifies Ras in intact cells, whereas exoenzyme S acts on Ras in cell lysates only.

In conclusion, C. sordellii lethal toxin (LT) has been identified as a glucosyltransferase that uses UDP-glucose as cosubstrate to modify Ras and Rac proteins. LT belongs to the novel family of clostridial glucosyltransferases that glucosylate the crucial threonine residue in the effector domain of low molecular mass GTP-binding proteins. Despite the high homology of C. sordellii lethal toxin to C. difficile ToxB, lethal toxin differs in its substrate specificity and the cofactor dependence. LT renders Ras inactive by glucosylation, resulting in inhibition of the EGF-stimulated MAP kinase pathway. LT is the first bacterial toxin that causes inactivation of Ras in intact cells.

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