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Transcytosis of cholera toxin subunits across model human intestinal epithelia

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ABSTRACT **Cholera toxin (CT) elicits a massive secretory response from intestinal epithelia by binding apical receptors (ganglioside G_{M1}) and ultimately activating basolateral effectors (adenylate cyclase). The mechanism of signal transduction from apical to basolateral membrane, however, remains undefined. We have previously shown that CT action on the polarized human intestinal epithelial cell line T84 requires endocytosis and processing in multiple intracellular compartments. Our aim in the present study was to test the hypothesis that CT may actually move to its site of action on the basolateral membrane by vesicular traffic. After binding apical receptors, CT entered basolaterally directed transcytotic vesicles. Both CT B subunits and to a lesser extent CT A subunits were delivered intact to the serosal surface of the basolateral membrane. The toxin did not traverse the monolayer by diffusion through intercellular junctions. Transcytosis of CT B subunits displayed nearly identical time course and temperature dependency with that of CT-induced Cl^- secretion—suggesting the two may be related. These data identify a mechanism that may explain the link between the toxin's apical receptor and basolateral effector.**

Colonization of the human small bowel with *Vibrio cholerae* results in a massive secretory diarrhea primarily through the action of the secreted enterotoxin cholera toxin (CT). CT acts directly on polarized intestinal epithelial cells by binding specific glycosphingolipid receptors on the apical (lumenal) membrane and subsequently activating adenylate cyclase on the cytoplasmic surface of the basolateral membrane (1). *In vivo*, the secretory response is strongly enhanced by effects on submucosal nerves (2) and other subepithelial cells present in the lamina propria (3). Remarkably, orally administered CT may also interact directly with subepithelial components of the mucosal immune system, as the toxin is recognized to be one of the most potent oral immunogens known (4). CT (84 kDa), however, is restricted from transepithelial diffusion by size exclusion from the paracellular pathway (5). The mechanism(s) by which CT gains access to basolateral enzymes (i.e., adenylate cyclase) within the surface epithelial cell itself and also breeches the epithelial barrier to interact with subepithelial components of the lamina propria remain uncharacterized.

CT consists of five identical B subunits that bind to cell membranes and a single enzymatic A subunit composed of two peptides linked by a single disulfide bond (6). Binding of the toxin to ganglioside G_{M1} on the cell surface is followed by translocation of the A subunit across the membrane and reduction of the A subunit to form the enzymatically active A_1 peptide. The reduced A_1 peptide functions inside the cell, catalyzing the ADP-ribosylation of the regulatory heterotrimeric GTPase $G_{s\alpha}$, which in turn activates adenylate cyclase (7); intracellular cAMP then increases and, in intestinal crypt epithelia, elicits the primary transport event of secretory diarrhea—electrogenic Cl^- secretion. The exact site of A-subunit translocation within the cell and whether the A_1 peptide eventually breaks free of the membrane to diffuse in the cytosol remain undefined. The B subunit does not penetrate the cell membrane and remains bound to G_{M1} on the exocytoplasmic membrane surface (8, 9).

The process by which signal transduction occurs takes 20–40 min in nonpolarized cells (7). Only after this unexplained lag phase, presumably corresponding to events leading to A-subunit translocation and transit of toxin (or possibly another messenger) to adenylate cyclase, do levels of cAMP increase. To define the events responsible for this "lag phase" in polarized epithelia, we have utilized the human intestinal cell line T84 (5, 10). T84 cells grown on permeable supports form confluent monolayers of columnar epithelia that exhibit high transepithelial resistance, polarized apical and basolateral membranes, and a cAMP-regulated Cl^- secretory pathway analogous to that found in intact intestine (11). This system is particularly relevant because the model requires that CT transduces a signal from the apical membrane and the response to CT in T84 cells reproduces the primary transport event of secretory diarrhea (electrogenic Cl^- secretion), which characterizes cholera in humans.

In polarized T84 cells, two sequential and distinct membrane transport "events" are essential for toxin action (5, 10). Both events are functionally defined but occur after CT binding to the apical cell surface and after toxin entry into an early apical endosomal compartment. The first event occurs early in the lag phase, is sensitive to the drug brefeldin A, and is essential for movement of CT into a subcellular compartment where translocation of the A_1 peptide occurs (10). Brefeldin A is known to reversibly disrupt normal patterns of vesicular transport in a wide variety of eukaryotic cell systems (12). The second event is temperature-sensitive and occurs late in the lag phase after the A subunit has been reduced and presumably translocated (5).

Our aim in the present study was to test the hypothesis that CT may actually move to its site of action on the basolateral membrane by vesicular traffic. Movement of CT B and A subunits from apical to basolateral cell surfaces was measured directly by using the method of selective cell surface biotinylation (13). Our data show that after binding apical cell surface receptors, CT entered a transcytotic pathway and both the B subunit and a smaller fraction of A subunit (presumably as holotoxin) were delivered to the exocytoplasmic (serosal) surface of the basolateral membrane. These data identify a mechanism that may explain the link between the toxin's apical receptor (G_{M1}) and basolateral effector (adenylate cyclase). Furthermore, these studies may explain the confounding observations that, in nature, this large toxin, while restricted from transepithelial diffusion, is able to transmit signals to subepithelial cells of the lamina propria.

MATERIALS AND METHODS

Selective Cell Surface Biotinylation. T84 cells were cultured and electrophysiologic measurements were performed as de-

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Abbreviations: CT, cholera toxin; sulfo-NHS, sulfosuccinimidyl.

scribed (5). Methods (13) were modified for use in the T84 cell system. Biotinylation was performed at pH 7.4 for B subunit and at pH 9.0 for A subunit. Apical cell surfaces were quenched with sulfosuccinimidyl (sulfo-NHS) acetate (0.5 mg/ml) during basolateral biotinylations. For A subunits, cells were lysed in the presence of 10 mM *N*-ethylmaleimide. Antisera were raised in rabbits against denatured CT A and B subunits. Immunoprecipitated proteins were analyzed under reducing conditions for B subunit and nonreducing conditions for A subunit. Ligand blots were analyzed by chemiluminescence (Amersham) and densitometry (Molecular Dynamics 300A).

Dissociation of CT from Cell Surface G_{M1}. ^{125}I-labeled CT (20 nM) was applied to T84 monolayers at 4°C and washed. After shifting to 37°C, 0.5-ml aliquots were removed and radioactivity was analyzed by γ counting of 10% (wt/vol) trichloroacetic acid precipitates.

RESULTS AND DISCUSSION

Since sulfo-NHS-biotin, the reactive agent used in selective cell surface biotinylation, is impermeable to cell membranes and intact tight junctions, it is possible to selectively label proteins containing free amino groups on either the apical or basolateral cell surface of polarized T84 cell monolayers. Thus, if CT enters transcytotic vesicles, the B subunit should become accessable to sulfo-NHS-biotin applied to basolateral cell surfaces after toxin transit through the cell. If, however, reduction and membrane translocation of the A subunit are not 100% efficient, movement of CT through the same (or alternative) transcytotic route would also result in the delivery of A subunits to a site accessible to sulfo-NHS-biotin on the exocytoplasmic surface of the basolateral membrane. The fraction of CT subunits labeled with biotin can then be detected by immunoprecipitating the subunits, followed by Western blot analysis with peroxidase-coupled streptavidin.

Transepithelial Transport of CT Across T84 Cell Monolayers Occurs After a Lag Phase. In initial experiments, we found that application of CT to apical cell surfaces was followed by the appearance of CT B subunits on basolateral membranes. Fig. 1 shows the representative results of one such experiment, a Western blot to demonstrate that each lane contains equivalent amounts of immunoprecipitated CT B subunit and an avidin blot. Lanes 1 and 2 are positive and negative controls, respectively. CT (20 nM) was applied apically to T84 monolayers and incubated for 120 min at 4°C, a temperature that completely inhibits vesicular traffic and CT-induced signal transduction (5). The monolayers were then biotinylated at the apical (lane 1) or basolateral (lane 2) cell surface. The large avidin-peroxidase signal (*Lower*, lane 1) demonstrates that CT B subunit can be labeled with biotin while bound at the cell surface. In the absence of vesicular traffic (at 4°C), however, the CT B subunit was not labeled by applying biotin to basolateral reservoirs (lane 2).

Lane 4 shows that in monolayers exposed to apical CT and incubated at 37°C for 2 h, basolaterally applied biotin has now labeled a fraction of the B subunit at the basolateral membrane indicating that CT had breeched the epithelial barrier. In contrast (lane 3), the CT B subunit was not labeled in monolayers incubated at 37°C for only 30 min, a point in the lag phase where a CT-induced secretory response was not yet detected. Thus, transepithelial transport of CT displays a lag phase characteristic of CT action. Approximately 1–3% of apically bound CT B subunit was present on the basolateral cell surface after 120 min, as assessed by serial dilutions of samples representing the total amount of CT initially bound to apical membranes at 4°C. The CT B subunit was also detected at the basolateral cell surface in monolayers pretreated with brefeldin A (5 μM) (Fig. 1, lane 5), a fungal metabolite that scrambles protein targeting (12) and blocks CT action by

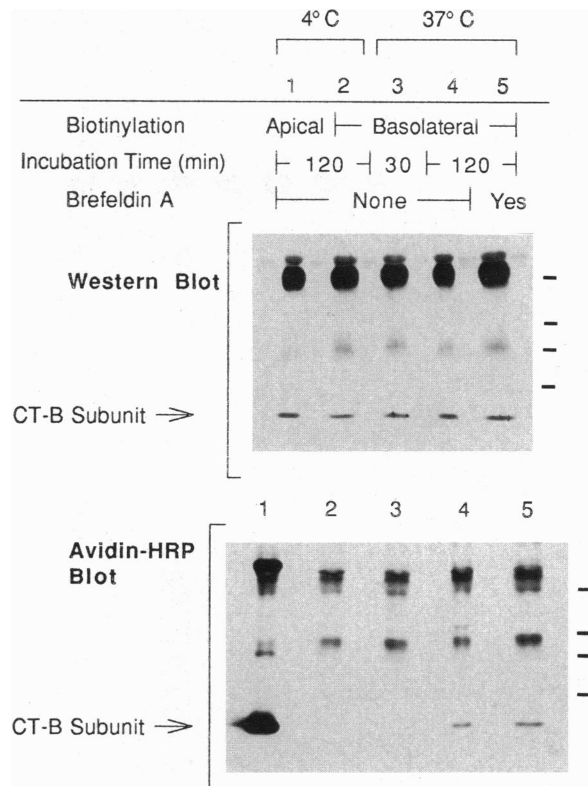

FIG. 1. Effect of time, temperature, and brefeldin A on transepithelial transport of CT B subunit across T84 cell monolayers. (*Upper*) Western blot of immunoprecipitated CT B subunit. (*Lower*) Horseradish peroxidase-avidin blot. Bars show the location of molecular mass markers: 18.5, 27.5, 32.5, and 49.5 kDa. Additional bands represent antibody and dissociated light chains.

preventing translocation of the A subunit (10, 14) (see below). Thus this maneuver permits one to dissociate transcytosis of CT from reduction of the A subunit and, as expected, results in prevention of the physiological signal (10, 14).

Transport of CT to the Basolateral Cell Surface Occurs by Transcytosis and Not by Diffusion Through the Paracellular Pathway. To show that CT was transported through the cell by vesicular traffic and not around the cell by diffusion through a paracellular route, we examined transepithelial transport of CT in monolayers exposed to a pulse of CT at the apical cell surface and then chased in the absence of a basolaterally directed chemical gradient of CT. To demonstrate that the B subunit was in fact labeled with biotin at the basolateral cell surface (and not during or after cell lysis), reactive sites on cell surface proteins were blocked with sulfo-NHS-acetate prior to biotinylation. Each of these experiments then have internal controls run in parallel with monolayers exposed first to non-biotin-containing blocking reagents and then to biotin, instead of biotin first and then blocking agent. Both monolayers of each experimental pair are treated with the same reagents but in a different order.

Fig. 2*A* shows the results of one of three representative experiments. Again, Fig. 2*A Upper* shows the Western blot demonstrating that each lane contains nearly equivalent amounts of immunoprecipitated CT B subunit. Fig. 2 *Lower* shows the avidin blot of three sets of monolayer pairs, each run in parallel. One monolayer of each pair was quenched with sulfo-NHS-acetate blocking reagent before biotinylation. Lanes 1 and 2 are positive and negative controls, respectively, as described above. Lanes 7 and 8 (termed "continuous uptake") show the results of basolateral biotinylation of monolayers exposed continuously to 20 nM CT at 37°C for 120 min. A fraction of cell-associated CT B subunit contains biotin (lane

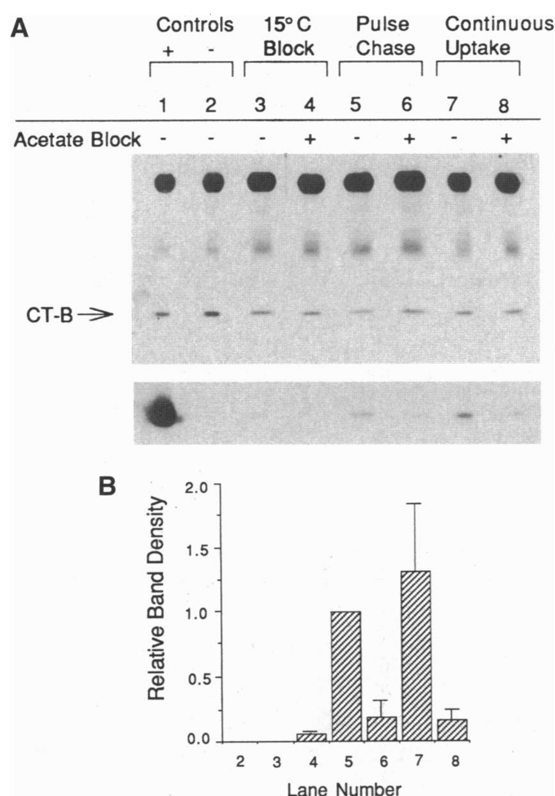

FIG. 2. Transcellular transport of CT by vesicular traffic. (*A*) Representative Western blot (*Upper*) and avidin blot (*Lower*) of three experiments. Monolayers treated with sulfo-NHS-acetate to block reactive sites prior to biotinylation are indicated as +. (*B*) Relative band densities for avidin blots (mean ± SEM; $n = 3$). Signals in lanes 5 and 2 provide two point calibrations.

7), and this signal was strongly inhibited by pretreating monolayers with the quenching reagent (lane 8). Lanes 5 and 6 (termed pulse chase) show the results of basolateral biotinylation of monolayers exposed to a pulse of CT at the apical cell surface. These monolayers were initially exposed to 20 nM CT at 4°C for 30 min but were then washed to remove unbound freely diffusable CT from apical reservoirs before shifting the monolayers to permissive temperatures. Separate experiments showed that this maneuver decreased the steady-state concentration of unbound diffusable CT in apical reservoirs from 20.0 to 0.15 nM or less. Thus, Fig. 2*A*, lane 5, shows that even after reducing the chemical gradient driving CT from apical to basolateral reservoirs by 45- to 130-fold, the CT B subunit was still detected in comparable amounts at the basolateral surface (compare with lane 7) and this signal was also quenched by pretreating monolayers with blocking agents (lane 6).

The results of three experiments are summarized in Fig. 2*B* (relative band densities, mean ± SEM, $n = 3$). These data provide strong evidence that CT B subunit enters basolaterally directed transcytotic vesicles and moves to the basolateral membrane by vesicular traffic. Further evidence in support of this view is supplied by our demonstration that movement of CT to basolateral reservoirs does not occur in monolayers exposed continuously to 20 nM CT at 4°C for 120 min (Figs. 1 and 2, lanes 2) or in monolayers exposed to a late lag phase 15°C temperature block (see below). Thus, these data effectively exclude the possibility that CT moves across the monolayer through intercellular tight junctions by paracellular diffusion.

Both Transcytosis and Signal Transduction by CT Display Sensitivity to a Late Lag Phase 15°C Temperature Block. We also examined, in parallel, transepithelial transport of CT in monolayers exposed to a nonpermissive 15°C temperature block applied 30 min after incubation at permissive temperatures (37°C). This maneuver inhibits vesicular traffic in T84 cells at a step late in the lag phase after the toxin has entered a compartment where reduction and translocation of the A_1 peptide occur (5). Reducing temperatures to nonpermissive levels at this late stage in the lag phase completely inhibits signal transduction by apically applied CT as described (5). Fig. 2*A*, lanes 3 and 4 (termed 15°C block), show that transcytosis of CT B subunits was also blocked in monolayers exposed to a late lag phase temperature block ($n = 3$ experiments). These data show that lowering temperatures to nonpermissive levels late in the lag phase completely inhibits both apical to basolateral signal transduction as assessed by inhibition of CT-induced Cl^- secretion (5) and apical to basolateral transcytosis as assessed by selective cell surface biotinylation.

Transcytosis of CT Depends Primarily on Binding Ganglioside G_{M1}. Transcytosis of CT B subunit did not require the enzymatic action of the CT A subunit. In these experiments, purified CT B subunit (20 nM) instead of CT holotoxin was applied to apical cell surfaces (data not shown). Purified CT B subunit also entered a transcytotic pathway. At 37°C, the kinetics of transport were similar to CT holotoxin (see below). Nearly 3-fold more CT B subunit, however, was labeled at the basolateral cell surface in comparison to that seen when monolayers were exposed to CT holotoxin (relative band optical densities, 295 ± 40% B subunit > holotoxin; mean ± SD, $n = 2$). Control experiments showed that the efficiency of cell surface biotinylation of CT B subunits was equal to or less than that of CT holotoxin. Preparations of CT B subunit did not contain sufficient amounts of contaminating holotoxin to elicit detectable increases in intracellular levels of cAMP as assessed by measurement of cAMP-dependent Cl^- secretion (buffer control, 6 ± 0.4; CT B subunit, 6 ± 0.5; CT holotoxin, 72 ± 0.2 $\mu A/cm^2$; mean ± SD, $n = 3$). These data provide evidence that the CT B subunit may facilitate transit of the toxin across polarized cells by exploiting structural and/or functional features of its cell surface receptor, ganglioside G_{M1}.

Our data do not exclude the possibility that the CT A subunit may also affect vesicular transport of toxin-containing membranes. In concurrent studies on CT transit through intermediary compartments of this signal transduction pathway (22),

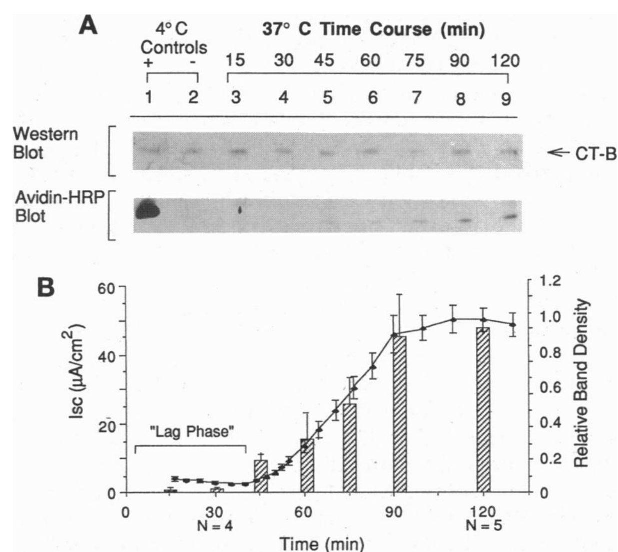

FIG. 3. Time course of CT transcytosis correlates closely with that of CT-induced Cl^- secretion. (*A*) Transcytosis of B subunit. (*B*) CT-induced Cl^- secretion. Relative band optical densities for avidin blots (mean ± SEM, $n = 3$) are superimposed at the appropriate time points. Signals in lanes 9 and 2 provide two point calibrations. Isc, short circuit current.

we find that CT and the closely related *Escherichia coli* labile toxin mimick endogenous signals for protein targeting via the KDEL amino acid motif present on the C terminus of the A_2 peptide. The KDEL sequence is the sorting signal that allows lumenal endoplasmic reticulum proteins to be retrieved from postendoplasmic reticulum compartments (15). The A subunit may also plausibly affect vesicular traffic either through its activity as an ADP-ribosyltransferase or through its interactions with the family of GTP-binding ADP-ribosylating factors.

The Time Course of CT Transcytosis Correlates Closely with the Time Course of CT-Induced Cl^- Secretion. The time courses of CT transcytosis and CT-elicited Cl^- secretion were examined in parallel (Fig. 3*A*). Lanes 1 and 2 show positive and negative controls as described above. Lanes 3–9 show that the CT B subunit was not detected on the basolateral cell surface until 45 min after shifting monolayers to permissive temperatures (lane 5). This corresponds exactly to a time at the end of the lag phase when a secretory response was first detected by electrophysiologic methods (Fig. 3*B*). The amount of transcytosis then increases progressively to 120 min and parallels the secretory response induced by apical CT exposed to monolayers of the same passage and studied in parallel. Relative optical densities of biotinylated B-subunit bands (mean ± SEM, n = 3) are summarized and represented superimposed at the appropriate time points on the time course of CT-induced Cl^- secretion (Fig. 3*B*). The appearance of the B subunit on the basolateral membrane coinsides temporally and quantitatively with CT-induced Cl^- secretion—suggesting the two are related.

These data raise the possibility that the A_1 peptide may not completely dissociate from the B subunit after translocation. Both subunits may move together as "second messenger" from the site of translocation to adenylate cyclase on the basolateral membrane. This interpretation is consistent with data from other groups that indicate that the A_1 peptide may remain membrane-associated during its transit through the cell (16, 17). The actual cellular events after reduction and translocation of the A subunit in both polarized and nonpolarized cells are not, in fact, known. The simple view that the A_1 peptide breaks free of the membrane and diffuses in the cytosol to its site of action is, although compellingly simple, likely incorrect in polarized intestinal epithelial cells. Our initial studies showed that lowering temperatures to 15°C or below, which should not substantially impair cytosolic diffusion but is known to ablate certain steps in vesicular transport, blocks CT signaling even when applied late in the lag phase after reduction and formation of the A_1 peptide has occurred (5). These data provide evidence that signal transduction may not be diffusion limited after formation of the A_1 peptide.

Brefeldin A Alters the Time Course and Transcytosis of CT B and A Subunits. The time course of CT transcytosis was also examined in monolayers pretreated with brefeldin A (5 μM). Brefeldin A scrambles protein targeting (12) and affects membrane traffic of CT in T84 cells (10). We found that transcytosis of CT occurred more rapidly (lag phase reduced to 15 min or less) and peaked more quickly (75 vs. 90 min) in monolayers treated with brefeldin A (data not shown). These results are consistent with those found for the receptor-mediated transcellular trafficking of transferrin, IgG, low density lipoprotein, and the toxin-lectin ricin in brefeldin A-treated MDCK epithelial cells (18–20). Thus brefeldin A may dissociate CT transcytosis from signal transduction by altering the route and possibly the mechanism of toxin transport. By preventing movement of CT through compartments necessary for translocation of the A subunit (10, 14), brefeldin A may alter vesicular traffic of toxin containing membranes in T84 cells so that the toxin is delivered to the basolateral membrane more quickly but in a nonfunctional configuration with the A subunit remaining on the exocytoplasmic surface.

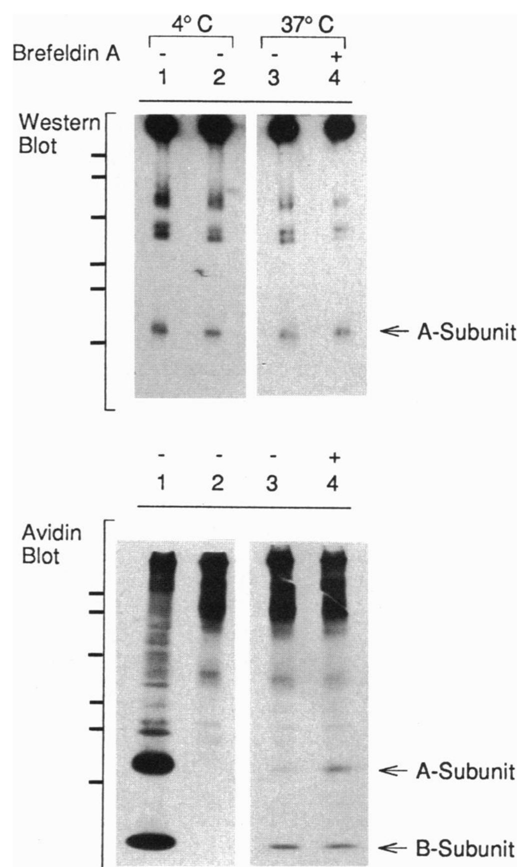

FIG. 4. Transcytosis of CT A subunit and effect of brefeldin A. (*Upper*) Western blot. Additional bands represent immunoglobulin and dissociated heavy chains. (*Lower*) Horseradish peroxidase-avidin blot. Both A and B subunits (arrows) are recognized. Bars indicate the position of molecular weight markers as described in Fig. 1. Additional bands represent immunoglobulin, bovine serum albumin, and nonspecific cross-reactive material as defined by immunoprecipitation from control monolayers.

This prediction was tested directly by examining transcytosis of the CT A subunit.

Fig. 4 shows that a fraction of CT A subunit moves across T84 cell monolayers by transcytosis. Fig. 4 *Upper* shows the Western blot to demonstrate that equal amounts of immunoprecipitated CT A subunit were present in each lane. Fig. 4 *Lower* shows the avidin blot. The antisera used for immunoprecipitation of CT A subunits cross react with CT B subunits, and both A and B subunits can be visualized on the avidin blot. Lanes 1 and 2 are positive and negative controls, respectively, as before. Lanes 3 and 4 show that after 150 min at 37°C, basolaterally applied biotin has labeled a small fraction of A subunit on the basolateral membrane. However, the fraction of A subunit delivered to the basolateral membrane was greater in monolayers treated with brefeldin A (lane 4). In contrast, equivalent amounts of B subunits were delivered to the basolateral cell surface under both experimental conditions (lanes 3 and 4, B subunit). Quantitatively less but otherwise nearly identical results were obtained in monolayers treated or not treated with brefeldin A and incubated at 37°C for 120 min. In a separate experiment, the A subunit was detected at the basolateral membrane after 90 min but only in monolayers treated with brefeldin A. The A subunit was not detected on the basolateral cell surface at earlier points in the time course of toxin action even though the B subunit was readily apparent.

CT A subunits contain ≈10-fold less reactive sites for coupling to sulfo-NHS-biotin. The assay for detection of A subunits is 30-fold less sensitive than for B subunits. Never-

theless, transcytosis of A subunits was detected in all monolayers incubated at 37°C for 150 min treated or not treated with brefeldin A ($n = 5$). These data provide direct evidence that under physiologic conditions a fraction of A subunit crosses the monolayer by transcytosis. As designed, our assay will detect only toxin bound to the exocytoplasmic cell surface. Binding is mediated exclusively by CT B subunits. Thus, the fraction of A subunit detected on the basolateral membrane likely represents transcytosis of intact and presumably functional holotoxin. Transcytosis and release of intact CT holotoxin from its high-affinity receptor into the basolateral reservoir, however, was not detected as assessed by transfer of conditioned buffer to fresh T84 cell monolayers (data not shown). These results were not surprising in that CT binds with high affinity ($<10^{-9}$ M) to its cell surface receptor G_{M1}. Release of toxin into basolateral buffer should occur only at extremely slow rates (estimated <0.09 fmol per cm^2 of apical surface area per min). *In vivo*, however, transfer of bound CT to subepithelial cells in the lamina propria may occur more readily by competitive displacement to empty receptors on adjacent cells [for example, retrograde interneuronal transfer of CT B subunit has been used to trace neural pathways in the rat (21)].

Brefeldin A increased the relative amount of A subunit delivered to the exocytoplasmic surface of the basolateral membrane in four of six experiments. The larger fraction of A subunit delivered to the exocytoplasmic surface of basolateral membranes in monolayers treated with brefeldin A (especially when viewed relative to the equal amounts of B subunits transcytosed across the same monolayer, lane 3 vs. lane 4) provides strong experimental evidence that translocation of A subunits occurs during transcytosis. These data fit well with the current view that brefeldin A blocks the movement of CT into compartments essential for translocation (10, 14).

In summary, the results of these studies show that CT moves through a transcytotic pathway in polarized human intestinal T84 cells and the B subunit and a smaller fraction of A subunit (presumably as holotoxin) are delivered to the serosal (exocytoplasmic) surface of the basolateral membrane. The time course of basolaterally directed transcytosis parallels quantitatively the time course of CT-induced Cl^- secretion, and both transcytosis and signal transduction display identical temperature sensitivities and identical lag phases. Thus, these data identify a mechanism that may explain the link between the toxin's apical receptor (G_{M1}) and basolateral effector (adenylate cyclase). These data show that movement of CT via vesicular carriers to the basolateral membrane may plausibly account for signal transduction in polarized intestinal epithelia. Further evidence in support of this hypothesis was recently obtained in separate studies (22) where we find that mutation in the C-terminal KDEL sequence of CT inhibits quantitatively both the rate of toxin induced Cl^- secretion and, in direct concordance with the proposed model, the rate of CT transcytosis.

These studies also suggest a cogent and plausible explanation for the mechanism by which CT may transduce a signal across the intestinal epithelial barrier to elicit physiologic effects on subepithelial cells within the lamina propria (2, 3). Our data indicate that both the CT B subunit and a smaller fraction of intact holotoxin may move by vesicular traffic across the epithelial cell *in vivo*. This would place both subunits in a position to interact directly with physiologically relevant cells of the lamina propria such as enteric nerves and cellular components of the mucosal immune system (4).

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1. Field, M. & Semrad, C. E. (1993) *Annu. Rev. Physiol.* **55,** 631–655.
2. Cassuto, J., Jodal, M. & Lundgren, O. (1981) *Scand. J. Gastroenterol.* **16,** 377–384.
3. Peterson, J. W. & Ochoa, L. G. (1989) *Science* **245,** 857–859.
4. Holmgren, J., Lycke, N. & Czerkinsky, C. (1993) *Vaccine* **11,** 1179–1184.
5. Lencer, W. I., Delp, C., Neutra, M. R. & Madara, J. L. (1992) *J. Cell Biol.* **117,** 1197–1209.
6. Sixma, T. K., Pronk, S. E., Kalk, H. H., Wartna, E. S., van Zanten, B. A. M., Witholt, B. & Hol, W. G. J. (1991) *Nature (London)* **351,** 371–377.
7. Moss, J. & Vaughan, M. (1979) *Annu. Rev. Biochem.* **48,** 581–600.
8. Tomasi, M. & Montecucco, C. (1981) *J. Biol. Chem.* **256,** 11177–11181.
9. Wisnieski, B. J. & Bramhall, J. S. (1981) *Nature (London)* **289,** 319–321.
10. Lencer, W. I., de Almeida, J. B., Moe, S., Stow, J. L., Ausiello, D. A. & Madara, J. L. (1993) *J. Clin. Invest.* **92,** 2941–2951.
11. Barrett, K. E. (1993) *Am. J. Physiol.* **265,** C859–C868.
12. Klausner, R. D., Donaldson, J. G. & Lippincott-Schwartz, J. (1992) *J. Cell Biol.* **116,** 1071–1080.
13. Sargiacomo, M., Lisanti, M., Graeve, L., Le Bivic, A. & Rodriguez-Boulan, E. (1989) *J. Membr. Biol.* **107,** 277–286.
14. Orlandi, P. A., Curran, P. K. & Fishman, P. H. (1993) *J. Biol. Chem.* **268,** 12010–12016.
15. Pelham, H. R. B. (1990) *Trends Biochem. Sci.* **15,** 483–486.
16. Goins, B. & Freire, E. (1988) *Biochemistry* **27,** 2046–2052.
17. Watkins, P. A., Moss, J. & Vaughan, M. (1981) *J. Biol. Chem.* **256,** 4895–4899.
18. Matter, K., Whitney, A., Yamamoto, E. M. & Mellman, I. (1993) *Cell* **74,** 1053–1064.
19. Prydz, K., Hansen, S. H., Sandvig, K. & van Deurs, B. (1992) *J. Cell Biol.* **119,** 259–272.
20. Wan, J., Taub, M. E., Shah, D. & Shen, W. C. (1992) *J. Biol. Chem.* **267,** 13446–13450.
21. Xuan-Cai, S. W., Trojanowski, J. Q. & Gonatas, J. O. (1982) *Brain Res.* **243,** 215–224.
22. Lencer, W. I., Constable, C., Moe, S., Jobling, M., Webb, H. M., Ruston, S., Madara, J. L., Hirst, T. & Holmes, R. (1995) *J. Cell Biol.*, in press.