MICROBIOLOGY REVIEWS, Dec. 1983, p. 596–620
0146-0749/83/040596-25$02.00/0

Vol. 47, No. 4

Membrane Receptors for Bacterial Toxins

LEON EIDELS,* RICHARD L. PROIA,† AND DAVID A. HART‡

Department of Microbiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

INTRODUCTION

A number of excellent reviews are available in which the structure-function relationship and the mechanism of action of an individual toxin, or pair of toxins with similar properties, are the central features. In the present article we have attempted to review the current literature on a number of bacterial toxins with emphasis on their receptors and on their toxin-receptor interactions. We have limited ourselves to those toxins that are polypeptides and have the generalized A-B structure (with the exception of *Escherichia coli* heat-stable toxin which has been included for comparative purposes). Some of these toxins are synthesized as single polypeptide chains and are proteolytically processed to the two-chain disulfide-linked A-B molecules (i.e., diphtheria toxin, *Pseudomonas* exotoxin A, tetanus toxin, and botulinum toxin). The other toxins are composed of subunits or protomers that are synthesized separately and then associate to form the A-B structure (i.e., cholera toxin, *E. coli* heat-labile toxin, pertussis toxin, and *Shigella* toxin). In addition, in the toxins that have been well characterized, the A component has an enzymatic activity that acts on a target site that is intracellular, located either in the cytosol or at the inner (cytosolic) surface of the plasma membrane. The enzymatic A components of those toxins that act in the cytosol (i.e., diphtheria, *Pseudomonas*, and *Shigella* toxins) do so by modifying a factor necessary for protein synthesis. The enzymatic components of the toxins that act at the inner surface of the plasma membrane do so by modifying plasma membrane enzymes; cholera toxin, *E. coli* heat-labile toxin, and pertussis toxin activate adenylate cyclase, and *E. coli* heat-stable toxin activates guanylate cyclase. No enzymatic activity has yet been associated with the A fragments of tetanus and botulinum toxins. The enzymatic activities described are properties of the free A chains and are demonstrable in cell-free preparations; however, these activities (in general) are cryptic in the intact toxin and are only expressed upon the appropriate activation of the toxin, which results in the release, or exposure, of the A chain or a fragment of it. This activation can be performed in vitro (usually, but not exclusively, by limited proteolysis and reduction), and it is widely assumed that the toxins are activated by similar mechanisms in vivo. In contrast, the free A chains are not toxic to animals or to cells, or they are several orders of magnitude less toxic than the intact toxin molecules. It has long been recognized that this dichotomy is due to the requirement that the A component be associated with a B component, which is necessary for the toxin to bind to cell surface receptors, thus initiating the intoxication process.

† Present address: Genetics and Biochemistry Branch, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, MD 20205.

‡ Present address: Immunological Sciences Research Group, Department of Microbiology and Infectious Diseases, University of Calgary Health Science Center, Calgary, Alberta, Canada T2N 4N1.

The lack of toxicity of the free A chains suggested to early toxin investigators that toxins do not enter cells via bulk-phase pinocytosis (though the low level of toxicity observed with free A chains is probably due to this nonspecific process), but rather that they enter by receptor-mediated pinocytosis (endocytosis). That the B component is responsible for receptor binding has been demonstrated with many, but not all, of these bacterial toxins. It has been shown that the free B components, or the nontoxic cross-reacting (CRM) proteins with enzymatically inactive A components and normal B components, can prevent the toxin-mediated process and can inhibit the binding of radioactively labeled toxin to cells by competing with the toxin for specific cell surface receptors.

These bacterial toxins, in all probability, utilize receptors and entry mechanisms for more biologically relevant macromolecules (e.g., hormones and growth factors) in an "illicit" fashion. If a particular receptor is a ubiquitous molecule, then the toxin that utilizes such a receptor may be able to affect (or at least bind to) a variety of tissues and cells from many species, including cells which the toxin is quite unlikely to encounter in nature; such is the case with cholera toxin, which binds to a membrane glycolipid, the ganglioside G_{M1}. With other toxins the species specificity observed in the whole animal is preserved in cells derived from that animal; for instance, rats and mice are quite resistant to diphtheria toxin and cells derived from these species are also resistant. This species specificity might be due to the lack of functional diphtheria toxin receptors, though binding components may be present but not be coupled to the entry process, or the entry process itself may be defective.

In the present review, the term functional or physiological receptor is used to define the binding component on the cell surface that is involved in the initial step which ultimately leads to the productive expression of the toxicity of a particular toxin. For a particular toxin-binding component to be considered the physiological receptor, it is necessary to demonstrate experimentally its involvement in the toxin-mediated process. One, or preferably both, of the following criteria should be met: (i) mutants lacking the particular toxin-binding component should be resistant to the effects of the toxin, and reconstitution of these mutants with the purified toxin-binding molecule should result in responsiveness to the toxin; and (ii) antibodies to the toxin-binding site of the particular toxin-binding component should result in inhibition of the toxin-mediated process.

In the next section we review the structure and mechanism of action of each toxin and the nature of the toxin-specific cell surface binding components that have hitherto been described. With many of the toxins these binding components represent candidates for toxin receptors since in most cases they have not been shown to be functional receptors. In the third section of the review we discuss the nature of the biochemical groups involved in the toxin-receptor interaction that leads to the entry of some selected toxins. In an upcoming review in this series, Middlebrook and Dorland (manuscript in preparation) discuss the mechanisms by which these toxins enter the cell to express their toxic action.

BACTERIAL TOXINS AND NATURE OF THEIR RECEPTORS

Cholera Toxin

Cholera toxin (choleragen) is the exotoxin produced by *Vibrio cholerae* that is responsible for the clinical manifestations of cholera. The toxin has a molecular weight of 84,000 and consists of two protomers, A ("light" subunit; molecular weight, 29,000) and B ("heavy" subunit; molecular weight, 55,000), that are associated by noncovalent bonds (94). The A protomer has been shown to be synthesized as a single polypeptide chain in a protease-deficient strain of *V. cholerae* (149); in non-protease-deficient strains it is normally isolated in the "nicked" form, though the unnicked form has been isolated by rapid purification of the toxin (47). The nicked A protomer consists of two polypeptide chains, A_1 (molecular weight, 23,000) and A_2 (molecular weight, 6,000), linked by a disulfide bond and by noncovalent interactions (149). The B protomer consists of five identical noncovalently associated polypeptide chains (B chains) (molecular weight, 11,600). Reduction of nicked toxin in the presence of 4 M urea releases the A_1 chain from the A_2-5B complex, indicating that the A protomer is attached to the B protomer via the A_2 chain (149). Higher concentrations of urea at low pH or sodium dodecyl sulfate (SDS) cause the dissociation of the unnicked toxin into A and B protomers (149). Neither of the free protomers is toxic to intact cells. The A protomer, specifically the A_1 polypeptide which is formed during activation of the toxin by mild trypsinization and reduction (130, 149), has the enzymatic activity of the toxin which ultimately results in the activation of adenyl cyclase via NAD-dependent ADP-ribosylation of the GTP-binding component of the cyclase (94, 170, 228). The B protomer has the toxin's receptor-binding domain since it, as well as choleragenoid (the naturally occurring cholera toxoid which is immunologically similar to B and is also composed of B chains [58, 97]), inhibits the binding of ^{125}I-

labeled cholera toxin to liver membranes (30), protects fat cells from the cytotoxic effect of the toxin (30), and inhibits the secretory response to cholera toxin in rabbit ileal loops (92, 182).

Although the natural targets for cholera toxin are the cells of the intestine, the toxin has been shown to activate adenylate cyclase in cells derived from a large variety of tissues (57). This observation suggested that the cholera toxin receptor might be a rather ubiquitous component of the plasma membrane of cells (28). Indeed, van Heyningen et al. (224) suggested that the receptor is a ganglioside since the effect of cholera toxin on isolated fat cells and on the intestinal loop of rabbits was blocked by incubation of the toxin with a crude preparation of gangliosides. Furthermore, extraction of glycolipids from cell membranes results in the loss of toxin binding; the binding activity can be recovered in the ganglioside fraction of the extracts (28). By using purified gangliosides, it has been demonstrated in a variety of systems that the monosialoganglioside G_{M1} (Table 1) is in fact the component that most strongly interacts with cholera toxin. G_{M1} (and to a much lesser degree, G_{M2} and G_{D1a}) inhibits the binding of ^{125}I-labeled cholera toxin to liver membranes and to fat cells (28), inhibits the toxin-induced effects on fat cells (29) and on mouse thymocytes (232), and also inhibits the toxin-induced accumulation of fluid in rabbit ligated ileal loops (92, 100, 182).

Cholera toxin and choleragenoid have been shown to bind directly to G_{M1} by a variety of methods: (i) by double diffusion in gels in which both cholera toxin and choleragenoid specifically reacted with G_{M1} (97, 100, 101); (ii) by precipitation with a G_{M1}-cerebroside complex (221); (iii) by binding to G_{M1}-coated tubes (93, 97, 98); (iv) by binding to G_{M1} covalently linked to silica beads (217); and (v) by binding to liposomes containing G_{M1} (163, 167). The binding to G_{M1} occurs between the toxin and the oligosaccharide component of the ganglioside (93, 102), and each toxin, or its B protomer, binds between five and six molecules of oligosaccharide, presumably one per B chain (62). In addition to the experimental evidence on cholera toxin binding to G_{M1} in vitro, it has been demonstrated that the toxin interacts with the G_{M1} ganglioside present on the surface of cells, since prebinding of toxin to fibroblasts (165) and to BALB/c 3T3 cells (27) prevents the oxidation of the unblocked terminal galactose residue of G_{M1} by galactose oxidase. Furthermore, there is a good correlation between the binding of, and sensitivity to, cholera toxin and the content of G_{M1} in a variety of cell types (91, 99).

Thus, there is good evidence for the specific binding of cholera toxin to G_{M1} both in vitro and in vivo; however, for G_{M1} to be considered the functional receptor for cholera toxin, it must be shown to be capable of mediating the response to the toxin. Indeed, it has been demonstrated that preincubation of a variety of toxin-sensitive cells with G_{M1}, which gets inserted into the plasma membrane, results in an enhanced capacity of these cells to bind ^{125}I-labeled cholera toxin and in an increased sensitivity to the toxin (29, 99). Furthermore, a line of transformed mouse fibroblasts (NCTC 2071) which cannot synthesize G_{M1} (63) does not respond to cholera toxin when grown on a chemically defined medium (63, 162). Integration of exogenously supplied G_{M1} into the plasma membrane of these cells results in the reconstitution of a maximal response to cholera toxin (162); other gangliosides are much less effective than G_{M1} in the reconstitution of cholera toxin sensitivity (63).

This latter reconstitution experiment with G_{M1}-deficient cells demonstrates that G_{M1} can serve as a functional receptor for cholera toxin and strongly supports the concept that G_{M1} is the toxin receptor. Nevertheless, the possibility remains that other molecules exist that can bind cholera toxin and could act as receptors. Indeed, Critchley et al. (25) labeled mouse fibroblasts

TABLE 1. Structure of gangliosides and related compounds[a]

Ganglioside	Structure
G_{A1}	Cer-Glc-Gal-GalNAc-Gal
G_{M3}	Cer-Glc-Gal | NANA
G_{M2}	Cer-Glc-Gal-GalNAc | NANA
G_{M1}	Cer-Glc-Gal-GalNAc-Gal | NANA
G_{D1a}	Cer-Glc-Gal-GalNAc-Gal | | NANA NANA
G_{D1b}	Cer-Glc-Gal-GalNAc-Gal | NANA-NANA
G_{T1a}	Cer-Glc-Gal-GalNAc-Gal | | NANA NANA-NANA
G_{T1b}	Cer-Glc-Gal-GalNAc-Gal | | NANA-NANA NANA
G_{Q1b}	Cer-Glc-Gal-GalNAc-Gal | | NANA-NANA NANA-NANA

[a] Cer, Ceramide; Glc, glucose; Gal, galactose; GalNac, *N*-acetylgalactosamine; NANA, *N*-acetylneuraminic acid (sialic acid).

(BALB/c 3T3) and a mouse lymphoid cell line (AT5), using the galactose oxidase and [^{3}H]$NaBH_4$ technique, followed by cholera toxin binding and solubilization of the toxin-receptor complexes with Nonidet P-40 and subsequent immunoprecipitation with antitoxin antibodies. The immune precipitates contained mostly labeled G_{M1} and minor amounts of a labeled glycoprotein of 80,000 to 90,000 molecular weight. These authors suggested that this glycoprotein might possess an oligosaccharide structure similar to that of G_{M1} which would permit it to interact with cholera toxin (25). Morita et al. (160), using rat small intestine epithelial cells and the same galactose oxidase-[^{3}H]$NaBH_4$ labeling method coupled with a toxin-antitoxin immunoprecipitation technique, identified cholera toxin-binding glycoproteins with molecular weights of 69,000, 90,000, 100,000, 114,000, and 132,000 (160); the same binding glycoproteins were localized after electrophoresis by incubation of SDS-polyacrylamide gels with ^{125}I-labeled cholera toxin (160). Subsequent reports by Critchley et al. (26, 27), in which they analyzed cholera toxin-binding components of rat intestinal brush-border membranes (26) and of BALB/c 3T3 cells (27), confirmed their original observations that labeling by the galactose oxidase-[^{3}H]$NaBH_4$ method, followed by solubilization and immunoprecipitation analysis, resulted mostly in the immunoprecipitation of G_{M1} and of a small amount of galactoproteins. However, they could not detect cholera toxin-binding glycoproteins either in detergent extracts of [^{35}S]methionine-labeled BALB/c 3T3 cell by immunoprecipitation (27) or when brush-border membrane proteins (26) or BALB/c 3T3 cell proteins (27) first were separated by SDS-polyacrylamide gel electrophoresis and then the gel was overlaid with ^{125}I-labeled cholera toxin (though binding to G_{M1} ganglioside was readily observed). They suggested that it is possible that these latter detection methods may be less sensitive than the method in which the cell surface is labeled with galactose oxidase, or that the galactoproteins detected may themselves not bind toxin but may be associated with G_{M1} in mixed micelles formed during solubilization and deteced by virtue of their association with G_{M1} (27). One of us has suggested (84) that these galactose-containing glycoproteins that bind cholera toxin may actually represent *E. coli* heat-labile enterotoxin receptors (see next section).

From the preceding experiments it is quite evident that G_{M1} ganglioside is the major cholera toxin-binding component of the cell surface and that, although cholera toxin-binding glycoproteins may exist, they probably represent (quantitatively) minor components.

E. coli Heat-Labile Toxin

The heat-labile enterotoxin (LT) produced by certain strains of *E. coli* is one of the toxins that has been associated with travelers diarrhea. This toxin resembles cholera toxin in a number of ways. *E. coli* LT has a molecular weight of approximately 91,000 (20) and is composed of an A protomer (molecular weight, 25,500 [32] to 29,000 [19, 20]) and a B protomer (approximate molecular weight, 59,000) (19, 73). The A protomer is synthesized as a single polypeptide chain (19) that upon limited treatment with trypsin can be converted to the nicked form, which consists of an enzymatically active A_1 polypeptide chain (molecular weight, 21,000) linked by a disulfide bond to an A_2-like chain (19, 32, 128). The B protomer consists of five noncovalently linked identical polypeptide chains (B chains; 73) (molecular weight, 11,800; 31) which remain associated in SDS at room temperature (19, 32).

Treatment of cells with *E. coli* LT, like treatment with cholera toxin, results in an increase of intracellular cyclic AMP (53, 89, 129). In broken pigeon erythrocyte preparations it has been demonstrated that the A_1 chain of *E. coli* LT catalyzes the NAD-dependent activation of adenylate cyclase (32, 74). This activation is the result of the ADP-ribosylation of the guanyl nucleotide-dependent regulatory component of the cyclase (75). The B protomer mediates the binding to cells since it is capable of blocking the fluid secretory action of the toxin on ligated rabbit intestinal loops (96), and it effectively competes for the binding of ^{125}I-labeled toxin to Y1 mouse adrenal cells (40).

Besides *E. coli* LT being similar to cholera toxin in subunit structure and in its mechanism of action, the A and B protomers share antigenic determinants with the corresponding protomers of cholera toxin (17, 18, 103, 191). The toxins also possess a large degree of sequence homology. For the B chains (31) and for the amino-terminal region of the A_1 chains (208) there is an 80% homology, whereas for the A_2 chain of cholera toxin and the corresponding region in *E. coli* LT (derived from the nucleotide sequence) the homology is less (31 [48] to 55% [208]). Furthermore, it has been reported that *E. coli* LT binds in vitro to plastic tubes coated with G_{M1} (Table 1) (92, 96, 212); this binding occurs between the B protomer and the oligosaccharide portion of G_{M1} (166). Low concentrations of G_{M1} block the toxin-induced effects of *E. coli* LT on mouse thymocytes (232) and on Y1 adrenal tumor cells (39). In contrast, Pierce (182) and Holmgren (92) reported that several orders of magnitude more G_{M1} were needed to inhibit the *E. coli* LT-mediated effects on ligated rabbit ileal loops than to inhibit the cholera toxin-

mediated effect on this same target; however, recently Holmgren et al. (96) reported that preincubation of low concentrations (1 μM) of G_{M1} with cholera toxin or with purified *E. coli* LT completely inhibited their activities in rabbit intestine.

Incorporation of exogenously supplied G_{M1} into G_{M1}-deficient rat glioma C6 cells results in a dramatic increase (~30-fold) in the binding of ^{125}I-labeled *E. coli* LT to these cells (166). Addition of G_{M1} to the cholera toxin- and *E. coli* LT-unresponsive mouse fibroblast cell line NCTC 2071, which cannot synthesize G_{M1} (63), followed by incubation with *E. coli* LT results in a significant (~3-fold) increase in the cyclic AMP content of these cells (164).

These experiments demonstrate that *E. coli* LT binds to G_{M1} and that the latter can function as a receptor for LT, as it does for cholera toxin; however, there are a number of experimental observations which suggest that cholera toxin and *E. coli* LT differ in their binding properties and that G_{M1} might not be the native or only receptor for *E. coli* LT. First, *E. coli* LT strongly adheres to galactose-containing supports (agarose) (19, 128), a property which has been exploited by Clements and Finkelstein (19) to purify *E. coli* LT to apparent homogeneity by affinity chromatography on agarose with subsequent elution with galactose. Under similar chromatographic conditions cholera toxin does not adhere to agarose columns, although it is slightly retarded (19). Second, if G_{M1} is the receptor for *E. coli* LT, the B protomer of cholera toxin (choleragenoid), which binds to G_{M1} and prevents cholera toxin action, should be able to block the action of *E. coli* LT on cells. There are conflicting reports on this matter. (i) Nalin and McLaughlin (172) reported that pretreatment with choleragenoid blocked both cholera toxin- and *E. coli* LT-mediated fluid secretion in ligated dog intestinal loops, and Donta et al. (40) reported that the binding of ^{125}I-labeled cholera toxin and of ^{125}I-labeled *E. coli* LT to Y1 adrenal cells was competitively inhibited by both toxins and by the B protomers of both toxins. (ii) In contrast, Pierce (182) and Holmgren (92) reported that preincubation of rabbit ileal loops with choleragenoid prevented the response to cholera toxin but did not prevent the response to *E. coli* LT, and Holmgren et al. (96) recently reported the reciprocal experiment, using the B protomer of *E. coli* LT, and demonstrated that this B protomer could block the fluid secretion induced by *E. coli* LT and that induced by cholera toxin. Holmgren et al. (96) also reported that all of the binding sites for cholera toxin were extractable by chloroform-methanol-water and were recovered in the monosialoganglioside fraction, whereas the binding sites for *E. coli* LT remained in the delipidated tissue residue. Since treating brush-border membranes with periodate completely inactivated the binding sites for both cholera toxin and *E. coli* LT, they suggested that the non-G_{M1} *E. coli* LT-binding components may be glycoproteins (96); however, no further characterization of this non-G_{M1} material as a glycoprotein (e.g., lectin-binding ability or molecular weight) was reported (96). Furthermore, Holmgren et al. (96) suggested that since the blocking of G_{M1} by cholera toxin B protomer did not give any detectable inhibition of the *E. coli* LT effect, these non-G_{M1} *E. coli* LT receptors either predominate in number or are more effective than G_{M1} as receptors for *E. coli* LT (96). The galactose-containing glycoproteins reported by Critchley et al. (25–27) and Morita et al. (160) (see previous section on cholera toxin), which were described as binding to cholera toxin but representing only minor cholera toxin-binding components, may conceivably be the non-G_{M1} *E. coli* LT receptors (84).

If indeed these non-G_{M1} *E. coli* LT binding sites (galactoproteins?) are the toxin receptors, then it would be necessary to explain why the G_{M1}-deficient cells, NCTC 2071 mouse fibroblasts (164) and rat glioma C6 cells (166), were found to be insensitive to *E. coli* LT action (164, 166). It is possible that these G_{M1}-deficient cells may also be deficient in these putative galactoproteins by virtue of the fact that the cells may have a pleiotropic mutation or may have more than one mutation; indeed, the NCTC 2071 cells are missing at least two glycosyl transferases, one of which is a galactosyl transferase (63) that could be involved in the synthesis of both G_{M1} and galactoproteins. In this case, the reconstitution of sensitivity to *E. coli* LT (and to cholera toxin) by the addition of exogenous G_{M1} (164, 166) would strongly suggest that G_{M1} can function as an alternate *E. coli* LT receptor. Another equally plausible explanation for the insensitivity of the G_{M1}-deficient cells to *E. coli* LT would be that the non-G_{M1} *E. coli* LT-binding components are present but their binding efficiency is strongly dependent on the presence of G_{M1}. In this case, addition of exogenous G_{M1} would reconstitute the *E. coli* LT-binding receptor system, but the G_{M1} would not function as the toxin receptor per se.

E. coli Heat-Stable Toxins

Heat-stable enterotoxins (STs) produced by some enterotoxigenic strains of *E. coli* have been shown to be associated with certain cases of travelers and childhood diarrhea (78). The *E. coli* STs bear no antigenic cross-reactivity with

E. coli LT or with cholera toxin (66, 72). ST-producing *E. coli* strains have been isolated from a variety of hosts, principally porcine and human (2, 210). Each strain may produce more than one type of ST which differ in their methanol solubility and animal species specificity (10, 11, 78). The methanol-soluble *E. coli* STs (ST_A) are active in infant mice and in neonatal piglets but not in weaned pigs, whereas the methanol-insoluble *E. coli* STs (ST_B) are active in weaned pigs but inactive in infant mice (10). Furthermore, *E. coli* ST_A and ST_B are immunologically (66, 72) and genetically (207) distinct. The *E. coli* ST_As have been the most extensively investigated; besides being methanol soluble and heat stable (100°C, 15 to 30 min) (2, 132), they are small proteins with molecular weights of approximately 2,000 (1, 131, 210) that are stable to acid treatment (2, 132, 143) and to treatment with a number of proteases, nucleases, lipases, phospholipase C, and amylase (2, 132). The ST_As from *E. coli* of porcine and human origin are immunologically cross-reactive (66, 72). They are acidic proteins which lack basic amino acids and are composed of 18 to 19 amino acid residues, 6 of which are half-cystines (1, 2, 13, 131, 210). The four amino acid residues at the amino-terminal end can be removed without loss of biological activity (13). The complete amino acid sequence of two human (1, 13) and one porcine (131) ST_A has been determined. There is a large degree of amino acid sequence homology among the human ST_As and between them and the porcine ST_A (1, 13, 131); the minor amino acid differences, which mostly occur at the amino-terminal end of the toxin, represent only conservative amino acid changes. Thus, the differences in host susceptibilities between different ST_A-producing strains may be due to these minor differences in the ST_A primary structure or, more likely, to virulence factors other than ST_A (e.g., colonization and adherence factors).

Treatment of intestinal tissues with *E. coli* ST_A results in increased fluid secretion which is preceded by an increase in cyclic GMP levels (56, 71, 79, 104, 176). This increase in cyclic GMP is believed to be due to the activation of guanylate cyclase since *E. coli* ST_A treatment of a crude membrane fraction from rabbit intestinal epithelial cells (56), and of homogenates of rat and rabbit intestinal mucosa (79), resulted in significant stimulation of this cyclase activity. In contrast to the effects of *E. coli* LT and cholera toxin on many tissues, the effect of *E. coli* ST_A appears to be quite tissue specific since the latter did not alter the activity of guanylate cyclase of rat liver, lung, heart, kidney, and cerebral cortex (79). In further contrast, although the mechanism by which *E. coli* LT and cholera toxin activates adenylate cyclase is well known, the mechanism whereby *E. coli* ST_A stimulates guanylate cyclase is not known.

Several lines of evidence suggest that the receptor for *E. coli* ST_A is distinct from the receptor for cholera toxin and *E. coli* LT: (i) neither choleragenoid (182) nor G_{M1} ganglioside (Table 1) (79, 182) has an effect on *E. coli* ST-induced intestinal fluid secretion, and (ii) neither *E. coli* LT nor mixed gangliosides alter the binding of ^{125}I-labeled *E. coli* ST_A to rat intestinal epithelial cells and to brush-border membranes (68).

Frantz and Robertson (67, 68) have recently demonstrated the specific binding of ^{125}I-labeled *E. coli* ST_A (porcine) to rat intestinal epithelial cells and to brush-border membranes. This binding was found to be saturable, time and temperature dependent, and mediated by a single class of high-affinity receptors (67, 68). Dissociation of the ^{125}I-labeled *E. coli* ST_A, however, was found to be neligible and not increased by a large excess of unlabeled toxin (67), an observation that is apparently inconsistent with the known reversibility of the toxin-induced effect on secretion obtained upon rinsing of ligated loops (78). *E. coli* ST_As purified from porcine-, human-, and bovine-derived strains inhibit the binding of ^{125}I-labeled porcine *E. coli* ST_A in a competitive manner, suggesting that they all can bind to the same rat intestinal receptor (67, 68). This latter result is not surprising in light of the almost identical amino acid sequence exhibited by these ST_As (1, 13, 131). The binding component on brush-border membranes appears to be a protein since treatment with pronase resulted in decreased binding of ^{125}I-labeled *E. coli* ST_A, whereas no decrease in binding was observed when the membranes were treated with phospholipase A_2 or C, neuraminidase, or endoglycosidase P (68).

Robertson's group has solubilized the *E. coli* ST_A-binding component from rat intestinal brush-border membranes with the detergent CHAPS and has found it to be a protein with an apparent molecular weight of 100,000 (44). The *E. coli* ST_A could not be dissociated from its binding protein by 0.1 M glycine (pH 3.5), by 4 M sodium thiocyanate, or by 4 M guanidine hydrochloride, but was dissociated by 0.5 M dithiothreitol, by 0.5 M acetic acid, or by boiling in 0.1% SDS (44). This later result, coupled with the negligible dissociation of ^{125}I-labeled *E. coli* ST_A from intestinal cells and brush-border membranes and with the lack of binding of carboxymethylated *E. coli* ST_A to the solubilized binding protein (44), suggests that the *E. coli* ST_A may be linked to this 100,000-molecular-weight binding protein via a disulfide bridge. It is not clear whether such a disulfide bond formation between the *E. coli* ST_A and this receptor candi-

date is a fortuitous reaction, or whether it is indeed the first step in a possible "cascade" of disulfide-exchange reactions that would ultimately result in the toxin-mediated activation of guanylate cyclase and the subsequent increased fluid secretion. This specific *E. coli* ST_A binding protein appears to be present only on the surface of intestinal cells and not on liver, spleen, lung, and kidney cells (44; D. C. Robertson, personal communication), an observation that correlates well with the unique tissue specificity of the toxin-mediated activation of guanylate cyclase (79).

Pertussis Toxin

Pertussis toxin (pertussigen) is a protein, produced by *Bordetella pertussis*, which is believed to be of major importance in the pathogenesis of whooping cough (230). It has recently become evident that a variety of activities described for protein factors produced by *B. pertussis* are all due to this same protein, the pertussis toxin; these activities are: histamine-sensitizing factor, lymphocytosis- or leukocytosis-promoting factor, and islet-activating protein (183, 230).

The structure of this toxin has recently been elegantly elucidated by Tamura et al. (215). The toxin, originally characterized and purified as islet-activating protein, is a hexameric protein (molecular weight, 117,000) and consists of an A protomer (S-1 subunit, molecular weight, 28,000) and a rather unusual B oligomer (215). This B oligomer is a pentamer composed of four dissimilar subunits held by noncovalent interactions: S-2 (molecular weight, 23,000), S-3 (molecular weight, 22,000), S-4 (molecular weight, 11,700), and S-5 (molecular weight, 9,300). The pentamer is formed by connecting two dimers (S-2/S-4 and S-3/S-4) by the smallest subunit (S-5; A_2-like subunit, by analogy with cholera toxin). This pentamer associates with the S-1 subunit (A_1-like subunit, by analogy with cholera toxin) to form the active toxin (215).

It has been demonstrated that pertussis toxin exerts its effects on the adenylate cyclase system in vivo (113); treatment of rats with the toxin results in enhanced insulin secretion and cyclic AMP accumulation in pancreatic islets (113). In vitro the toxin has been shown to (i) reverse the α-adrenergic inhibition of insulin release from rat pancreatic islet cells in culture (114); (ii) potentiate the β-adrenergic stimulation in isolated rat heart cells (86) and C6 glioma cells (112), resulting in increased cyclic AMP levels; and (iii) enhance the GTP-dependent activation of adenylate cyclase in membrane preparations of C6 glioma cells (115). It appears that the toxin exerts its effect on adenylate cyclase by the NAD-dependent ADP-ribosylation of a 41,000-molecular-weight membrane protein which is believed to be a regulatory (inhibitory) subunit of the cyclase (115). In contrast, cholera toxin ADP-ribosylates the 45,000-molecular-weight GTP-binding regulatory (stimulatory) subunit (G/F) of the cyclase and does not modify this 41,000-molecular-weight GTP-binding subunit (6, 115).

The free A protomer of pertussis toxin has the enzymatic activity since it is capable of ADP-ribosylating the 41,000-molecular-weight membrane protein in broken-cell preparations of C6 glioma cells, although the free A protomer has no biological activity in vivo (215). Unlike other bacterial toxins, the intact toxin was found to be active in a broken-cell preparation; it ADP-ribosylated the 41,000-molecular-weight protein (215). It is possible, however, that under the conditions investigated a free A protomer was liberated (215).

The B oligomer is assumed to be the component involved in binding to cell surface receptors (107, 215). It remains to be demonstrated that addition of the B oligomer to cells prevents the biological effect of the toxin. There is no information on the nature of the cell surface receptor for pertussis toxin; it has been suggested that it may be a sialic acid-containing molecule since the toxin and the purified B protomer bind to the sialoprotein haptoglobin (107, 215). However, if indeed pertussis toxin binds to sialic acid residues, it is possible that the toxin receptor may be a ganglioside.

Tetanus Toxin

Tetanus toxin is a potent neurotoxin produced by *Clostridium tetani*. The toxin, as synthesized by the organism (intracellular toxin), consists of a single polypeptide chain with a molecular weight of approximately 160,000 which can be nicked by an endogenous clostridial protease upon its release into the culture medium or in vitro by mild trypsinization (144–146). The nicked toxin consists of two protein components, one component with an approximate molecular weight of 55,000 (light or α-chain), and one component with an approximate molecular weight of 105,000 (heavy or β-chain), held together by at least one disulfide bond and by noncovalent bonds dissociable by SDS or urea (144–146). Neither component alone is toxic (144–146). The light chain constitutes the original amino-terminal region of the intact toxin (173). As with a number of other toxins, it is the heavy chain that possesses the toxin's determinants involved in receptor binding (88, 222). Treatment of the nicked toxin with papain (which cleaves approximately in the middle of the heavy chain) results in two fragments, fragment B (molecular weight, 95,000) and fragment

C (molecular weight, 47,000). The B fragment is composed of one-half of the heavy chain (β-2) and the entire light chain held by a disulfide bond, and the C fragment consists of the remaining (carboxy-terminal) portion of the heavy chain (β-1) (83, 87, 174). The C fragment contains the receptor-binding domain of the heavy chain since it inhibits the binding of ^{125}I-labeled tetanus toxin to neural and thyroid membranes (76, 161). Tetanus toxin has been demonstrated to bind to gangliosides, specifically to sialidase-sensitive disialosyl (G_{D1b}) and trisialosyl (G_{T1b}) gangliosides containing two sialic acid residues attached to the inner galactose (i.e., lactose) moiety (Table 1) (223). Tetanus toxin has been shown to bind to G_{D1b} and G_{T1b} by a variety of techniques: (i) adsorption of toxin by an insolubilized ganglioside-cerebroside complex (223); (ii) direct binding of ^{125}I-labeled tetanus toxin to ganglioside (G_{D1b})-containing liposomes (134); and (iii) direct binding of ^{125}I-labeled tetanus toxin to specific gangliosides adsorbed to polystyrene, with G_{T1b}, G_{Q1b} (a tetrasialosyl ganglioside), and G_{D1b} possessing the greatest affinity (95). Furthermore, the binding of ^{125}I-labeled toxin to bovine thyroid (133) and to bovine brain (192) plasma membranes is specifically inhibited by G_{D1b} and G_{T1b}. All of these observations suggest that the tetanus toxin receptor may be a ganglioside (G_{D1b} or G_{T1b}) or, at least, a ganglioside-like molecule.

Binding of ^{125}I-labeled tetanus toxin to bovine thyroid plasma membranes is blocked by unlabeled toxin and by thyrotropin (TSH) and, conversely, the binding of ^{125}I-labeled TSH is inhibited by unlabeled TSH and by tetanus toxin (133). Based on this observation and on the fact that the binding of both ^{125}I-labeled tetanus toxin and ^{125}I-labeled TSH to thyroid plasma membranes is strongly inhibited by G_{D1b} and G_{T1b} (133), it has been suggested that the tetanus toxin receptor on neural tissue resembles the TSH receptor on thyroid tissue (133). Furthermore, membranes from a rat thyroid tumor with a TSH receptor defect are deficient in binding of both TSH and tetanus toxin (82). On the other hand, a TSH receptor from bovine thyroid plasma membranes has been solubilized and shown to be a glycoprotein (216) to which ^{125}I-labeled tetanus toxin can bind (134).

The strictly reciprocal inhibition (described above) of tetanus toxin and TSH binding is a property of bovine (133) and rat (135) thyroid plasma membranes that is not completely shared with rat brain plasma membranes (135). In the latter case, ^{125}I-labeled TSH binding is inhibited by both unlabeled TSH and tetanus toxin; however, ^{125}I-labeled tetanus toxin binding is inhibited by unlabeled toxin but, surprisingly, is enhanced by TSH (135). The experiments on the relationship between the TSH receptor and the tetanus toxin receptor merit re-examination in light of the report (4a) that under physiological conditions gangliosides do not serve as TSH receptors. Nevertheless, the experimental evidence so far reported suggests that the tetanus toxin receptor may be either a ganglioside (G_{D1b} or G_{T1b}) or a glycoprotein with ganglioside-like polysaccharide structures.

Botulinum Toxin

Botulinum toxin is a potent neurotoxin produced by *Clostridium botulinum*. There are eight types of botulinum toxins (A, B, C_α, C_β, D, E, F, G) which are immunologically distinct but produce the same pharmacological effect: they block the release of acetylcholine from cholinergic nerve endings (200–204). Most strains of *C. botulinum* synthesize only one type of toxin (200, 204). All toxin types have a similar molecular weight of approximately 150,000 and are synthesized as a single polypeptide chain (unnicked) that is nicked by endogenous proteases in the proteolytic strains (e.g., types A and B) and can be nicked by exogenously added proteases in the non-proteolytic strains (e.g., type E) (200, 201, 204). Proteolytic cleavage of unnicked botulinum toxin results in activation of the toxin (i.e., increased cytotoxicity) and the formation of a two-chain molecule composed of a heavy chain (approximate molecular weight, 100,000) and a light chain (approximately molecular weight, 50,000) held by at least one disulfide bond (204, 213). Reduction of the two-chain molecule results in the formation of free chains which are individually nontoxic (200). By analogy with other bacterial toxins, it has been suggested that one of the chains is involved in binding to cell surface receptors and that the other chain is involved in producing the lytic effect of the toxin (202); it has not yet been demonstrated whether the receptor-binding domain is located in the heavy chain, as is the case with other bacterial toxins, or in the light chain. There is no known enzymatic activity associated with either one of the two chains or for that matter with the whole toxin itself (203). Treatment of botulinum toxin with trypsin or chymotrypsin resulted in the formation of two fragments, one of approximately 100,000 molecular weight and one of approximately 50,000 molecular weight. The smaller fragment corresponds to one-half of the heavy chain (H_1), whereas the larger fragment contains the remainder of the heavy chain (H_2) disulfide-linked to the light chain (33, 204). The receptor-binding capacity and the pharmacological activity of these fragments have not been tested. Treatment of botulinum toxin with Formalin (toxoid formation)

results in destruction of its receptor-binding activity (202, 203).

Gangliosides have been shown to interact with botulinum toxin. Preincubation of the toxin with gangliosides greatly decreased its in vivo toxicity (i.e., increased survival times for mice) as well as its toxicity in an in vitro system which utilizes rat phrenic nerve-diaphragm preparations (i.e., lengthened paralysis time) (205, 206). The trisialoganglioside G_{T1} was the most potent inactivator (205, 206) followed by the disialogangliosides (G_{D1a} and G_{D1b}) (Table 1) (206). Treatment of the G_{T1} ganglioside with neuraminidase resulted in the loss of the ability of the ganglioside to inhibit botulinum toxin-mediated toxicity (206). A recent quantitative study utilizing purified gangliosides has demonstrated that G_{T1b} is the ganglioside most capable of detoxifying botulinum toxin (123); G_{T1b} was followed in effectiveness by G_{Q1b} (a tetrasialoganglioside of the 1b series) and by G_{D1b} (123). ^{125}I-labeled botulinum toxin has been shown to bind to synaptosomes from monkey brain (123) and rat brain (80), but pretreatment of the synaptosomes with neuraminidase resulted in the loss of their ability to bind botulinum toxin (81). G_{T1b} was the only ganglioside that effectively inhibited the binding of ^{125}I-labeled botulinum toxin to synaptosomes (123); furthermore, this inhibition of binding was due to the direct interaction of G_{T1b} with the ^{125}I-labeled toxin (123).

These binding experiments, together with the results of the detoxification experiments, show that: (i) two sialic acid residues bound to the inner galactose of the gangliosides (as found in G_{T1b}, G_{Q1b}, and G_{D1b}) are essential for binding of botulinum toxin (123); and (ii) an additional sialic acid at the nonreducing end of the ganglioside also aids in the binding of the toxin (e.g., G_{T1b} being more effective than G_{D1b}) (123). Thus, G_{T1b}, or a minor (as yet unidentified) ganglioside, or a glycoprotein with a similar distribution of sialic acid residues should be considered as possible botulinum toxin receptor candidates.

Diphtheria Toxin

Diphtheria toxin is the exotoxin responsible for clinical diphtheria. It is produced by those *Corynebacterium diphtheriae* organisms which are lysogenic for corynephage β that carry the *tox* gene (21, 24, 180). The toxin is secreted as a single polypeptide chain (molecular weight, 62,000) with two disulfide bridges and no free sulfhydryl groups. Upon limited proteolysis with trypsin (nicking), diphtheria toxin is cleaved into an amino-terminal A fragment (molecular weight, 22,000) and a carboxy-terminal B fragment (molecular weight, 40,000) which remain associated by a disulfide bond and by noncovalent interactions (nicked toxin). Both nicked and unnicked toxin are cytotoxic (21, 180). Recent studies (155, 196) have shown that the nicked toxin is more cytotoxic. It is not yet clear whether the lower cytotoxicity of the unnicked toxin is due to a decreased ability to bind to the toxin receptor or to a slow rate-limiting nicking system of the cell; however, it is clear from the experiments of Sandvig and Olsnes (196) that the nicking of the toxin must occur before the toxin penetrates the cell membrane.

Diphtheria toxin acts by inhibiting protein synthesis (21, 180). This is due to the ADP-ribosylation of elongation factor 2 (EF-2), catalyzed by the A fragment after it reaches the cytosol of the cell, which results in an altered EF-2 that is inactive in protein synthesis (21, 180). There is a single site on EF-2 that gets ADP-ribosylated; this is an unusual amino acid, 2-[3-carboxyamido-3-(trimethylammonio)propyl]histidine, which has been named diphthamide (225). Diphthamide is the result of a posttranslational modification of a histidine residue (156, 225). The isolated A and B fragments are not cytotoxic to intact cells. In cell-free systems, in contrast to intact cells, the toxin is inactive, whereas the isolated A fragment catalyzes the inhibition of protein synthesis; this inhibition has a strict requirement for NAD as a cofactor for the ADP-ribosylation of EF-2 (21, 180). The B fragment carries the receptor-binding domain of the toxin since the isolated B fragment protects cells against the cytotoxic effect of the toxin (153, 231) and prevents the binding of ^{125}I-labeled toxin to cells (153). Furthermore, not only the isolated B fragment is protective, but also a functional B fragment linked to an inactive A fragment, as in CRM 197 protein (a ~62,000-molecular-weight, nontoxic, immunologically cross-reactive form of the toxin [219]), protects cells (54, 108) and prevents binding of ^{125}I-labeled diphtheria toxin to cells (153), presumably by binding to the same toxin receptor. It has been concluded that the receptor-binding domain of the toxin is located within the 17,000-molecular-weight carboxy-terminal portion of the B fragment. This conclusion is based on the observation that the nontoxic CRM 45 protein (45,000 molecular weight), which has an enzymatically active A fragment and a B fragment that lacks this 17,000-molecular-weight carboxy-terminal portion, fails to block the cytotoxic action of intact toxin on cells (219, 220). Furthermore, monoclonal antibodies to this 17,000-molecular-weight carboxy-terminal region block the cytotoxic action of intact toxin by preventing its binding to cells (85).

Recently, Collier and co-workers (4, 142) have discovered that a large population of the diph-

theria toxin molecules bear a tightly but noncovalently bound endogenous nucleotide, adenylyl-(3′,5′)-uridine 3′-monophosphate (ApUp). Thus far the presence of a small molecule bound to a bacterial toxin appears to be unique to diphtheria toxin. The toxin is secreted from *Corynebacterium diphtheriae* predominantly in the form bearing the nucleotide (4). The role of the endogenous nucleotide in toxin synthesis and secretion remains unclear at this time. We have demonstrated that this form of the toxin, the nucleotide-bound form (DTN_b) (188), is much less cytotoxic than the nucleotide-free form (DTN_f) of the toxin when the binding step in the cytotoxicity assay is performed at 4°C, suggesting that DTN_b binds less effectively to the toxin receptor on cells (185). However, since the two forms of the toxin are equally cytotoxic at 37°C, it appears that the nucleotide can readily dissociate or be degraded at this temperature or both, permitting the binding of the toxin to the cell surface receptor (185). Recently, Collins and Collier (personal communication) measured the rate of dissociation of ApUp and found it to have a $t_{1/2}$ of 1.6 s at 37°C and 64 min at 5.5°C. DTN_b also binds less effectively than DTN_f to a solubilized diphtheria toxin-binding cell surface glycoprotein (described below) (185). Thus, it appears that the nucleotide-binding site, or part of it, may correspond to (185, 188), or may modify (140), the receptor-binding site on the toxin.

Diphtheria toxin is highly lethal to most animal species; however, rats and mice are quite resistant (21, 180). Primary cell cultures and established cell lines derived from toxin-sensitive animals are sensitive to the cytotoxic action of the toxin, whereas cells derived from toxin-resistant animals are quite resistant (70, 150). This difference in sensitivity is not due to a difference in EF-2 susceptibility to ADP-ribosylation by the toxin (21, 180) but rather to the lack of specific receptors, or to differences in the internalization process, or both. The experimental evidence on this matter is not conclusive. Middlebrook and co-workers (153) reported that the most toxin-sensitive cell lines tested (monkey derived) demonstrated the highest specific binding of ^{125}I-labeled toxin (roughly proportional to their sensitivities), whereas cells of intermediate sensitivity (human HeLa and WI38) or resistant cells (mouse L cells) did not demonstrate specific binding. Didsbury et al. (36), on the other hand, have recently reported specific binding of ^{125}I-labeled diphtheria toxin to mouse L cells. Chang and Neville (14) reported the specific binding of ^{125}I-labeled toxin to membranes from rat liver and rat mammary glands. Keen et al. (116) reported that rhodamine-labeled diphtheria toxin bound to both toxin-sensitive WI38 human cells and toxin-resistant Swiss mouse 3T3 cells. We have reported (187) that we were unable to detect specific binding of toxin to solubilized membrane components of mouse L cells; however, we have since been able to demonstrate a number of toxin-binding glycoproteins from toxin-resistant rat NRK9 cells and from rat and mouse thymocytes (C. P. Robles and L. Eidels, unpublished data).

As a means of understanding toxin-receptor interactions, a number of compounds have been tested for their ability to protect cells against the cytotoxic action of diphtheria toxin. Such polyanionic molecules as nucleotides and polyphosphates were shown by Middlebrook and coworkers (152, 153) to inhibit toxin-mediated cytotoxicity; they demonstrated that this protection was due to the inhibition of toxin binding to cells. These polyanionic molecules exert their protective effect by binding directly to the toxin rather than to the receptor (140, 142, 186, 188). A number of polyamines, polyornithine (109, 151), ruthenium red (151), and polylysine (49), also protect cells. We have shown that polylysine exerts its effect by interfering with the toxin-receptor interaction, and we have suggested that the polylysine binds to the receptor, although this has not yet been directly demonstrated (49). In contrast, mono and diamines (e.g., ammonium chloride and chloroquine) protect cells (109, 122, 137, 151) not by inhibiting toxin binding but rather by inhibiting a subsequent step in the intoxication process (43, 122, 137). Concanavalin A and wheat germ agglutinin (WGA) have been shown to block diphtheria toxicity (42); this effect is also not due to inhibition of toxin binding, as originally suggested (42), but rather to interference with toxin internalization (154). Neither gangliosides nor galactosides (180) and other monosaccharides (42) have any effect on diphtheria intoxication. Moehring and Crispell (157) have reported that treatment of KB cells with trypsin, pronase, or phospholipase C renders them less sensitive to diphtheria toxin. Treatment of Chinese hamster ovary (CHO) cells with bromelain also results in decreased sensitivity (K. Hranitzky and L. Eidels, unpublished data). Treatment of sensitive cells with neuraminidase, from a variety of sources, resulted in a small increase in sensitivity to the cytotoxic action of the toxin (147, 197). The results with proteases suggest that the toxin receptor may have a protein component, whereas the results with neuraminidases suggest that a carbohydrate component which becomes exposed on neuraminidase treatment may be involved in toxin binding. However, these effects might be on components, other than the receptor, which are also involved in the cytotoxic process (i.e., entry). Correlative studies using

direct binding of ^{125}I-labeled toxin should help resolve this issue.

Several approaches have been used to attempt to characterize the nature of the toxin receptor. Chin and Simon (15) have extracted and partially purified a protein from rabbit liver plasma membranes by a method know to extract peripheral membrane proteins. This protein protected Vero (monkey kidney) cells from diphtheria toxicity and also prevented the binding of ^{125}I-labeled toxin to these cells. The protein was specific for diphtheria toxin since it did not protect Vero cells from the toxic effects of either abrin or *Pseudomonas aeruginosa* exotoxin A (15). Furthermore, they demonstrated that this extracted protein is an acidic protein to which ^{125}I-labeled diphtheria toxin can bind. This protein, due to the method of extraction, may be only part of a larger molecule; nevertheless, it has the appropriate characteristics of a candidate for the diphtheria toxin receptor. Alving et al. (3) reported that diphtheria toxin binds to the phosphate moiety of certain phospholipids incorporated into liposomes. This binding was inhibited by ATP and UTP, which have been shown to bind to the toxin and prevent its binding to the physiological receptor on cells (see above). They suggested that the cellular receptor may be a membrane phospholipid (such as phosphatidic acid or phosphatidylinositol phosphate); however, this phosphate-specific binding may merely be a reflection of the high affinity of diphtheria toxin for phosphate-containing compounds (140, 142, 186, 188). In contrast, Boquet and Duflot (7) reported that both DTN_f (which binds polyphosphates [142, 188]) and DTN_b (which does not bind polyphosphates [142, 189]) are able to bind to phospholipid-containing vesicles and concluded that the phosphate-binding site (P site) of the toxin does not participate in the ability of the toxin to bind liposomes. Friedman et al. have reported (69) that monoclonal antibodies which reacted with phosphate groups of phospholipids, and of other molecules, protected CHO cells from the cytotoxic action of diphtheria toxin. However, it is possible that the protective effect of these antibodies is not at the level of toxin-receptor interaction but at a step subsequent to it. Therefore, it would be of interest to determine whether these monoclonal antibodies inhibit the binding of ^{125}I-labeled toxin to cells.

In our laboratory we have identified and characterized specific diphtheria toxin-binding cell surface glycoproteins from guinea pig lymph node cells (187) and from hamster lymph node and thymus cells (184). These glycoproteins are isolated from radioiodinated cells lysed in the nonionic detergent Nonidet P-40, partially purified by lentil lectin affinity chromatography, and are detected by an immunoprecipitation system using toxin and antitoxin (184, 187). These are high-molecular-weight glycoproteins (~150,000) that show an anomalous behavior on SDS-polyacrylamide gel electrophoresis, which is probably a reflection of a high carbohydrate content (184). The specificity of these diphtheria toxin-binding glycoproteins is identical to that of the physiological receptor on cells: (i) besides binding to diphtheria toxin, they bind to the B fragment and to CRM 197 but not to the A fragment or to diphtheria toxoid (184, 185, 187); (ii) the binding of toxin and of its B fragment to the ^{125}I-labeled cell surface glycoproteins is inhibited by nucleotides and polyphosphates (185, 186), their relative effectiveness being the same as their effectiveness in protecting cells and in inhibiting the binding of ^{125}I-labeled toxin to cells (152, 153); (iii) the binding is also inhibited by polycationic molecules (185), among which polyornithine (109, 151), ruthenium red (151), and polylysine (49) have been shown to protect cells, the latter by interfering with toxin-receptor interactions (49); (iv) DTN_f binds to the solubilized binding glycoproteins and its binding is inhibited by nucleotides, whereas DTN_b does not bind to these glycoproteins as it does not bind to the functional cell surface receptor (185). In addition, these diphtheria toxin-binding cell surface glycoproteins exhibit a number of other properties expected for a diphtheria toxin receptor. (i) The binding of toxin to these glycoproteins is saturable with respect to both toxin and the glycoproteins (184, 187), and the interaction is reversible; that is, the radioiodinated glycoproteins can be displaced from a [glycoprotein-toxin] complex by an excess of partially purified nonradioactive glycoproteins (L. L. Ross and L. Eidels, unpublished data). (ii) Removal of the nonionic detergent from these glycoprotein-enriched preparations results in precipitation of the diphtheria toxin-binding cell surface glycoproteins, a result that suggests that these binding glycoproteins are integral membrane proteins (Ross and Eidels, unpublished data). (iii) Treatment of ^{125}I-labeled hamster thymocytes with bromelain releases a glycoprotein of approximately 75,000 molecular weight that binds toxin, and this binding is inhibitable by polyphosphates (J. M. Conrad and L. Eidels, unpublished data), suggesting that the polyphosphate-sensitive diphtheria toxin-binding domain of these glycoproteins is exposed to the cell surface. Utilizing the same radioiodination and immunoprecipitation system with mammalian cell lines (Vero and BHK), several toxin-binding glycoproteins have been detected, rather than a single 150,000-molecular-weight toxin-binding glycoprotein as is the case with lymphoid cells; these glycoproteins have apparent molecular weights of 185,000, 154,000, 135,000, 110,000, and 85,000

(C. P. Robles, D. A. Hart, and L. Eidels, Fed. Proc. **41**:1392, 1982). A number of these diphtheria toxin-binding glycoproteins appear to be components of fetal calf serum (used to supplement the growth medium of the cells) that are adsorbed onto the cells and become available for surface radioiodination; however, if the cells (Vero and CHO) are biosynthetically labeled with either [^{3}H]leucine or [^{35}S]methionine, only two diphtheria toxin-binding glycoproteins are detected, with apparent molecular weights of ~140,000 and ~70,000, and these glycoproteins appear to be exposed to the cell surface (C. P. Robles, D. A. Hart, and L. Eidels, Fed. Proc. **42**:1809, 1983).

Although the toxin-binding glycoproteins described above are likely candidates for the physiological diphtheria toxin receptor(s), this remains to be demonstrated. Definitive proof will require either the selection of toxin-resistant mutants that lack a particular toxin-binding glycoprotein or the preparation of monoclonal antibodies that are specific for a particular toxin-binding glycoprotein. These antibodies should at least be protective; preferably, they should be both protective and able to block the binding of ^{125}I-labeled toxin to cells. Monoclonal antibodies have been successfully utilized for the study of such other systems as epidermal growth factor-receptor interactions (199) and insulin-receptor interactions (193).

Pseudomonas Toxin

Pseudomonas exotoxin A is one of a number of extracellular products secreted by clinical isolates of *P. aeruginosa* and is believed to be an important virulence factor during infections of humans and experimental animals (23, 24). Similar to diphtheria toxin, this toxin is secreted as a single polypeptide chain (molecular weight, 66,000) containing four disulfide bridges and no free sulfhydryl groups (136). This intact form of the toxin is toxic to animals and to animal cells in culture.

Pseudomonas toxin acts very similarly to diphtheria toxin: it inhibits protein synthesis in a variety of cells (150) and in organs of sensitive species (22, 181). It was first shown by Iglewski and Kabat (105) that, like diphtheria toxin, *Pseudomonas* toxin inhibits protein synthesis by an NAD-dependent ADP-ribosylation of EF-2. The intact *Pseudomonas* toxin, like intact diphtheria toxin, is not active in the ADP-ribosylation reaction in cell-free systems; however, this ADP-ribosyltransferase activity can be elicited ("toxin activation") by either reduction in the presence of denaturing agents (136, 227) or proteolytic cleavage (16, 141, 227). This is in contrast to diphtheria toxin activation where both reduction and limited proteolysis are necessary to release the enzymatically active A fragment (21, 180). It is quite clear that the reduced and denatured 66,000-molecular-weight form of *Pseudomonas* toxin, unlike diphtheria toxin, is the ADP-ribosyltransferase and that this activity is not due to a smaller fragment generated during the in vitro enzyme assay (136, 141, 227). This reduced and denatured *Pseudomonas* toxin, however, appears to be significantly less toxic to cells and to animals than the intact toxin (141, 227). The activation by proteolytic cleavage, which results in a 26,000-molecular-weight enzymatically active fragment, can occur during storage of intact toxin (227) and during secretion of toxin into the medium (16), apparently due to the presence of *Pseudomonas* proteases; in addition, this 26,000-molecular-weight A-like fragment can be generated by treatment of the intact toxin with chymotrypsin in the presence of NAD (141). This enzymatically active A-like fragment lacks free sulfhydryl groups or disulfide bonds and is not cytotoxic (16, 141, 227), presumably due to the need for a B-like binding fragment. This B-like fragment has not yet been isolated, though a 45,000-molecular-weight fragment has been detected in toxin preparations containing the A-like fragment (227). By analogy with diphtheria toxin, it has been assumed that this A-like fragment represents the amino-terminal end of the *Pseudomonas* toxin (23, 24); however, automated amino acid sequence analysis of the reduced 66,000-molecular-weight intact toxin has revealed that three of the eight cysteine residues of the toxin are located within the first 39 amino-terminal residues (M. Vasil and G. Gray, personal communication). This result suggests that the enzymatically active fragment, which lacks cystine or cysteine residues (16, 141), is not at the amino-terminal end of the molecule and either is an internal peptide or is derived from the carboxy-terminal end.

There is good evidence that the activated *Pseudomonas* toxin and its 26,000-molecular-weight active fragment ADP-ribosylate the same site on EF-2 as the A fragment of diphtheria toxin since: (i) the reaction can be reversed by either the homologous or the heterologous toxin in the presence of excess nicotinamide (16, 106); (ii) when labeled NAD is utilized for the ADP-ribosylation of EF-2, the same tryptic peptide of EF-2 gets labeled by both toxins (105); and (iii) mutant cells resistant to diphtheria toxin due to an EF-2 that cannot be ADP-ribosylated by diphtheria toxin, presumably because they lack diphthamide (225), are also resistant to *Pseudomonas* toxin (41, 156). However, it remains to be demonstrated directly that it is the diphthamide residue in EF-2 that gets ADP-ribosylated by *Pseudomonas* toxin. In addition, the 26,000-molecular-weight fragment of *Pseudomonas*

toxin and the A fragment of diphtheria toxin have similar kinetic constants for NAD and its analogs (16, 141). In spite of these similarities in EF-2- and NAD-binding sites and the fact that the two catalyze the same intracellular reaction, the two toxins differ in the following ways. (i) They have a different amino acid composition (16, 136). (ii) They show either no immunological cross-reactivity (16, 105) or some rather restricted cross-reactivity (194). (iii) Intact diphtheria toxin (DTN_f) binds ATP (142) and has NAD-glycohydrolase activity (139), whereas intact *Pseudomonas* toxin does not bind ATP (142) and lacks this enzymatic activity (141).

Pseudomonas toxin is cytotoxic to cells in culture derived from toxin-sensitive species (150). Middlebrook and Dorland (150) investigated 21 cell lines from a variety of species for sensitivity to *Pseudomonas* toxin and to diphtheria toxin. They found a different spectrum of sensitivities for each toxin; most notably, mouse- and rat-derived cells were quite sensitive to *Pseudomonas* toxin and insensitive to diphtheria toxin. Since the intracellular mechanism by which the two toxins act is the same, these results suggest that the difference in toxicities is due to a difference in either their receptors or their entry mechanisms. Similar conclusions have been derived from the study of a class of mutants, isolated from cell lines sensitive to both toxins, which are resistant to diphtheria toxin, while having normal EF-2 (i.e., ADP-ribosylatable by both toxins), but which remain sensitive to *Pseudomonas* toxin (41, 158). Furthermore, such polyamines as ruthenium red and polyornithine, which protect cells from diphtheria toxin (151) apparently by preventing diphtheria toxin-receptor interaction (49), have no effect on *Pseudomonas* toxin-induced cytotoxicity (151). On the other hand, mono- and diamines (ammonium chloride, methylamine, chloroquine), which protect cells from diphtheria toxin by preventing toxin internalization (43, 122, 137), also protect cells against *Pseudomonas* toxin by preventing its entry (64). These results further suggest that the receptors for the two toxins are different while their internalization mechanism may be similar. That the receptor for these two toxins are indeed distinct has been demonstrated by Vasil and Iglewski (226). With chicken embryo fibroblasts that are equally sensitive to both toxins, they showed that CRM 197 protein (the nontoxic immunologically cross-reactive form of diphtheria toxin which binds to the diphtheria toxin receptor [153]) protects these cells against the cytotoxic effect of diphtheria toxin but does not protect them from the cytotoxic effect of *Pseudomonas* toxin.

Except for the fact that it is different from the diphtheria toxin receptor, there is little information on the actual nature of the receptor for *Pseudomonas* toxin. Treatment of cells with neuraminidase has been reported to increase their sensitivity to *Pseudomonas* toxin (as well as to diphtheria toxin) (147). As discussed earlier for diphtheria toxin, this increased sensitivity could be an effect on the toxin receptor or on a component other than the receptor; if the former is the case, then the result with neuraminidase suggests that the *Pseudomonas* toxin receptor may have a carbohydrate component.

Shigella Toxin

Shigella (Shiga) toxin is a protein, produced by *Shigella dysenteriae* 1 and other organisms of the genus *Shigella*, that is believed to be an important virulence factor in shigellosis (119). Purified toxin preparations show cytotoxic activity (HeLa cells in culture) and enterotoxic activity (fluid accumulation in ligated ileal loops), as well as neutrotoxic activity (lethality in mice, rabbits, and monkeys) (119, 177). Eiklid and Olsnes (52) have recently shown that all three activities are due to the same purified protein toxin, since all three activities had identical chromatographic and electrophoretic properties, and various physical and chemical treatments of the toxin resulted in the concomitant modification of all three activities. The structure of the toxin has only recently been established (179). The toxin has a molecular weight of approximately 68,000 and consists of two components: the A component with a molecular weight of 30,000 and the B component whose exact molecular weight is not known but which is composed of six to seven B chains of ~5,000 molecular weight each (179). Mild treatment of the toxin with trypsin results in nicked toxin in which the A chain is converted into two polypeptide chains, A_1 (molecular weight, 27,500) and A_2 (molecular weight, 3,000), linked by noncovalent interactions and by a disulfide bond (179). Similar to diphtheria toxin, the isolated A and B chains of *Shigella* toxin are not toxic to intact cells (179).

The first demonstrable effect in the cytotoxic process (with HeLa cells) is the inhibition of protein synthesis (8, 190). This inhibition appears to be at the level of peptide chain elongation (9, 190). In contrast to the mechanism by which the A chain of diphtheria toxin and *Pseudomonas* toxin inhibit protein synthesis, no evidence was obtained for the ADP-ribosylation of EF-2 (9) and no cofactor requirement could be found for the *Shigella* A_1-catalyzed inhibition of cell-free protein synthesis (190). The inhibition of peptide chain elongation has been shown to be due to the A_1-catalyzed inactivation of the 60S ribosomal subunit (190). However, it is not

yet known which function of the 60S ribosomal subunit is actually inhibited or the mechanism (enzymatic?) by which the A_1 chain accomplishes this inhibition (190). As with other bacterial toxins, it is presumed that the B chains are involved in binding to cell surface receptors; however, this has not been experimentally demonstrated. In fact, it has been reported that the isolated B chains (molecular weight, ~5,000) did not bind to HeLa cells (179). It is possible that the isolated B chains have undergone a conformational change which reduces their receptor-binding capacity or that the B chains need to be associated into a B oligomer (which as such has not yet been isolated) to form one receptor binding site (179).

During the *Shigella* toxin-mediated enterotoxic process (fluid accumulation in ligated rabbit ileal loops) there appears to be no increase in the intracellular cyclic AMP levels (38). It has been postulated that the enterotoxic activity (the watery phase of shigellosis), in analogy with the cytotoxic activity, could be the result of the toxin binding, entering, and inhibiting protein synthesis in intestinal epithelial cells (52, 119). Furthermore, Keusch and co-workers (37, 119) have suggested that the dysenteric phase of shigellosis (which is initiated by invasion of colonic epithelial cells by *shigellae* that then multiply intracellularly) is the result of the intracellular production of toxin by the bacterium, with the resultant inhibition of protein synthesis and eventual cellular death.

Only a limited number of cultured cell lines have been found to be sensitive to *Shigella* toxin (51, 121, 178). Keusch and Jacewicz (121) found that HeLa cells were very sensitive, whereas WI-38 and Y1 adrenal cells were resistant. These resistant cells were not able to adsorb toxin from the medium (i.e., the medium remained fully cytotoxic to HeLa cells), suggesting that these resistant cells lacked cell surface receptors for the toxin (121). Eiklid and Olsnes (51) investigated a large number of cell lines for sensitivity to *Shigella* toxin; only Vero (monkey kidney) cells and a few human carcinoma cell lines of epithelial origin were found to be sensitive to the toxin (51). They found that the HeLa S_3 subline was extremely sensitive to the toxin, whereas other HeLa cells were resistant (51, 178). These authors found that it took 10 times more resistant HeLa cells than sensitive HeLa cells to adsorb equivalent amounts of toxin (51). Furthermore, they were able to demonstrate direct binding of ^{125}I-labeled *Shigella* toxin to a number of resistant cell lines, though the sensitive cells bound somewhat more toxin than the resistant cells (10^6 versus 10^5 binding sites per cell) (51). They concluded that this difference in binding could not account for resistance and that the difference between sensitive and resistant cells resides in the ability to internalize surface-bound toxin (51).

The biochemical nature of the receptor for *Shigella* toxin has been investigated by Keusch and co-workers (118, 119, 121). They have demonstrated the specific binding of toxin to rat liver membranes and to HeLa cells, utilizing an indirect assay which measures the amount of cytotoxin remaining after adsorption (121). The binding to the rat liver membranes was sensitive to pretreatment of the membranes with proteolytic enzymes or with lysozyme, and the binding to intact HeLa cells was sensitive to lysozyme pretreatment (118, 120, 121). The binding to HeLa cells was also inhibited by the chitin-derived short-chain oligosaccharides containing β-1,4-linked *N*-acetyl-D-glucosamines (glcNAc), chitotriose and chitotetraose being the best inhibitors (118, 120, 121). This binding was not inhibited by a large number of mono-, di-, and trisaccharides or by gangliosides (121). Furthermore, the binding of toxin to rat liver membranes and to HeLa cells was inhibited by their pretreatment with the lectin WGA, which strongly binds glcNAc oligosaccharides such as chitotriose, but not by pretreatment with concanavalin A or phytohemagglutinin (118, 120, 121). Based on these results obtained with enzyme treatments, oligosaccharide inhibition, and receptor blockade with WGA, Keusch and co-workers (118–121) postulated that the receptor for *Shigella* toxin (cytotoxin) is a glycoprotein bearing an oligosaccharide with exposed (terminal) β-1,4-linked glcNAc residues as the toxin-binding component. They have also reported (117) that some of these receptors might be masked in both toxin-sensitive and toxin-resistant cells. Treatment of toxin-sensitive HeLa cells with β-galactosidase resulted in (i) enhancement of sensitivity to toxin, (ii) potentiation of the action of lysozyme on the receptor, and (iii) augmentation of the receptor blockade afforded by WGA (117). In addition, when the toxin-resistant Y1 adrenal cells were treated with β-galactosidase they became sensitive to the toxin (117). These results with β-galactosidase lend support to the notion that the toxin-binding domain is localized on the β-1,4-linked glcNAc residues of the oligosaccharide(s) of an asparagine-linked glycoprotein; these glcNAc residues may be exposed or "masked" by terminal galactose and possibly by terminal sialic acid residues. This type of oligosaccharide structure is present in a large number of glycoproteins (125), and thus far the specific glycoprotein receptor for *Shigella* toxin has not been isolated and characterized. Since this type of oligosaccharide structure is widespread, the existence of toxin-resistant cells may not necessarily be due

to the absence of this carbohydrate configuration but may relate to a defect in the internalization process, a conclusion supported by the work of Eiklid and Olsnes discussed above (51).

BIOCHEMICAL BASIS OF TOXIN-RECEPTOR INTERACTIONS

From the preceding section on the structure of bacterial toxins and the nature of their receptors, it is apparent that the receptors which have been extensively studied are carbohydrate-containing macromolecules, either glycolipids or glycoproteins. In the majority of the toxins described it is quite clear that the carbohydrate component itself is intimately involved in toxin binding (*Shigella* toxin, cholera toxin, *E. coli* LT, tetanus toxin, and botulinum toxin), whereas in a few cases the role of carbohydrate is less well defined (diphtheria toxin, *Pseudomonas* toxin, pertussis toxin, and *E. coli* ST). In this section we discuss an example of the former case, cholera toxin, and an example of the latter case, diphtheria toxin, and present the experimental evidence on the nature of the biochemical groups in the receptor-binding domain(s) of these toxins that interact with the toxin-binding domain(s) of their receptors. Information on the nature of the receptor-binding domains of the other bacterial toxins is at this point minimal or nonexistent.

Carbohydrate-Mediated Binding

Cholera toxin. It has been demonstrated that the monosialoganglioside G_{M1} is the ganglioside that most strongly interacts with cholera toxin (28, 60, 93, 100), and it is the oligosaccharide portion of this ganglioside that is responsible for the binding of cholera toxin (61, 62, 93, 102). Both the terminal galactose residue and the sialic acid residue (linked to the internal galactose) are essential for binding. This conclusion is based on the comparison of G_{M1} with other gangliosides with respect to their toxin binding capacity. Even minor changes in the carbohydrate structure of G_{M1} have a dramatic effect on toxin binding. G_{M2}, which lacks the terminal galactose of G_{M1}, and G_{A1}, which lacks the sialic acid bound to the internal galactose of G_{M1} (Table 1), have ~1,000-fold-lower binding capacity than G_{M1} (60, 93, 100). Addition of a sialic acid residue at the nonreducing terminal galactose of G_{M1}, as in G_{D1a}, also results in ~1,000-fold-less reactivity (60, 93, 100). Therefore, the structural requirements for efficient interaction of cholera toxin with the carbohydrate moieties of gangliosides are fairly stringent. This is in contrast to the finding (discussed in a previous section) that *E. coli* LT binds strongly to galactose-containing supports (19, 128).

Cholera toxin has been treated with a number of group-specific chemical modification reagents to investigate the nature of the residues involved in its toxicity. Lönnroth and Holmgren reported (98, 138) that modification of amino groups (with picrylsulfonic acid) results in loss of both binding capacity towards G_{M1} and toxicity, and they suggested that lysine residues may be involved in binding to the acidic G_{M1}. They also reported that modification of arginine groups with 1,2-cyclohexanedione (0.1 to 10 mM) results in loss of biological activity without loss of binding to G_{M1} and suggested that arginine residues are not involved in receptor binding (98, 138). In contrast, Duffy and Lai (46), also utilizing 1,2-cyclohexanedione, demonstrated that modification of two of the three arginine residues (arg 73 and arg 35) of the B chain of cholera toxin results in loss of binding to G_{M1}. However, 150 mM 1,2-cyclohexanedione was required for this modification; modification of only one arginine (arg 73) had no effect on binding (46). Thus, these results suggest that arg 35 of the B chain is near or at the binding site for G_{M1}, and possibly this arginine and the neighboring lysine (lys 34) interact with the negatively charged sialic acid residue of G_{M1}. De Wolf et al. (34) have shown that modification of the single tryptophan residue of the B chains, by treatment of the intact cholera toxin or its B protomer with 2-nitrophenylsulfenyl chloride or 2,4-dinitrophenylsulfenyl chloride, results in complete loss of binding activity towards G_{M1} ganglioside and its oligosaccharide. Furthermore, De Wolf et al. (35) have reported that when the B protomer interacts with G_{M1} this tryptophan residue is very near the oligosaccharide moiety of G_{M1} and is located in a positively charged microenvironment (near arg 35 and lys 91). Thus, arginine, lysine, and tryptophan residues are part of the receptor-binding domain of cholera toxin.

Non-Carbohydrate-Mediated or Undefined Binding

Diphtheria toxin. The experimental evidence for the involvement of carbohydrates in the binding of diphtheria toxin to its cell surface receptor is not conclusive. Most of the studies implicating carbohydrates involve treatment of cells with enzymes (147, 197), lectins (42, 154), or oligosaccharides (42), and when an effect on cytotoxicity has been observed, it has been concluded that this is an effect on the toxin receptor. Unfortunately, in most cases these observations have not been followed by direct toxin binding measurements. These studies are as follows. (i) Treatment of cells with neuraminidase resulted in a small increase in sensitivity to the cytotoxic action of the toxin (147, 197). This

increased sensitivity could be due to the exposure of carbohydrate binding components normally covered by sialic acid (147); however, it could be an effect on a component (other than the receptor) involved in entry, or it could merely be a reflection of the removal of negatively charged groups that contribute to the nonspecific (i.e., nonproductive) binding of toxin to cells, resulting in a greater effective toxin concentration. (ii) Treatment of cells with concanavalin A or WGA also blocked cytotoxicity (42), and in this case it has been demonstrated that these lectins do not interfere with the initial toxin-receptor interaction since they have no effect on the binding of ^{125}I-labeled toxin to cells (154). (iii) An ovalbumin glycopeptide and a polysaccharide from *Salmonella cholerae-suis* have been reported to have a small protective effect, whereas monosaccharides had no effect (42).

Diphtheria toxin-binding cell surface glycoproteins have been identified and characterized in our laboratory (184, 187), and we have suggested that these glycoproteins (~150,000 molecular weight) might be the receptors for the toxin. To test whether the carbohydrate moieties of cell surface glycoproteins are involved in diphtheria toxin binding, we have recently tested mutant CHO cell lines, possessing alterations in the polysaccharide structure of their asparagine-linked glycoproteins, for their susceptibility to diphtheria toxin (D. L. Durham, D. A. Hart, and L., Eidels, manuscript in preparation). WG^r, a mutant resistant to a number of lectins due to the loss of a specific *N*-acetylglucosaminyl transferase activity (209) which results in the lack of synthesis of complex-type oligosaccharides, was as sensitive to diphtheria toxin as the wild-type and parental cells. This result suggests that such carbohydrate residues as sialic acid, galactose, glcNAc, and fucose, which are unique to the complex-type oligosaccharides, are not involved in toxin binding or in internalization of the toxin. Two other mutants that are defective in the synthesis of high-mannose oligosaccharides (B4-2-1 which is unable to synthesize mannosylphosphoryldolichol [211] and B211 which is unable to transfer glucose from glucosylphosphoryldolichol to the lipid-bound growing oligosaccharide chain [126]) but that do synthesize complex-type oligosaccharides (127, 211) were also found to be as sensitive to toxin as the corresponding parental and wild-type cells. This latter result suggests that the high-mannose oligosaccharides are also not involved in diphtheria intoxication. Taken together, the results with these mutants suggest that if the diphtheria toxin receptor is a glycoprotein with asparagine-linked oligosaccharides, then the toxin either binds to carbohydrate components that are common (internal residues) to the complex and to the high-mannose-type glycoproteins, or it binds to the peptide backbone of the receptor. Since there is indirect evidence that the toxin-binding domain on the receptor may be an anionic region (185, 186) (see below) and since the internal carbohydrate residues of glycoproteins are neutral sugars (125), this anionic toxin-binding region is probably located on the peptide backbone.

A number of chemical modification reagents have been used to investigate the role of particular amino acid residues of diphtheria toxin in the cytotoxic process. Modification of histidines, lysines, and tyrosines resulted in loss of toxicity and loss of enzymatic activity; however, the effect on the B fragment or on binding of toxin was not tested (54). S-sulfonated B fragment was tested and no longer inhibited the action of intact toxin on cells, presumably due to the introduction of the negatively charged sulfonyl groups (54). In contrast, modification of tyrosine groups with tetranitromethane resulted in complete loss of toxicity and enzymatic activity but no loss in the capacity to bind to toxin receptors (231), suggesting that tyrosine groups are not involved in receptor binding. Treatment of nucleotide-free diphtheria toxin with diketene, which modifies lysine residues, or with 1,2-cyclohexanedione, which modifies arginine residues, resulted in inhibition of binding to ATP-Sepharose (unpublished data), suggesting that these basic amino acid residues are involved in binding to polyphosphates and possibly to an anionic region of the receptor (185, 186, 188).

Lory and Collier (142) discovered that diphtheria toxin interacts with nucleotides and that the binding of ATP to the nucleotide-binding site on the toxin is strongly dependent on the phosphate content of the nucleotide, although the nucleoside moiety participates to some extent in the interaction. This nucleotide-binding site is composed of two subsites: a nucleoside-binding subsite located on the A fragment and a polyphosphate-binding subsite, P site, located on the B fragment (12, 140, 188). We have demonstrated (188) that the P site is located within the cationic carboxy-terminal region (8,000-molecular-weight peptide) of the B fragment; this conclusion was derived from affinity labeling experiments with ADP-ribose and [^{3}H]$NaBH_4$, in which the affinity label was localized on a lysine close to a cluster of arginine residues at the amino-terminal end of this peptide (55, 188). In addition, the peptide also contains a number of lysine residues at its carboxy-terminal end (55) which may further contribute to the binding of polyphosphates.

Lory and Collier (142) and our laboratory (185, 186, 188) suggested that the cationic P site

may correspond to, or be part of, the receptor-binding site on the toxin. This suggestion was based on two main facts. First, that binding of polyphosphates to the P site results in (i) protection of cells from diphtheria toxin action (152, 153) and (ii) inhibition of ^{125}I-labeled toxin binding to cells (152, 153) and inhibition of toxin binding to a solubilized diphtheria toxin-binding glycoprotein (185, 186). Second, CRM 45, which lacks the 17,000-molecular-weight carboxy-terminal receptor binding region of the toxin, does not bind polyphosphates (142). However, the fact that CRM 197 (which protects cells [54, 108] and inhibits binding of ^{125}I-labeled toxin to cells [153] by binding to the toxin receptor) does not bind ATP (140) has prompted Collier and coworkers to suggest that the P site does not correspond to the receptor-binding site and that the binding of polyphosphates at the P site alters the receptor-binding site of the toxin in an allosteric fashion (140).

We have recently reported (50) that when the solubilized 150,000-molecular-weight diphtheria toxin-binding cell surface glycoprotein, whose binding to toxin is inhibited by inositol hexaphosphate (a P-site ligand), is treated with papain, 88,000- and 74,000-molecular-weight diphtheria toxin-binding glycoproteins are generated, glycoproteins whose binding to toxin is no longer inhibited by inositol hexaphosphate. This result suggests that the toxin receptor has one binding site (P′ site) which interacts with the P site of the toxin in a polyphosphate-sensitive manner, and a second binding site (X′ site) which can interact with the toxin at a site distinct from the P site (the X site) in a polyphosphate-insensitive manner. The X site is probably located within the 17,000-molecular-weight carboxy-terminal region of the B fragment (50). According to this model, with intact toxin the P-P′ interaction prevails (since polyphosphates almost completely inhibit toxin-receptor interactions), whereas with CRM 197 it is possible that the P site is not accessible and the X-X′ interaction prevails (50). This would be consistent with CRM 197 not binding ATP and yet binding to the toxin receptor, at the X′ site, and sterically preventing toxin binding to the same receptor. Alternatively, this X-X′ interaction may not be involved in the initial (polyphosphate-sensitive) toxin-receptor interaction but may be part of a subsequent step in the translocation process of the toxin (50). A similar conclusion regarding a second function for the toxin receptor, or a second site, was derived by Didsbury et al. (36), based on a distinct class of diphtheria toxin-resistant CHO mutants (Dip^r). These mutants have normal toxin binding (as tested with ^{125}I-labeled toxin) but are 10,000-fold more resistant to toxin and are not protected by CRM 197. These authors suggested that this class of mutants has a block at a step in the intoxication process that is between binding of toxin and the pH-dependent entry into the cytosol from an intracellular vesicle (36). Interestingly, they also reported that the naturally toxin-resistant mouse L cells have the same phenotype as the Dip^r mutants, suggesting that their resistance may also be due to the lack of such a second component (X′ site?).

CONCLUDING REMARKS

There are a number of similarities, as well as differences, in the structures and mechanisms of action of the A-B structured polypeptide toxins reviewed herein (Table 2). Of the four toxins synthesized as a single polypeptide chain, diphtheria toxin and *Pseudomonas* toxin inhibit protein synthesis by inactivation of EF-2, whereas the mechanism by which tetanus toxin and botulinum toxin act has not yet been defined. Of the four toxins synthesized as separate A and B components, *Shigella* toxin inhibits protein synthesis by inactivation of the 60S ribosomal subunit, whereas cholera toxin, *E. coli* LT, and pertussis toxin activate adenylate cyclase. In all cases in which the enzymatic mechanism is known, the toxins act by ADP-ribosylation of a macromolecular target: diphtheria toxin and *Pseudomonas* toxin ADP-ribosylate EF-2, whereas cholera toxin and *E. coli* LT ADP-ribosylate a GTP-binding regulatory (stimulatory, G_s) subunit of adenylate cyclase and pertussis toxin ADP-ribosylates another GTP-binding regulatory (inhibitory, G_i) subunit of the same cyclase. Diphtheria toxin ADP-ribosylates diphthamide, a posttranslationally modified histidine residue; it is presumed, but remains to be directly demonstrated, that *Pseudomonas* toxin modifies the same residue. The amino acid residue(s) ADP-ribosylated by cholera toxin and *E. coli* LT has not been determined, though it has been assumed to be a basic residue since both toxins can ADP-ribosylate free arginine and iodinated *N*-guanyltyramine (148, 168, 169). The chemical relationship, if any, between diphthamide and the ADP-ribosyl acceptor residue(s) on the regulatory components of the cyclase (and the amino acid sequence surrounding these acceptor sites) should be investigated.

Cholera toxin and *E. coli* LT are similar in size and subunit composition, are immunologically cross-reactive, and exhibit a large degree of sequence homology. It will be of interest to determine the amino acid sequence homology of the NAD-binding sites and the cyclase regulatory subunit-binding sites on the A_1 chains of these two toxins. Furthermore, though both cholera toxin and *E. coli* LT bind to G_{M1}, from

TABLE 2. Properties of bacterial toxins and their reported binding components[a]

Toxin	Mol wt						Intracellular target	Reported toxin-binding component
	Toxin		A		B			
Cholera	84,000		29,000		55,000		GTP-binding subunit of adenylate cyclase	G_{M1}
		A_1	23,000		$(11,600)_5$			
		A_2	6,000					
E. coli LT	91,000		25,500–29,000		59,000		GTP-binding subunit of adenylate cyclase	G_{M1}, galactoproteins
		A_1	21,000		$(11,800)_5$			
		A_2	—[b]					
E. coli ST	~2,000						Guanylate cyclase	100,000-mol-wt protein
Pertussis	117,000	S_1	28,000	S_2	23,000		GTP-binding subunit of adenylate cyclase	Sialic acid-containing molecule (?)[c]
				S_3	22,000			
				S_4	11,700			
				S_5	9,300			
Tetanus	160,000		55,000		105,000		—	G_{D1b}, G_{T1b}
Botulinum	150,000		50,000		100,000		—	G_{T1b}
Diphtheria	62,000		22,000		40,000		EF-2 (diphthamide)	150,000-mol-wt glycoprotein
Pseudomonas	66,000		26,000		45,000[d]		EF-2	—
Shigella	68,000		30,000		—		60S ribosomal subunit	Glycoprotein with terminal β-1,4-linked glcNAc residues
		A_1	27,500		$(5,000)_{6-7}$			
		A_2	3,000					

[a] Details and references are given in the text.
[b] —, No available information.
[c] ?, Suggested but not directly demonstrated.
[d] Detected on SDS-polyacrylamide gel electrophoresis but not yet isolated.

the experiments discussed in the appropriate sections above it appears that there are distinct differences in their binding specificities. Most notable is the much higher binding affinity of *E. coli* LT than that of cholera toxin for galactose-containing supports. This observation suggests that the receptors for these two toxins may be different and is consistent with the proposal that a galactoprotein may be the *E. coli* LT receptor (96). It will thus be of interest to determine the structure of the receptor-binding domain(s) on the B chains for these two toxins. In contrast, despite the fact that diphtheria toxin and *Pseudomonas* toxin catalyze the identical reaction, they show almost no immunological cross-reactivity, they have quite different amino acid compositions, and their receptors are distinct. Nevertheless, it would be expected that their respective NAD- and EF-2-binding sites would share some similarities. Possibly, the NAD-binding sites and the enzymatic sites on the A chains of all five of these ADP-ribosylating toxins might share some common sequences.

There are a number of interesting similarities and differences between these bacterial toxins and polypeptide hormones, e.g., luteinizing hormone, chorionic gonadotropin, and thyroid-stimulating hormone. These polypeptide hormones also have an A-B structure (α and β subunits). Their α subunits mediate the biological activity by increasing cellular cyclic AMP levels. Unlike the A chains of those toxins (described above) which have a similar enzymatic activity but a different amino acid sequence, the α chains of the polypeptide hormones have an identical amino acid sequence (5). The β subunits of these hormones, like the B chains of the toxins, recognize specific cell surface receptors on their respective target cells (214). Interestingly, the receptor for thyroid-stimulating hormone has been reported to be the oligosaccharide moiety of the ganglioside G_{D1b} (171) or that of a high-molecular-weight membrane glycoprotein (216), or a complex of both the ganglioside and the glycoprotein (124). The receptor for human chorionic gonadotropin-luteinizing hormone has been reported to be the ganglioside G_{T1} (124) or a ~190,000-molecular-weight glycoprotein (45). From these latter reports, in which both specific ganglioside and glycoprotein binding components have been characterized, it is not clear which component represents the physiological hormone receptor. It is possible that only one of these carbohydrate-containing molecules is the physiological receptor and that the binding observed with the other is due to similar oligosaccharide structures being present on both molecules. Indeed, it has been shown that there are structural similarities in the carbohydrate sequences of glycoproteins and glycolipids (59,

189, 218). These structural similarities may also explain the fact that both glycoproteins and glycolipids have been proposed as candidates for toxin receptors.

As discussed in the section on tetanus toxin, there appears to be a similarity between the thyroid-stimulating hormone receptor and the tetanus toxin receptor. It is likely that the other bacterial toxins also utilize receptors intended for other macromolecules (e.g., hormones and growth factors).

Bacterial toxins enter cells by receptor-mediated processes. Diphtheria toxin (116) and *Pseudomonas* toxin (64, 65) have been reported to be internalized by receptor-mediated endocytosis through coated pits, specialized areas of the plasma membrane that invaginate to form intracellular coated vesicles (77); it has been suggested that *Shigella* toxin is also internalized via this pathway (118). A variety of ligands (proteins and peptides) are internalized by receptor-mediated endocytosis (77), and the receptors that have so far been characterized for these ligands have been shown to be high-molecular-weight cell surface glycoproteins. These ligands are low-density lipoprotein (198), transferrin (229), insulin (110), epidermal growth factor (90), asialoglycoproteins (175), lysosomal enzymes (195), and chorionic gonadotropin (45). Interestingly, glycoproteins have been implicated as receptors for diphtheria toxin (184–187) and for *Shigella* toxin (121). In contrast, it has been reported that with cholera toxin and tetanus toxin, whose receptors are believed to be membrane glycolipids, the initial binding and subsequent internalization occurs via noncoated surface microinvaginations (111, 159). It thus appears that the pathway of endocytosis of a ligand may depend on the chemical nature of its receptor.

Teleologically, a particular toxin will usurp a receptor for a ligand that normally is internalized to the cellular compartment containing the target of the toxin. That is, it is possible that toxins that act in the cytosol will utilize a glycoprotein receptor, whereas those toxins that act at the cytosolic face of the plasma membrane will utilize a glycolipid receptor. This hypothesis will be proved, or disproved, when it is demonstrated which of the presently known (or others yet to be described) toxin-binding glycoproteins and glycolipids corresponds to the actual physiological receptors.

One or more of several approaches will be necessary to demonstrate which binding candidate is the physiological receptor. Toxin-resistant mutants lacking the particular binding component should be isolated, and appropriate antibodies to the receptor should be prepared; antibodies to the toxin-binding site should prevent toxin binding and be protective, whereas those against other sites may or may not be protective. The purified receptor should be used to reconstitute responsiveness in receptor-deficient mutants, as has been done with the cholera toxin response by G_{M1} addition to G_{M1}-deficient cells (162). Ultimately, one would like to reconstitute completely an in vitro receptor system (in a monolayer or bilayer) in which the biological effect can be reproduced, i.e., binding of toxin, translocation, and activation of adenylate cyclase or inhibtion of protein synthesis, or, more modestly, ADP-ribosylation of the appropriate macromolecular target.

ACKNOWLEDGMENTS

We thank Robert S. Munford for critical review of the manuscript, Eleanor R. Eidels for editorial assistance, and Annetta Weatherby and Daisi Tucker for secretarial help. We thank those investigators who sent reprints and preprints and those who did not; the former made our endeavor more complete, the latter made it easier.

The National Institutes of Health provided support for our research under Public Health Service grant AI-16805.

LITERATURE CITED

1. **Aimoto, S., T. Takao, Y. Shimonishi, S. Hara, T. Takeda, Y. Takeda, and T. Miwatani.** 1982. Amino-acid sequence of heat-stable enterotoxin produced by human enterotoxigenic *Escherichia coli*. Eur. J. Biochem. **29**:257–263.
2. **Alderete, J. F., and D. C. Robertson.** 1978. Purification and chemical characterization of the heat-stable enterotoxin produced by porcine strains of enterotoxigenic *Escherichia coli*. Infect. Immun. **19**:1021–1030.
3. **Alving, C. R., B. H. Iglewski, K. A. Urban, J. Moss, R. L. Richards, and J. C. Sadoff.** 1980. Binding of diphtheria toxin to phospholipids in liposomes. Proc. Natl. Acad. Sci. U.S.A. **77**:1986–1990.
4. **Barbieri, J. T., S. F. Carroll, R. J. Collier, and J. A. McCloskey.** 1981. An endogenous dinucleotide bound to diphtheria toxin. Adenylyl-(3',5')-uridine 3'-monophosphate. J. Biol. Chem. **256**:12247–12251.

4a. **Beckner, S. K., R. O. Brady, and P. A. Fishman.** 1981. Reevaluation of the role of gangliosides in the binding and action of thyrotropin. Proc. Natl. Acad. Sci. U.S.A. **78**:4848–4852.

5. **Bellsario, R., R. B. Carlsen, and O. P. Bahl.** 1973. Human gonadotropin: amino acid sequence of α-subunit. J. Biol. Chem. **248**:6796–6809.
6. **Bokoch, G. M., T. Katada, J. K. Northup, E. L. Hewlett, and A. G. Gilman.** 1983. Identification of the predominant substrate for ADP-ribosylation of islet activating protein. J. Biol. Chem. **258**:2072–2075.
7. **Boquet, P., and E. Duflot.** 1981. Studies on the role of a nucleoside-phosphate-binding site of diphtheria toxin in the binding of toxin to Vero cells or liposomes. Eur. J. Biochem. **121**:93–98.
8. **Brown, J. E., S. W. Rothman, and B. P. Doctor.** 1980. Inhibition of protein synthesis in intact HeLa cells by *Shigella dysenteriae* 1 toxin. Infect. Immun. **29**:98–107.
9. **Brown, J. E., M. A. Ussery, S. H. Leppla, and S. W. Rothman.** 1980. Inhibition of protein synthesis by Shiga toxin. Activation of the toxin and inhibition of peptide elongation. FEBS Lett. **117**:84–88.
10. **Burgess, M. N., R. J. Bywater, C. M. Cowley, N. A. Mullan, and P. M. Newsome.** 1978. Biological evaluation of a methanol-soluble, heat-stable *Escherichia coli* enterotoxin in infant mice, pigs, rabbits, and calves. Infect. Immun. **21**:526–531.
11. **Burgess, M. N., N. A. Mullan, and P. M. Newsome.** 1980. Heat-stable enterotoxins from *Escherichia coli* P16. In-

fect. Immun. **28**:1038–1040.
12. **Carroll, S. F., S. Lory, and R. J. Collier.** 1980. Ligand interactions of diphtheria toxin. III. Direct photochemical cross-linking of ATP and NAD to toxin. J. Biol. Chem. **255**:12020–12024.
13. **Chan, S.-K., and R. A. Giannella.** 1981. Amino acid sequence of heat-stable enterotoxin produced by *Escherichia coli* pathogenic for man. J. Biol. Chem. **256**:7744–7746.
14. **Chang, T., and D. M. Neville, Jr.** 1978. Demonstration of diphtheria toxin receptors on surface membranes from both toxin-sensitive and toxin-resistant species. J. Biol. Chem. **253**:6866–6871.
15. **Chin, D., and M. I. Simon.** 1981. Diphtheria toxin interaction with an inhibitory activity from plasma membranes, p. 53–64. *In* J. L. Middlebrook and L. D. Kohn (ed.), Receptor-mediated binding and internalization of toxins and hormones. Academic Press, Inc., New York.
16. **Chung, D. W., and R. J. Collier.** 1977. Enzymatically active peptide from the adenosine diphosphate-ribosylating toxin of *Pseudomonas aeruginosa*. Infect. Immun. **16**:832–841.
17. **Clements, J. D., and R. A. Finkelstein.** 1978. Immunological cross-reactivity between a heat-labile enterotoxin(s) of *Escherichia coli* and subunits of *Vibrio cholerae* enterotoxin. Infect. Immun. **21**:1036–1039.
18. **Clements, J. D., and R. A. Finkelstein.** 1978. Demonstration of shared and unique immunological determinants in enterotoxins from *Vibrio cholerae* and *Escherichia coli*. Infect. Immun. **22**:709–713.
19. **Clements, J. D., and R. A. Finkelstein.** 1979. Isolation and characterization of homogeneous heat-labile enterotoxins with high specific activity from *Escherichia coli* cultures. Infect. Immun. **24**:760–769.
20. **Clements, J. D., R. J. Yancey, and R. A. Finkelstein.** 1980. Properties of homogeneous heat-labile enterotoxin from *Escherichia coli*. Infect. Immun. **29**:91–97.
21. **Collier, R. J.** 1975. Diphtheria toxin: mode of action and structure. Bacteriol. Rev. **39**:54–85.
22. **Collier, R. J.** 1977. Inhibition of protein synthesis by exotoxins from *Corynebacterium diphtheriae* and *Pseudomonas aeruginosa*, p. 69–98. *In* P. Cautrecasas (ed.), The specificity and action of animal, bacterial and plant toxins. Chapman and Hall, London.
23. **Collier, R. J., D. G. Gilliland, and S. Lory.** 1979. Structure-activity relationships in diphtheria toxin and exotoxin A from *Pseudomonas aeruginosa*, p. 751–759. *In* M. Bitensky, R. J. Collier, D. F. Steiner, and C. F. Fox (ed.), Transmembrane signaling, progress in clinical and biological research, vol. 31. Alan R. Liss, Inc., New York.
24. **Collier, R. J., and J. J. Mekalanos.** 1980. ADP-ribosylating exotoxins, p. 261–291. *In* H. Bisswanger and E. Schmincke-Ott (ed.), Multifunctional proteins. John Wiley & Sons, Inc., New York.
25. **Critchley, D. R., S. Ansell, R. Perkins, S. Dilks, and J. Ingram.** 1979. Isolation of cholera toxin receptors from a mouse fibroblast and lymphoid cell line by immune precipitation. J. Supramol. Struct. **12**:273–291.
26. **Critchley, D. R., J. L. Magnani, and P. H. Fishman.** 1981. Interaction of cholera toxin with rat intestinal brush border membranes. Relative roles of gangliosides and galactoproteins as toxin receptors. J. Biol. Chem. **256**:8724–8731.
27. **Critchley, D. R., C. H. Streuli, S. Kellie, S. Ansell, and B. Patel.** 1982. Characterization of the cholera toxin receptor on BALB/c 3T3 cells as a ganglioside similar to, or identical with, ganglioside G_{M1}. Biochem. J. **204**:209–219.
28. **Cuatrecasas, P.** 1973. Interaction of *Vibrio cholerae* enterotoxin with cell membranes. Biochemistry **12**:3547–3558.
29. **Cuatrecasas, P.** 1973. Gangliosides and membrane receptors for cholera toxin. Biochemistry **12**:3558–3566.
30. **Cuatrecasas, P.** 1973. *Vibrio cholerae* choleragenoid. Mechanism of inhibition of cholera toxin action. Biochemistry **12**:3577–3581.
31. **Dallas, W. S., and S. Falkow.** 1980. Amino acid sequence homology between cholera toxin and *Escherichia coli* heat-labile toxin. Nature (London) **288**:499–501.
32. **Dallas, W. S., D. M. Gill, and S. Falkow.** 1979. Cistrons encoding *Escherichia coli* heat-labile toxin. J. Bacteriol. **139**:850–858.
33. **Dasgupta, B. R.** 1979. Botulinum neurotoxin: studies on the structure and structure-biological activity relation. Toxicon **17**(Suppl.):33.
34. **De Wolf, M. J. S., M. Fridkin, M. Epstein, and L. D. Kohn.** 1981. Structure-function studies of cholera toxin and its A and B protomers. Modification of tryptophan residues. J. Biol. Chem. **256**:5481–5488.
35. **De Wolf, M. J. S., M. Fridkin, and L. D. Kohn.** 1981. Tryptophan residues of cholera toxin and its A and B protomers. Intrinsic fluorescence and solute quenching upon interacting with the ganglioside G_{M1}, or dansylated oligo-G_{M1}. J. Biol. Chem. **256**:5489–5496.
36. **Didsbury, J. R., J. M. Moehring, and T. J. Moehring.** 1983. Binding and uptake of diphtheria toxin by toxin-resistant Chinese hamster ovary and mouse cells. Mol. Cell. Biol. **3**:1283–1294.
37. **Donohue-Rolfe, A., and G. T. Keusch.** 1983. *Shigella dysenteriae* 1 cytotoxin: periplasmic protein releasable by polymyxin B and osmotic shock. Infect. Immun. **39**:270–274.
38. **Donowitz, M., G. T. Keusch, and H. J. Binder.** 1975. Effect of *Shigella* enterotoxin on electrolyte transport in rabbit ileum. Gastroenterology **69**:1230–1237.
39. **Donta, S. T.** 1976. Interactions of choleragenoid and G_{M1} ganglioside with enterotoxins of *Vibrio cholerae* and *Escherichia coli* in cultured adrenal cells. J. Infect. Dis. **133**(Suppl.):S115–S119.
40. **Donta, S. T., N. J. Poindexter, and B. H. Ginsberg.** 1982. Comparison of the binding of cholera and *Escherichia coli* enterotoxins to Y1 adrenal cells. Biochemistry **21**:660–664.
41. **Draper, R. K., D. Chin, D. Eurey-Owens, I. E. Scheffler, and M. I. Simon.** 1979. Biochemical and genetic characterization of three hamster cell mutants resistant to diphtheria toxin. J. Cell Biol. **83**:116–125.
42. **Draper, R. K., D. Chin, and M. I. Simon.** 1978. Diphtheria toxin has the properties of a lectin. Proc. Natl. Acad. Sci. U.S.A. **75**:261–265.
43. **Draper, R. K., and M. I. Simon.** 1980. The entry of diphtheria toxin into the mammalian cell cytoplasm: evidence of lysosomal involvement. J. Cell Biol. **87**:849–854.
44. **Dreyfus, L. A., and D. C. Robertson.** 1982. Detergent solubilization and partial characterization of the *E. coli* enterotoxin (STa) receptor. Abstr. Annu. Meet. Am. Soc. Microbiol., B138, p. 41.
45. **Dufau, M. L., E. H. Charreau, and K. J. Catt.** 1973. Characteristics of soluble gonadotropin receptor from the rat testis. J. Biol. Chem. **248**:6973–6982.
46. **Duffy, L. K., and C.-Y. Lai.** 1979. Involvement of arginine residues in the binding site of cholera toxin subunit B. Biochem. Biophys. Res. Commun. **91**:1005–1010.
47. **Duffy, L. K., J. W. Peterson, and A. Kurosky.** 1981. Isolation and characterization of a precursor form of the "A" subunit of cholera toxin. FEBS Lett. **126**:187–190.
48. **Duffy, L. K., J. W. Peterson, and A. Kurosky.** 1981. Covalent structure of the γ chain of the A subunit of cholera toxin. J. Biol. Chem. **256**:12252–12256.
49. **Eidels, L., and D. A. Hart.** 1982. Effects of polymers of L-lysine on the cytotoxic action of diphtheria toxin. Infect. Immun. **37**:1054–1058.
50. **Eidels, L., L. L. Ross, and D. A. Hart.** 1982. Diphtheria toxin-receptor interaction: a polyphosphate-insensitive diphtheria toxin-binding domain. Biochem. Biophys. Res. Commun. **109**:493–499.
51. **Eiklid, K., and S. Olsnes.** 1980. Interaction of *Shigella*

shigae cytotoxin with receptors on sensitive and insensitive cells. J. Recept. Res. **1**:199–213.
52. **Eiklid, K., and S. Olsnes.** 1983. Animal toxicity of *Shigella dysenteriae* cytotoxin: evidence that the neurotoxic, enterotoxic, and cytotoxic activities are due to one toxin. J. Immunol. **130**:380–384.
53. **Evans, D. J., Jr., L. C. Chen, G. T. Curlin, and D. G. Evans.** 1972. Stimulation of adenyl cyclase by *Escherichia coli* enterotoxin. Nature (London) New Biol. **236**:137–138.
54. **Everse, J., D. A. Lappi, J. M. Beglau, C.-L. Lee, and N. O. Kaplan.** 1977. Investigations into the relationship between structure and function of diphtheria toxin. Proc. Natl. Acad. Sci. U.S.A. **74**:472–476.
55. **Falmagne, P., C. Capiau, J. Zanen, G. Kayser, and J.-M. Ruysschaert.** 1982. Structure-activity relationship of the B fragment of diphtheria toxin: the lipid-binding domains. Toxicon **20**:243–246.
56. **Field, M., L. H. Graf, Jr., W. J. Laird, and P. L. Smith.** 1978. Heat-stable enterotoxin of *Escherichia coli*: *in vitro* effects on guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. Proc. Natl. Acad. Sci. U.S.A. **75**:2800–2804.
57. **Finkelstein, R. A.** 1973. Cholera. Crit. Rev. Microbiol. **2**:553–623.
58. **Finkelstein, R. A., and J. J. LoSpalluto.** 1969. Pathogenesis of experimental cholera. Preparation and isolation of choleragen and choleragenoid. J. Exp. Med. **130**:185–202.
59. **Finne, J., T. Krusius, and H. Rauvala.** 1977. Occurrence of disialosyl groups in glycoproteins. Biochem. Biophys. Res. Commun. **74**:405–410.
60. **Fishman, P. H.** 1982. Role of membrane gangliosides in the binding and action of bacterial toxins. J. Membr. Biol. **69**:85–97.
61. **Fishman, P. H., and E. E. Atikkan.** 1980. Mechanism of action of cholera toxin: effect of receptor density and multivalent binding on activation of adenylate cyclase. J. Membr. Biol. **54**:51–60.
62. **Fishman, P. H., J. Moss, and J. C. Osborne, Jr.** 1978. Interaction of choleragen with the oligosaccharide of ganglioside G_{M1}: evidence for multiple oligosaccharide binding sites. Biochemistry **17**:711–716.
63. **Fishman, P. H., J. Moss, and M. Vaughan.** 1976. Uptake and metabolism of gangliosides in transformed mouse fibroblasts. Relationship of ganglioside structure to choleragen response. J. Biol. Chem. **251**:4490–4494.
64. **FitzGerald, D., R. E. Morris, and G. B. Saelinger.** 1980. Receptor-mediated internalization of *Pseudomonas* toxin by mouse fibroblasts. Cell **21**:867–873.
65. **FitzGerald, D. J. P., R. Padmanabhan, I. Pastan, and M. C. Willingham.** 1983. Adenovirus-induced release of epidermal growth factor and *Pseudomonas* toxin into the cytosol of KB cells during receptor-mediated endocytosis. Cell **32**:607–617.
66. **Frantz, J. C., and D. C. Robertson.** 1981. Immunological properties of *Escherichia coli* heat-stable enterotoxins: development of a radioimmunoassay specific for heat-stable enterotoxins with suckling mouse activity. Infect. Immun. **33**:193–198.
67. **Frantz, J. C., and D. C. Robertson.** 1982. Binding kinetics and partial characterization of a receptor for *Escherichia coli* heat-stable enterotoxin (STa). Abstr. Annu. Meet. Am. Soc. Microbiol., B137, p. 40.
68. **Frantz, J. C., and D. C. Robertson.** 1983. High affinity receptor for heat-stable enterotoxins (ST1) on rat intestinal epithelial cells, p. 247–257. *In* S. Kuwahara and N. F. Pierce (ed.), Advances in research on cholera and related diarrheas. Martinus Nijhoff Publ., Boston.
69. **Friedman, R. L., B. H. Iglewski, F. Roerdink, and C. R. Alving.** 1982. Suppression of cytotoxicity of diphtheria toxin by monoclonal antibodies against phosphatidylinositol phosphate. Biophys. J. **37**:23–24.
70. **Gabliks, J., and M. Solotorovsky.** 1962. Cell culture reactivity to diphtheria, staphylococcus, tetanus and *Escherichia coli* toxins. J. Immunol. **88**:505–512.
71. **Giannella, R. A., and K. W. Drake.** 1979. Effect of purified *Escherichia coli* heat-stable enterotoxin on intestinal cyclic nucleotide metabolism and fluid secretion. Infect. Immun. **24**:19–23.
72. **Giannella, R. A., K. W. Drake, and M. Luttrell.** 1981. Development of a radioimmunoassay for *Escherichia coli* heat-stable enterotoxin: comparison with the suckling mouse bioassay. Infect. Immun. **33**:186–192.
73. **Gill, D. M., J. D. Clements, D. C. Robertson, and R. A. Finkelstein.** 1981. Subunit number and arrangement in *Escherichia coli* heat-labile enterotoxin. Infect. Immun. **33**:677–682.
74. **Gill, D. M., D. J. Evans, Jr., and D. G. Evans.** 1976. Mechanism of activation of adenylate cyclase *in vitro* by polymyxin-released, heat-labile enterotoxin of *Escherichia coli*. J. Infect. Dis. **133**(Suppl.):S103–S107.
75. **Gill, D. M., and S. H. Richardson.** 1980. Adenosine diphosphate-ribosylation of adenylate cyclase catalyzed by heat-labile enterotoxin of *Escherichia coli*: comparison with cholera toxin. J. Infect. Dis. **141**:64–70.
76. **Goldberg, R. L., T. Costa, W. H. Habig, L. D. Kohn, and M. C. Hardegree.** 1981. Characterization of fragment C and tetanus toxin binding to rat brain membranes. Mol. Pharmacol. **20**:565–570.
77. **Goldstein, J. L., R. G. W. Anderson, and M. S. Brown.** 1979. Coated pits, coated vesicles, and receptor-mediated endocytosis. Nature (London) **279**:679–685.
78. **Greenberg, R. N., and R. L. Guerrant.** 1981. *E. coli* heat-stable enterotoxin. Pharmacol. Ther. **13**:507–531.
79. **Guerrant, R. L., J. M. Hughes, B. Chang, D. C. Robertson, and F. Murad.** 1980. Activation of intestinal guanylate cyclase by heat-stable enterotoxin of *Escherichia coli*: studies of tissue specificity, potential receptors, and intermediates. J. Infect. Dis. **142**:220–228.
80. **Habermann, E.** 1974. ^{125}I-labeled neurotoxin from *Clostridium botulinum* A: preparation, binding to synaptosomes and ascent to the spinal cord. Naunyn-Schmiedeberg's Arch. Pharmacol. **281**:47–56.
81. **Habermann, E., and I. Heller.** 1975. Direct evidence for the specific fixation of *Clostridium botulinum* A neurotoxin to brain matter. Naunyn-Schmiedeberg's Arch. Pharmacol. **287**:97–106.
82. **Habig, W. H., E. F. Grollman, F. D. Ledley, M. F. Meldolesi, S. M. Aloj, M. C. Hardegree, and L. D. Kohn.** 1978. Tetanus toxin interactions with the thyroid: decreasd toxin binding to membranes from a thyroid tumor with a thyrotropin receptor defect and *in vivo* stimulation of thyroid function. Endocrinology **102**:844–851.
83. **Habig, W. H., L. D. Kohn, and M. C. Hardegree.** 1982. Tetanus toxin: structure and function, p. 48–53. *In* J. B. Robbins, J. C. Hill, and J. C. Sadoff (ed.), Seminars in infectious disease, vol. 4. Bacterial vaccines. Thieme-Stratton Inc., New York.
84. **Hart, D. A.** 1980. Lectins in biological systems: applications to microbiology. Am. J. Clin. Nutr. **33**:2416–2425.
85. **Hayakawa, S., T. Uchida, E. Mekada, M. R. Moynihan, and Y. Okada.** 1983. Monoclonal antibody against diphtheria toxin. Effect on toxin binding and entry into cells. J. Biol. Chem. **258**:4311–4317.
86. **Hazeki, O., and M. Ui.** 1981. Modification by islet-activating protein of receptor-mediated regulation of cyclic AMP accumulation in isolated rat heart cells. J. Biol. Chem. **256**:2856–2862.
87. **Helting, T. B., and O. Zwisler.** 1977. Structure of tetanus toxin. I. Breakdown of the toxin molecule and discrimination between polypeptide fragments. J. Biol. Chem. **252**:187–193.
88. **Helting, T. B., O. Zwisler, and H. Wiegandt.** 1977. Structure of tetanus toxin. II. Toxin binding to ganglioside. J. Biol. Chem. **252**:194–198.
89. **Hewlett, E. L., R. L. Guerrant, D. J. Evans, Jr., and W. B. Greenough III.** 1974. Toxins of *Vibrio cholerae* and *Escherichia coli* stimulate adenyl cyclase in rat fat cells. Nature (London) **249**:371–373.

90. **Hock, R. A., E. Nexo, and M. D. Hollenberg.** 1979. Isolation of the human placenta receptor for epidermal growth factor-urogastrone. Nature (London) **277**:403–405.
91. **Hollenberg, M. D., P. H. Fishman, V. Bennett, and P. Cuatrecasas.** 1974. Cholera toxin and cell growth: role of membrane gangliosides. Proc. Natl. Acad. Sci. U.S.A. **71**:4224–4228.
92. **Holmgren, J.** 1973. Comparison of the tissue receptors for *Vibrio cholerae* and *Escherichia coli* enterotoxins by means of gangliosides and natural cholera toxoid. Infect. Immun. **8**:851–859.
93. **Holmgren, J.** 1973. Immunological and receptor-binding properties of cholera toxin and its subunits, p. 196–213. *In* Proceedings of the 9th Joint Conference on Cholera, U.S.-Japan Cooperative Medical Science Program. Publ. 8762, U.S. Department of State, Washington, D.C.
94. **Holmgren, J.** 1981. Actions of cholera toxin and the prevention and treatment of cholera. Nature (London) **292**:413–417.
95. **Holmgren, J., H. Elwing, P. Fredman, and L. Svennerholm.** 1980. Polystyrene-adsorbed gangliosides for investigation of the structure of tetanus-toxin receptor. Eur. J. Biochem. **106**:371–379.
96. **Holmgren, J., P. Fredman, M. Lindblad, A.-M. Svennerholm, and L. Svennerholm.** 1982. Rabbit intestinal glycoprotein receptor for *Escherichia coli* heat-labile enterotoxin lacking affinity for cholera toxin. Infect. Immun. **38**:424–433.
97. **Holmgren, J., and I. Lönnroth.** 1975. Oligomenic structure of cholera toxin: characteristics of the H and L subunits. J. Gen. Microbiol. **86**:49–65.
98. **Holmgren, J., and I. Lönnroth.** 1976. Cholera toxin and the adenylate cyclase-activating signal. J. Infect. Dis. **133**(Suppl.):S64–S74.
99. **Holmgren, J., I. Lönnroth, J.-E. Mansson, and L. Svennerholm.** 1975. Interaction of cholera toxin and membrane G_{M1} ganglioside of small intestine. Proc. Natl. Acad. Sci. U.S.A. **72**:2520–2524.
100. **Holmgren, J., I. Lönnroth, and L. Svennerholm.** 1973. Tissue receptor for cholera exotoxin: postulated structure from studies with G_{M1} ganglioside and related glycolipids. Infect. Immun. **8**:208–214.
101. **Holmgren, J., I. Lönnroth, and L. Svennerholm.** 1973. Fixation and inactivation of cholera toxin by G_{M1} ganglioside. Scand. J. Infect. Dis. **5**:77–78.
102. **Holmgren, J., J-E. Mansson, and L. Svennerholm.** 1974. Tissue receptor for cholera exotoxin: structural requirements of G_{M1} ganglioside in toxin binding and inactivation. Med. Biol. **52**:229–233.
103. **Honda, T., Y. Takeda, and T. Miwatani.** 1982. Isolation of special antibodies which react only with homologous enterotoxins from *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. Infect. Immun. **34**:333–336.
104. **Hughes, J. M., F. Murad, B. Chang, and R. L. Guerrant.** 1978. Role of cyclic GMP in the action of heat-stable enterotoxin of *Escherichia coli*. Nature (London) **271**:755–756.
105. **Iglewski, B. H., and D. Kabat.** 1975. NAD-dependent inhibition of protein synthesis by *Pseudomonas aeruginosa* toxin. Proc. Natl. Acad. Sci. U.S.A. **72**:2284–2288.
106. **Igleswki, B. H., P. V. Liu, and D. Kabat.** 1977. Mechanism of action of *Pseudomonas aeruginosa* exotoxin A: adenosine diphosphate-ribosylation of mammalian elongation factor 2 in vitro and in vivo. Infect. Immun. **15**:138–144.
107. **Irons, L. I., and A. P. MacLennan.** 1979. Isolation of the lymphocytosis promoting factor haemagglutinin of *Bordetella pertussis* by affinity chromatography. Biochim. Biophys. Acta **580**:175–185.
108. **Ittelson, T. R., and D. M. Gill.** 1973. Diphtheria toxin: specific competition for cell receptors. Nature (London) **242**:330–332.
109. **Ivins, B., C. B. Saelinger, P. F. Bonventre, and C. Woscinski.** 1975. Chemical modulation of diphtheria toxin action on cultured mammalian cells. Infect. Immun. **11**:665–674.
110. **Jacobs, S., Y. Schechter, K. Bissel, and P. Cuatrecasas.** 1977. Purification and properties of insulin receptors from rat liver membranes. Biochem. Biophys. Res. Commun. **77**:981–988.
111. **Joseph, K. C., A. Stieber, and N. K. Gonatas.** 1979. Endocytosis of cholera toxin in Gerl-like structures of murine neuroblastoma cells pretreated with G_{M1} ganglioside. J. Cell Biol. **81**:543–554.
112. **Katada, T., T. Anamo, and M. Ui.** 1982. Modulation of islet-activating protein of adenylate cyclase activity in C6 glioma cells. J. Biol. Chem. **257**:3739–3746.
113. **Katada, T., and M. Ui.** 1979. Islet activating protein. Enhanced insulin secretion and cyclic AMP accumulation in pancreatic islets due to activation of native calcium ionophores. J. Biol. Chem. **254**:469–479.
114. **Katada, T., and M. Ui.** 1980. Slow interaction of islet-activating protein with pancreatic islets during primary culture to cause reversal of α-adrenergic inhibition of insulin secretion. J. Biol. Chem. **255**:9580–9588.
115. **Katada, T., and M. Ui.** 1982. ADP-ribosylation of the specific membrane protein of C6 cells by islet-activating protein associated with modification of adenylate cyclase activity. J. Biol. Chem. **257**:7210–7216.
116. **Keen, J. H., F. R. Maxfield, M. C. Hardegree, and W. H. Habig.** 1982. Receptor-mediated endocytosis of diphtheria toxin by cells in culture. Proc. Natl. Acad. Sci. U.S.A. **79**:2912–2916.
117. **Keusch, G. T.** 1979. Specific membrane receptors: pathogenetic and therapeutic implications in infectious diseases. Rev. Infect. Dis. **1**:517–529.
118. **Keusch, G. T.** 1981. Receptor-mediated endocytosis of *Shigella* cytotoxin, p. 95–112. *In* J. L. Middlebrook and L. D. Kohn (ed.), Receptor-mediated binding and internalization of toxins and hormones. Academic Press, Inc., New York.
119. **Keusch, G. T., A. Donohue-Rolfe, and M. Jacewicz.** 1982. *Shigella* toxin(s): description and role in diarrhea and dysentery. Pharmacol. Ther. **15**:403–438.
120. **Keusch, G. T., and M. Jacewicz.** 1976. Membrane receptor (MR) for *Shigella* toxin (ST): role of N-acetyl glucosamine (NAG). Clin. Res. **24**:347A.
121. **Keusch, G. T., and M. Jacewicz.** 1977. Pathogenesis of *Shigella* diarrhea. VII. Evidence of a cell membrane toxin receptor involving β1→4-linked N-acetyl-D-glucosamine oligomers. J. Exp. Med. **146**:535–546.
122. **Kim, K., and N. B. Groman.** 1965. Mode of inhibition of diphtheria toxin by ammonium chloride. J. Bacteriol. **90**:1557–1562.
123. **Kitamura, M., M. Iwamori, and Y. Nagai.** 1980. Interaction between *Clostridium botulinum* neurotoxin and gangliosides. Biochim. Biophys. Acta **628**:328–335.
124. **Kohn, L. D.** 1978. Relationships in the structure and function of receptors for glycoprotein hormones, bacterial toxins, and interferon, p. 133–212. *In* P. Cuatrecasas and M. F. Greaves (ed.), Receptors and recognition. Chapman and Hall, London.
125. **Kornfeld, R., and S. Kornfeld.** 1980. Structure of glycoproteins and their oligosaccharide units, p. 1–34. *In* W. J. Lennarz (ed.), The biochemistry of glycoproteins and proteoglycans. Plenum Press, New York.
126. **Krag, S. S.** 1979. A concanavalin A-resistant Chinese hamster ovary cell line is deficient in the synthesis of (^{3}H)glucosyl oligosaccharide-lipid. J. Biol. Chem. **254**:9167–9177.
127. **Krag, S. S., and A. R. Robbins.** 1982. A Chinese hamster ovary cell mutant deficient in glycosylation of lipid-linked oligosaccharide synthesizes lysosomal enzymes of altered structure and function. J. Biol. Chem. **257**:8424–8431.
128. **Kunkel, S. L., and D. C. Robertson.** 1979. Purification and chemical characterization of the heat-labile enterotoxin produced by enterotoxigenic *Escherichia coli*. Infect. Immun. **25**:586–596.

129. **Kwan, C. N., and R. M. Wishnow.** 1974. *Escherichia coli* enterotoxin-induced steroidogenesis in cultured adrenal tumor cells. Infect. Immun. **10**:146–151.
130. **Lai, C.-Y., F. Cancedda, and L. K. Duffy.** 1981. ADP-ribosyl transferase activity of cholera toxin polypeptide A_1 and the effect of limited trypsinolysis. Biochem. Biophys. Res. Commun. **102**:1021–1027.
131. **Lallier, R., F. Bernard, M. Gendreau, C. Lazure, N. G. Seidah, M. Chretien, and S. A. St.-Pierre.** 1982. Isolation and purification of *Escherichia coli* heat-stable enterotoxin of porcine origin. Anal. Biochem. **127**:267–275.
132. **Lallier, R., S. Lariviere, and S. St.-Pierre.** 1980. *Escherichia coli* heat-stable enterotoxin: rapid method of purification and some characteristics of the toxin. Infect. Immun. **28**:469–474.
133. **Ledley, F. D., G. Lee, L. D. Kohn, W. H. Habig, and M. C. Hardegree.** 1977. Tetanus toxin interactions with thyroid plasma membranes. J. Biol. Chem. **252**:4049–4055.
134. **Lee, G., E. Consiglio, W. Habig, S. Dyer, C. Hardegree, and L. D. Kohn.** 1978. Structure:function studies of receptors for thyrotropin and tetanus toxin: lipid modulation of effector binding to the glycoprotein receptor component. Biochem. Biophys. Res. Commun. **83**:313–320.
135. **Lee, G., E. F. Grollman, S. Dyer, F. Beguinot, L. D. Kohn, W. H. Habig, and M. C. Hardegree.** 1979. Tetanus toxin and thyrotropin interactions with rat brain membrane preparations. J. Biol. Chem. **254**:3826–3832.
136. **Leppla, S. H.** 1976. Large-scale purification and characterization of the exotoxin of *Pseudomonas aeruginosa*. Infect. Immun. **14**:1077–1086.
137. **Leppla, S. H., R. B. Dorland, and J. L. Middlebrook.** 1980. Inhibition of diphtheria toxin degradation and cytotoxic action by chloroquine. J. Biol. Chem. **255**:2247–2250.
138. **Lönnroth, I., and J. Holmgren.** 1975. Protein reagent modification of cholera toxin: characterization of effects on antigenic, receptor-binding and toxin properties. J. Gen. Microbiol. **91**:263–277.
139. **Lory, S., S. F. Carroll, P. D. Bernard, and R. J. Collier.** 1980. Ligand interactions of diphtheria toxin. I. Binding and hydrolysis of NAD. J. Biol. Chem. **255**:12011–12015.
140. **Lory, S., S. F. Carroll, and R. J. Collier.** 1980. Ligand interactions of diphtheria toxin. II. Relationships between the NAD site and the P site. J. Biol. Chem. **255**:12016–12019.
141. **Lory, S., and R. J. Collier.** 1980. Expression of enzymic activity by exotoxin A from *Pseudomonas aeruginosa*. Infect. Immun. **28**:494–501.
142. **Lory, S., and R. J. Collier.** 1980. Diphtheria toxin: nucleotide binding and toxin heterogeneity. Proc. Natl. Acad. Sci. U.S.A. **77**:267–271.
143. **Madsen, G. L., and F. C. Knoop.** 1980. Physicochemical properties of a heat-stable enterotoxin produced by *Escherichia coli* of human origin. Infect. Immun. **28**:1051–1053.
144. **Matsuda, M., and M. Yoneda.** 1974. Two antigenically active, complementary polypeptide fragments of tetanus neurotoxin, p. 363–373. *In* A. Ohsaka, K. Hayashi, and Y. Sawai (ed.), Animal, plant and microbial toxins, vol. I. Biochemistry. Plenum Press, New York.
145. **Matsuda, M., and M. Yoneda.** 1974. Dissociation of tetanus neurotoxin into two polypeptide fragments. Biochem. Biophys. Res. Commun. **57**:1257-1262.
146. **Matsuda, M., and M. Yoneda.** 1977. Antigenic substructure of tetanus neurotoxin. Biochem. Biophys. Res. Commun. **77**:268–274.
147. **Mekada, E., T. Uchida, and Y. Okada.** 1979. Modification of the cell surface with neuraminidase increases the sensitivities of cells to diphtheria toxin and *Pseudomonas aeruginosa* exotoxin. Exp. Cell Res. **123**:137–146.
148. **Mekalanos, J. J., R. J. Collier, and W. R. Romig.** 1979. Enzymic activity of cholera toxin. I. New method of assay and the mechanism of ADP-ribosyl transfer. J. Biol. Chem. **254**:5849–5854.
149. **Mekalanos, J. J., R. J. Collier, and W. R. Romig.** 1979. Enzymic activity of cholera toxin. II. Relationships to proteolytic processing, disulfide bond reduction, and subunit composition. J. Biol. Chem. **254**:5855–5861.
150. **Middlebrook, J. L., and R. B. Dorland.** 1977. Response of cultured mammalian cells to the exotoxins of *Pseudomonas aeruginosa* and *Corynebacterium diptheriae*: differential cytotoxicity. Can. J. Microbiol. **23**:183–189.
151. **Middlebrook, J. L. and R. B. Dorland.** 1977. Differential chemical protection of mammalian cells from the exotoxins of *Corynebacterium diphtheriae* and *Pseudomonas aeruginosa*. Infect. Immun. **16**:232–239.
152. **Middlebrook, J. L., and R. B. Dorland.** 1979. Protection of mammalian cells from diphtheria toxin by exogenous nucleotides. Can. J. Microbiol. **25**:285–290.
153. **Middlebrook, J. L., R. B. Dorland, and S. H. Leppla.** 1978. Association of diptheria toxin with Vero cells. Demonstration of a receptor. J. Biol. Chem. **253**:7325–7330.
154. **Middlebrook, J. L., R. B. Dorland, and S. H. Leppla.** 1979. Effects of lectins on the interaction of diphtheria toxin with mammalian cells. Exp. Cell Res. **121**:95–101.
155. **Middlebrook, J. L., and D. L. Leatherman.** 1982. Differential sensitivity of reticulocytes to nicked and unnicked diphtheria toxin. Exp. Cell Res. **138**:175–182.
156. **Moehring, J. M., T. J. Moehring, and D. E. Danley.** 1980. Posttranslational modification of elongation factor 2 in diphtheria toxin-resistant mutants of CHO-Kl cells. Proc. Natl. Acad. Sci. U.S.A. **77**:1010–1014.
157. **Moehring, T. J., and J. P. Crispell.** 1974. Enzyme treatment of KB cells: the altered effect of diphtheria toxin. Biochem. Biophys. Res. Commun. **60**:1446–1452.
158. **Moehring, T. J., and J. M. Moehring.** 1977. Selection and characterization of cells resistant to diphtheria toxin and *Pseudomonas* exotoxin A: Presumptive translational mutants. Cell **11**:447–454.
159. **Montesano, R., J. Roth, A. Robert, and L. Orci.** 1982. Non-coated membrane invaginations are involved in binding and internalization of cholera and tetanus toxins. Nature (London) **296**:651–653.
160. **Morita, A., D. Tsao, and Y. S. Kim.** 1980. Identification of cholera toxin binding glycoproteins in rat intestinal microvillus membranes. J. Biol. Chem. **255**:2549–2553.
161. **Morris, N. P., E. Consiglio, L. D. Kohn, W. H. Habig, M. C. Hardegree, and T. B. Helting.** 1980. Interaction of fragments B and C of tetanus toxin with neural and thyroid membranes and with gangliosides. J. Biol. Chem. **255**:6071–6076.
162. **Moss, J., P. H. Fishman, V. C. Manganiello, M. Vaughan, and R. O. Brady.** 1976. Functional incorporation of ganglioside into intact cells: induction of choleragen responsiveness. Proc. Natl. Acad. Sci. U.S.A. **73**:1034–1037.
163. **Moss, J., P. H. Fishman, R. L. Richards, C. R. Alving, M. Vaughan, and R. O. Brady.** 1976. Choleragen-mediated release of trapped glucose from liposomes containing ganglioside G_{M1}. Proc. Natl. Acad. Sci. U.S.A. **73**:3480–3483.
164. **Moss, J., S. Garrison, P. H. Fishman, and S. H. Richardson.** 1979. Gangliosides sensitize unresponsive fibroblasts to *Escherichia coli* heat-labile enterotoxin. J. Clin. Invest. **64**:381–384.
165. **Moss, J., V. C. Manganiello, and P. H. Fishman.** 1977. Enzymatic and chemical oxidation of gangliosides in cultured cells: effect of choleragen. Biochemistry **16**:1876–1881.
166. **Moss, J., J. C. Osborne, Jr., P. H. Fishman, S. Nakaya, and D. C. Robertson.** 1981. *Escherichia coli* heat-labile enterotoxin. Ganglioside specificity and ADP-ribosyltransferase activity. J. Biol. Chem. **256**:12861–12865.
167. **Moss, J., R. L. Richards, C. R. Alving, and P. H. Fishman.** 1977. Effect of the A and B protomers of choleragen on release of trapped glucose from liposomes containing or lacking ganglioside G_{M1}. J. Biol. Chem.

252:797–798.

168. **Moss, J., and S. H. Richardson.** 1978. Activation of adenylate cyclase by heat-labile *Escherichia coli* enterotoxin. Evidence for ADP-ribosyltransferase activity similar to that of choleragen. J. Clin. Invest. **62**:281–285.
169. **Moss, J., and M. Vaughan.** 1977. Mechanism of action of choleragen. Evidence for ADP-ribosyltransferase activity with arginine as an acceptor. J. Biol. Chem. **252**:2455–2457.
170. **Moss, J., and M. Vaughan.** 1979. Activation of adenylate cyclase by choleragen. Annu. Rev. Biochem. **48**:581–600.
171. **Mullin, B. R., P. H. Fishman, G. Lee, S. M. Aloj, F. D. Ledley, R. J. Winand, L. D. Kohn, and R. O. Brady.** 1976. Thyrotropin-ganglioside interactions and their relationship to the structure and function of thyrotropin receptors. Proc. Natl. Acad. Sci. U.S.A. **73**:842–846.
172. **Nalin, D. R., and J. C. McLaughlin.** 1978. Effects of choleragenoid and glucose on the response of dog intestine to *Escherichia coli* enterotoxins. J. Med. Microbiol. **11**:177–186.
173. **Neubauer, V., and T. B. Helting.** 1979. Structure of tetanus toxin. N-terminal amino acid analysis of the two molecular forms of tetanus toxin and its composite chains. Biochem. Biophys. Res. Commun. **86**:635–642.
174. **Neubauer, V., and T. B. Helting.** 1981. Structure of tetanus toxin. The arrangement of papain digestion products within the heavy chain-light chain framework of extracellular toxin. Biochim. Biophys. Acta **668**:141–148.
175. **Neufeld, E. F., and G. Ashwell.** 1980. Carbohydrate recognition systems for receptor-mediated pinocytosis, p. 241–266. *In* W. J. Lennarz (ed.), The biochemistry of glycoproteins and proteoglycans. Plenum Press, New York.
176. **Newsome, P. M., M. N. Burgess, and N. A. Mullan.** 1978. Effect of *Escherichia coli* heat-stable enterotoxin on cyclic GMP levels in mouse intestine. Infect. Immun. **22**:290–291.
177. **O'Brien, A. D., G. D. Laveck, D. E. Griffin, and M. R. Thompson.** 1980. Characterization of *Shigella dysenteriae* 1 (Shiga) toxin purified by anti-shiga toxin affinity chromatography. Infect. Immun. **30**:170–179.
178. **Olsnes, S., and K. Eiklid.** 1980. Isolation and characterization of *Shigella shigae* cytotoxin. J. Biol. Chem. **255**:284–289.
179. **Olsnes, S., R. Reisbig, and K. Eiklid.** 1981. Subunit structure of *Shigella* cytotoxin. J. Biol. Chem. **256**:8732–8738.
180. **Pappenheimer, A. M., Jr.** 1977. Diphtheria toxin. Annu. Rev. Biochem. **46**:69–94.
181. **Pavlovskis, O. R., B. H. Iglewski, and M. Pollack.** 1978. Mechanism of action of *Pseudomonas aeruginosa* exotoxin A in experimental mouse infections: adenosine diphosphate ribosylation of elongation factor 2. Infect. Immun. **19**:29–33.
182. **Pierce, N. F.** 1973. Differential inhibitory effects of cholera toxoids and ganglioside on the enterotoxins of *Vibrio cholerae* and *Escherichia coli*. J. Exp. Med. **137**:1009–1023.
183. **Pittman, M.** 1979. Pertussis toxin: the case of the harmful effects and prolonged immunity of whooping cough. A hypothesis. Rev. Infect. Dis. **1**:401–412.
184. **Proia, R. L., L. Eidels, and D. A. Hart.** 1979. Diphtheria toxin-binding glycoproteins on hamster cells: candidates for diphtheria toxin receptors. Infect. Immun. **25**:786–791.
185. **Proia, R. L., L. Eidels, and D. A. Hart.** 1981. Diphtheria toxin:receptor interaction. Characterization of the receptor interaction with the nucleotide-free toxin, the nucleotide-bound toxin, and the B-fragment of the toxin. J. Biol. Chem. **256**:4991–4997.
186. **Proia, R. L., D. A. Hart, and L. Eidels.** 1979. Interaction of diphtheria toxin with phosphorylated molecules. Infect. Immun. **26**:942–948.
187. **Proia, R. L., D. A. Hart, R. K. Holmes, K. V. Holmes, and L. Eidels.** 1979. Immunoprecipitation and partial characterization of diphtheria toxin-binding glycoproteins from the surface of guinea pig cells. Proc. Natl. Acad. Sci. U.S.A. **76**:685–689.
188. **Proia, R. L., S. K. Wray, D. A. Hart, and L. Eidels.** 1980. Characterization and affinity labeling of the cationic phosphate-binding (nucleotide-binding) peptide located in the receptor-binding region of the B-fragment of diphtheria toxin. J. Biol. Chem. **255**:12025–12033.
189. **Rauvala, H., and J. Finne.** 1979. Structural similarity of the terminal carbohydrate sequence of glycoproteins and glycolipids. FEBS Lett. **97**:1–8.
190. **Reisbig, R., S. Olsnes, and K. Eiklid.** 1981. The cytotoxic activity of *Shigella* toxin. Evidence for catalytic inactivation of the 60S ribosomal subunit. J. Biol. Chem. **256**:8739–8744.
191. **Robb, M., J. C. Nichols, S. K. Whoriskey, and J. R. Murphy.** 1982. Isolation of hybridoma cell lines and characterization of monoclonal antibodies against cholera enterotoxin and its subunits. Infect. Immun. **38**:267–272.
192. **Rodgers, T. B., and S. H. Snyder.** 1981. High affinity binding of tetanus toxin to mammalian brain membranes. J. Biol. Chem. **256**:2402–2407.
193. **Roth, R. A., D. J. Cassell, K. Y. Wong, B. A. Maddux, and I. D. Goldfine.** 1982. Monoclonal antibodies to the human insulin receptor block insulin binding and inhibit insulin action. Proc. Natl. Acad. Sci. U.S.A. **79**:7312–7316.
194. **Sadoff, J. C., G. A. Buck, B. H. Iglewski, M. J. Bjorn, and N. B. Groman.** 1982. Immunological cross-reactivity in the absence of DNA homology between *Pseudomonas* toxin A and diphtheria toxin. Infect. Immun. **37**:250–254.
195. **Sahagian, G. G., J. Distler, and G. W. Jourdian.** 1981. Characterization of a membrane-associated receptor from bovine liver that binds phosphomannosyl residues of bovine testicular β-galactosidase. Proc. Natl. Acad. Sci. U.S.A. **78**:4289–4293.
196. **Sandvig, K., and S. Olsnes.** 1981. Rapid entry of nicked diphtheria toxin into cells at low pH. Characterization of the entry process and effect of low pH on the toxin molecule. J. Biol. Chem. **256**:9068–9076.
197. **Sandvig, K., S. Olsnes, and A. Pihl.** 1978. Binding, uptake and degradation of the toxic proteins abrin and ricin by toxin-resistant cell variants. Eur. J. Biochem. **82**:13–23.
198. **Schneider, W. J., V. Beisiegel, J. L. Goldstein, and M. S. Brown.** 1982. Purification of the low density lipoprotein receptor, an acidic glycoprotein of 164,000 molecular weight. J. Biol. Chem. **257**:2664–2673.
199. **Schreiber, A. B., I. Lax, Y. Yarden, Z. Eshhar, and J. Schlessinger.** 1981. Monoclonal antibodies against receptor for epidermal growth factor induce early and delayed effects of epidermal growth factor. Proc. Natl. Acad. Sci. U.S.A. **78**:7535–7539.
200. **Simpson, L. L.** 1979. Studies on the mechanism of action of botulinum toxin. Adv. Cytopharmacol. **3**:27–34.
201. **Simpson, L. L.** 1979. The action of botulinal toxin. Rev. Infect. Dis. **1**:656–662.
202. **Simpson, L. L.** 1980. Kinetic studies on the interaction between botulinum toxin type A and the cholinergic neuromuscular junction. J. Pharmacol. Exp. Ther. **212**:16–21.
203. **Simpson, L. L.** 1981. Pharmacological studies on the cellular and subcellular effects of botulinum toxin, p. 35–46. *In* G. E. Lewis, Jr. (ed.), Biomedical aspects of botulism. Academic Press, Inc., New York.
204. **Simpson, L. L.** 1981. The origin, structure, and pharmacological activity of botulinum toxin. Pharmacol. Rev. **33**:155–188.
205. **Simpson, L. L., and M. M. Rapport.** 1971. Ganglioside inactivation of botulinum toxin. J. Neurochem. **18**:1341–1343.
206. **Simpson, L. L., and M. M. Rapport.** 1971. The binding of

botulinum toxin to membrane lipids: sphingolipids, steroids, and fatty acids. J. Neurochem. **18**:1751–1759.

207. **So, M., and B. J. McCarthy.** 1980. Nucleotide sequence of the bacterial transposon Tn1681 encoding a heat-stable (ST) toxin and its identification in enterotoxigenic *Escherichia coli* strains. Proc. Natl. Acad. Sci. U.S.A. **77**:4011–4015.
208. **Spicer, E. K., W. M. Kavanaugh, W. S. Dallas, S. Falkow, W. H. Kinigsberg, and D. E. Schafer.** 1981. Sequence homologies between A subunits of *Escherichia coli* and *Vibrio cholerae* enterotoxins. Proc. Natl. Acad. Sci. U.S.A. **78**:50–54.
209. **Stanley, P., S. Narasimhan, L. Siminovitch, and H. Schachter.** 1975. Chinese hamster ovary cells selected for resistance to the cytotoxicity of phytohemagglutnin are deficient in a UDP-N-acetylglucosaminyl-glycoprotein N-acetylglucosaminyltransferase activity. Proc. Natl. Acad. Sci. U.S.A. **72**:3323–3327.
210. **Staples, S. J., S. E. Asher, and R. A. Giannella.** 1980. Purification and characterization of heat-stable enterotoxin produced by a strain of *E. coli* pathogenic for man. J. Biol. Chem. **255**:4716–4721.
211. **Stoll, J., A. R. Robbins, and S. S. Krag.** 1982. Mutant of Chinese hamster ovary cells with altered mannose 6-phosphate receptor activity is unable to synthesize mannosylphosphoryldolichol. Proc. Natl. Acad. Sci. U.S.A. **79**:2296–2300.
212. **Svennerholm, A.-M., and J. Holmgren.** 1978. Identification of *Escherichia coli* heat-labile enterotoxin by means of a ganglioside immunosorbent assay (G_{M1}-ELISA) procedure. Curr. Microbiol. **1**:19–23.
213. **Syuto, B., and S. Kubo.** 1981. Separation and characterization of heavy and light chains from *Clostridium botulinum* type C toxin and their reconstitution. J. Biol. Chem. **256**:3712–3717.
214. **Tager, H. S., and D. F. Steiner.** 1974. Peptide hormones. Annu. Rev. Biochem. **43**:509–538.
215. **Tamura, M., K. Nogimori, S. Murai, M. Yajima, K. Ito, T. Katada, M. Ui, and S. Ishii.** 1982. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. Biochemistry **21**:5516–5522.
216. **Tate, R. L., J. M. Holmes, L. D. Kohn, and R. J. Winand.** 1975. Characteristics of a solubilized thyrotropin receptor from bovine thyroid plasma membranes. J. Biol. Chem. **250**:6527–6533.
217. **Tayot, J.-L., J. Holmgren, L. Svennerholm, M. Lindblad, and M. Tardy.** 1981. Receptor-specific large-scale purification of cholera toxin on silica beads derivatized with lyso-G_{M1} ganglioside. Eur. J. Biochem. **113**:249–258.
218. **Tonegawa, Y., and S. Hakomori.** 1977. "Ganglioprotein and globoprotein": the glycoproteins reacting with anti-ganglioside and anti-globoside antibodies and the ganglioprotein change associated with transformation. Biochem. Biophys. Res. Commun. **76**:9–17.
219. **Uchida, T., A. M. Pappenheimer, Jr., and R. Greany.** 1973. Diphtheria toxin and related proteins. I. Isolation and properties of mutant proteins serologically related to diphtheria toxin. J. Biol. Chem. **248**:3838–3844.
220. **Uchida, T., A. M. Pappenheimer, Jr., and A. A. Harper.** 1972. Reconstitution of diphtheria toxin from two nontoxic crossreacting mutant proteins. Science **175**:901–903.
221. **van Heyningen, S.** 1974. Cholera toxin: interaction of subunits with ganglioside G_{M1}. Science **183**:656–657.
222. **van Heyningen, S.** 1976. Binding of ganglioside by the chains of tetanus toxin. FEBS Lett.**68**:5–7.
223. **van Heyningen, W. E.** 1974. Gangliosides as membrane receptors for tetanus toxin, cholera toxin and serotonin. Nature (London) **249**:415–417.
224. **van Heyningen, W. E., C. C. J. Carpenter, N. F. Pierce, and W. B. Greenough III.** 1971. Deactivation of cholera toxin by gangliosides. J. Infect. Dis. **124**:415–418.
225. **Van Ness, B. G., J. B. Howard, and J. W. Bodley.** 1980. ADP-ribosylation of elongation factor 2 by diphtheria toxin. NMR spectra and proposed structures of ribosyldiphthamide and its hydrolysis products. J. Biol. Chem. **225**:10710–10716.
226. **Vasil, M. L., and B. H. Iglewski.** 1978. Comparative toxicities of diphtherial toxin and *Pseudomona aeruginosa* exotoxin A: evidence for different cell receptors. J. Gen. Microbiol. **108**:333–337.
227. **Vasil, M. L., D. Kabat, and B. H. Iglewski.** 1977. Structure-activity relationships of an exotoxin of *Pseudomonas aeruginosa*. Infect. Immun. **16**:353–361.
228. **Vaughan, M., and J. Moss.** 1981. The mechanism of action of choleragen, p. 69–77. *In* K. Bloch, L. Bolis, and D. C. Tosteson (ed.), Membranes, molecules, toxins, and cells. John Wright, PSG Inc., Boston.
229. **Wada, H. G., P. E. Hass, and H. H. Sussman.** 1979. Transferrin receptor in human placental brush border membranes. Studies on the binding of transferrin to placental membrane vesicles and the identification of a placental brush border glycoprotein with high affinity for transferrin. J. Biol. Chem. **254**:12629–12635.
230. **Wardlaw, A. C., and R. Parton.** 1983. *Bordetella pertussis* toxins. Pharmacol. Ther. **19**:1–53.
231. **Zanen, J., G. Muyldermans, and N. Beugnier.** 1976. Competitive antagonists of the action of diphtheria toxin in HeLa cells. FEBS Lett. **66**:261–263.
232. **Zenser, T. V., and J. F. Metzger.** 1974. Comparison of the action of *Escherichia coli* eneterotoxin on the thymocyte adenylate cyclase-cyclic adenosine monophosphate system to that of cholera toxin and prostaglandin E_1. Infect. Immun. **10**:503–509.