# Shiga Toxin: Purification, Structure, and Function

**Arthur Donohue-Rolfe, David W. K. Acheson, and Gerald T. Keusch**

*From the Division of Geographic Medicine and Infectious Diseases, Department of Medicine, New England Medical Center, and Tufts University School of Medicine, Boston, Massachusetts*

**Shiga toxin is a potent toxin produced by *Shigella dysenteriae* type 1 strains. The toxin has three biologic activities–cytotoxicity, enterotoxicity, and neurotoxicity–and one known biochemical effect: inhibition of protein synthesis. It consists of two polypeptide chains, an A chain (molecular weight, 32,225) and a B chain (molecular weight, 7,691). These two peptides associate with a stoichiometry of one A and five B subunits to form the holotoxin. The A chain is responsible for the biochemical effect of the holotoxin: cleavage of the *N*-glycosidic bond of adenine at nucleotide position 4324 in the 28*S* rRNA of the 60*S* ribosomal subunit. The B chain mediates binding of toxin to cell surface receptors. Shiga toxin is the prototype of a family of toxin molecules that have been termed Shiga-like in terms of both structural and functional analysis.**

Since the turn of the century, it has been known that strains of *Shigella dysenteriae* type 1 (Shiga bacillus) produce a toxin [1]. The original observation was that parenteral injection of crude cell-free extracts of *S. dysenteriae* into rabbits resulted in paralysis of the extremities, followed by death. Because of this biologic activity, the toxin was initially termed a neurotoxin. Later, cell-free extracts of *S. dysenteriae* type 1 were also shown to be cytotoxic to certain tissue culture cells [2] and to be enterotoxic, resulting in fluid secretion when administered in vivo into ligated segments of rabbit ileum [3]. The discovery of the latter two activities was particularly exciting because it suggested that the toxin may be directly involved in producing the two major clinical manifestations of shigellosis: diarrhea and dysentery. Until the toxin was purified, it was unclear whether its multiple biologic activities were due to the presence of multiple toxins or to a single toxin molecule with multiple activities. However, recent purification of the toxin and neutralization studies with polyclonal and monoclonal antibodies to the purified toxin have demonstrated that a single toxin molecule is in fact responsible for the multiple activities [4–7]. This molecule has been termed Shiga toxin.

In 1977 a toxin was reported in *Escherichia coli* that was cytotoxic to Vero cells and distinct from the well-known heat-labile and heat-stable *E. coli* toxins (LT and ST) [8]. This toxin has been called Verotoxin by some groups. *E. coli* strains producing the toxin have been associated with the hemolytic-uremic syndrome and hemorrhagic colitis [9]. It was later found that the cytotoxin produced by *E. coli* O157:H7, a strain associated with hemorrhagic colitis, was neutralized by antiserum to purified Shiga toxin [10]. On the basis of this neutralization, it was proposed that this toxin be called Shiga-like toxin. Further studies on *E. coli* O157:H7 strain 933 revealed the presence of two toxin-converting phages, termed 933J and 933W, that carried the structural genes for two cytotoxins, one neutralized and the other not neutralized by antiserum to Shiga toxin [11, 12]. These two toxins have been designated Shiga-like toxin I (SLT I) and Shiga-like toxin II (SLT II), respectively [13]. The genes for Shiga toxin and the two Shiga-like toxins have been sequenced, and, from the predicted amino acid sequence, SLT I has been shown to be nearly identical [14] or identical [15] to Shiga toxin and SLT II to be 56% homologous with Shiga toxin [16]. These Shiga-like toxins have the three biologic activities of Shiga toxin: neurotoxicity, cytotoxicity, and enterotoxicity [17]. Compared with Shiga toxin, they bind to a similar set of receptors [18, 19] and have an identical mode of action [20]. In addition to SLT I and SLT II, extracts of other *Shigella* species and strains of a variety of other bacterial genera display toxic activity similar to that of Shiga toxin [21, 22].

Hence, it is now clear that Shiga toxin is the prototype of a family of toxins termed Shiga-like toxins. Until proven otherwise, Shiga toxin's basic structure and the functional role of its subunits should be considered a model for the other members of the family.

## Shiga Toxin Purification

Shiga toxin has been purified by several procedures involving a variety of chromatographic techniques, including molecular sieve and/or ion-exchange chromatography, affinity chromatography on acid-washed chitin or over antibody-containing columns, and isoelectric focusing [7, 23, 24]. Since <5% of toxin is recovered and since under optimal conditions toxin represents only ∼0.1% of total protein, these methods yield only small quantities of purified protein.

Grant support: National Institute of Allergy and Infectious Diseases (AI-20325 and AI-16242) and the National Institute of Diabetes and Digestive and Kidney Diseases (DK-34928). D.W.K.A. is supported by the Wellcome Trust, London, UK.

Reprints and correspondence: Dr. Arthur Donohue-Rolfe, New England Medical Center, Box 041, 750 Washington Street, Boston, Massachusetts 02111.

**Reviews of Infectious Diseases 1991;13(Suppl 4):S293–7**
© 1991 by The University of Chicago. All rights reserved.
0162–0886/91/1302–0045$02.00

Downloaded from http://cid.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 13, 2016

In our own laboratory we have purified Shiga toxin by a relatively simple three-step procedure [5], with yields routinely at 50% of the initial amount of toxin. In this review we present the scheme in outline; details are published elsewhere [5, 25]. For toxin purification we use a nonpathogenic rough mutant strain of *S. dysenteriae* type 1, strain 60R, first described by Dubos and Geiger in 1946 [26]. Since toxin production is regulated by the iron level [27], for optimal yields the culture medium should be low in iron. We use a modified syncase broth with an iron ($Fe^{3+}$) concentration of 0.1–0.15 µg/mL. Cultures are grown aerobically at 37°C with vigorous (300 rpm) shaking. The toxin is produced during the logarithmic phase of growth, and bacteria are therefore harvested when the stationary phase is reached. Since Shiga toxin is a periplasmic protein, the greatest starting yield is obtained by making a sonic lysate of the bacteria. The crude cell lysate, in low ionic-strength buffer, is then applied to a column containing the dye Cibacron Blue F3G-A coupled to Sepharose CL-6B (Blue Sepharose; Pharmacia Fine Chemicals, Piscataway, NJ). Shiga toxin and many other proteins bind to Blue Sepharose and, after extensive washing, can be separated from Blue Sepharose by elution in buffer containing 0.5 *M* NaCl. The Blue Sepharose salt eluate, which contains 80%–90% of the original cytotoxic activity, is then subjected to chromatofocusing. Shiga toxin elutes from the column at an eluting buffer pH of 7.0–7.1. The final step in the purification process is molecular sieve chromatography in Bio-Gel P-60 (Bio-Rad, Richmond, CA). The primary purpose of this step is to remove the ampholytes from the chromatofocusing step. The three steps – Blue Sepharose, chromatofocusing, and molecular sieve chromatography – result in a final yield of toxin of 50% and an increase in toxin-specific activity (cytotoxin activity per milligram of protein) of 1,300-fold. A 4-L culture volume yields ~1 mg of purified Shiga toxin (figure 1).

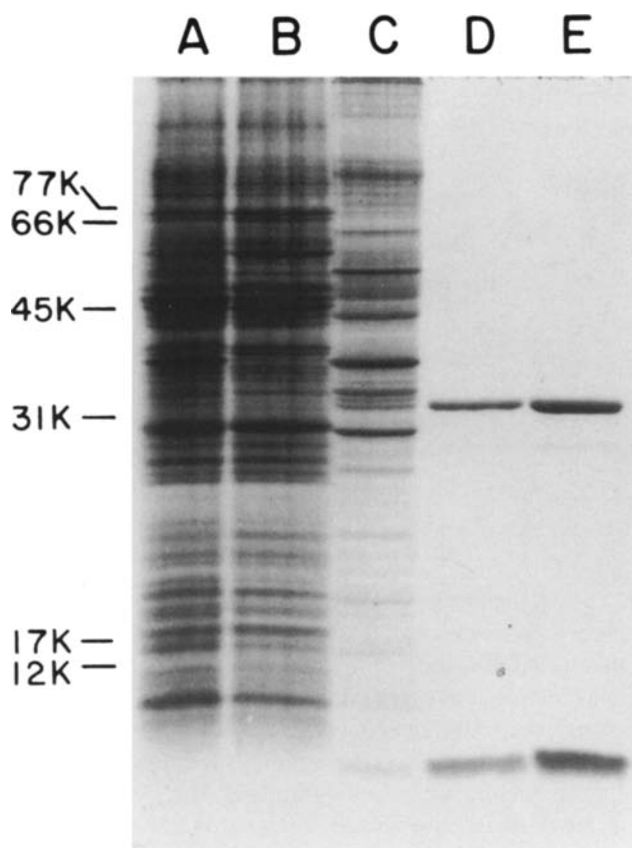


**Figure 1.** SDS-PAGE of the different stages of toxin purification. All samples were dissolved in SDS sample buffer containing β-mercaptoethanol. Samples were heated in boiling water for 10 minutes before being applied to a 15% acrylamide gel. (*A*) Whole-cell lysate of *S. dysenteriae* 1. (*B*) Blue Sepharose flow-through. (*C*) Blue Sepharose salt eluate. (*D*) The pI 7.1 region from the chromatofocusing step. (*E*) The final product after chromatography on Bio-Gel P-60. The molecular weight markers were transferrin (77K), bovine serum albumin (66K), ovalbumin (45K), carbonic anhydrase (31K), myoglobin (17K), and cytochrome C (12K). Reprinted from the *Journal of Experimental Medicine* [5] by copyright permission of the Rockefeller University Press.

An alternative purification scheme recently developed by our laboratory [28] takes advantage of the carbohydrate specificity of the toxin's binding domain. The P blood group system described by Landsteiner and Levine [29] consists of three antigens: P1, P, and Pk [30]. Hydatid cysts isolated from sheep infected with *Echinococcus granulosus* contain material, identified as a glycoprotein [31], with P1 blood group reactivity. The P1 glycoprotein's antigenic determinant consists of a trisaccharide, Gal-α1→4Gal-β1→4GlcNAc, identical to the nonreducing end of the P1 glycolipid on human erythrocytes. Since toxin binds to terminal Gal-α1→4Gal disaccharide of glycolipids, the P1 glycolipid is a good binding site for the toxin [32]. We have observed that the P1 glycoprotein in hydatid cyst fluid interacts directly with Shiga toxin and inhibits its binding and cytotoxicity to tissue culture cells. By covalent coupling of the hydatid cyst glycoprotein to Sepharose 4B, a solid-phase system for the capture of toxin can be generated. Shiga toxin binds strongly to the matrix and is resistant to elution by buffers of high and low pH (2.5–11), 6*M* urea, 50% ethylene glycol, and solutions of up to 3*M* NaCl. Toxin can be eluted by buffer containing 4.5*M* $MgCl_2$. Thus, to purify Shiga toxin, an *S. dysenteriae* type 1 bacterial lysate is applied to a column containing the coupled matrix. To remove nonspecifically or weakly attached proteins, the column is washed with buffer containing 1*M* NaCl. Finally, toxin is eluted with buffer containing 4.5 *M* $MgCl_2$.

This method results in an increase in specific activity (cytotoxin activity per milligram of protein) of >1,000-fold, with yields of toxin >80%. Besides its high yield, high degree of purity, and simplicity, this receptor analogue affinity chromatography offers an additional advantage over other methods of Shiga toxin purification: because it is based on the toxin's recognition of its receptor, it can be applied to the purification of many Shiga-like toxins that bind to similar

Downloaded from http://cid.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 13, 2016

receptors. To date, we have used receptor analogue affinity chromatography to purify both SLT I and SLT II from *E. coli*. In addition, we have used the crude P1 glycoprotein material in an ELISA format to measure toxin antigen, using polyclonal antibodies to Shiga toxin or SLT II (authors' unpublished observations). This ELISA is nearly as sensitive as the assay using a monoclonal B subunit-specific antibody for Shiga toxin previously described by our laboratory [25]. The clear advantage of this ELISA is that in many parts of the world hydatid cyst disease occurs naturally in animals, providing a rich source for the capture step, so that only the production of polyclonal antibodies is required for the complete ELISA system. This assay is comparable in sensitivity to any assay using Gb3, which is time-consuming to prepare and expensive when purchased commercially.

## Toxin Structure and Function Relationships

On SDS-polyacrylamide gels purified Shiga toxin migrates as two polypeptide chains, an A subunit (mol wt, ~32,000) and a B subunit (mol wt, ~7,700) (figure 1). The stoichiometric characteristics of toxin subunits have been determined by means of homobifunctional cross-linking agents that covalently link polypeptide chains in association with one another. The cross-linking studies indicate that Shiga toxin actually consists of one A subunit in association with five B subunits [5, 33].

The structure of most well-characterized bacterial toxins conforms to a general two-domain (A-B) model in which the toxin molecule is subdivided into a domain responsible for biologic activity (the A domain) and a domain mediating the binding of the toxin to cell surface receptor(s) (the B domain). In some cases (e.g., that of diphtheria toxin), the two domains are located in different regions of a single polypeptide chain; in others (e.g., that of cholera toxin), the two domains are located on two polypeptide chains: the A domain on the A subunit and the B domain on the B subunit. Shiga toxin is no exception to this general A-B structural model. The two subunits can be dissociated by treatment with urea or formic acid and isolated by molecular sieve chromatography [33,34]. The toxin A chain is responsible for the biochemical effect of holotoxin, i.e., inhibition of protein synthesis [34]. This chain contains a region that is proteolytically sensitive. After cleavage with trypsin the chain consists of two fragments, $A_1$ (mol wt, ~29,000) and $A_2$ (mol wt, ~3,000), which are held together by a disulfide linkage. After chemical reduction it is the larger ($A_1$) fragment that catalytically inactivates the eukaryotic ribosomal subunit in vitro. The end result of this inactivation is the inhibition of nascent polypeptide chain elongation [34].

The A subunit of the plant toxin ricin is a polypeptide similar in size to the A subunit of Shiga toxin. In addition, like the A subunit of Shiga toxin, the A subunit of ricin catalytically inactivates the eukaryotic 60*S* ribosomal subunit. When the amino acid sequences of the two A subunits were compared, striking homology was found (9.3 SD above the mean of randomly permuted sequences) [35]. Although convergent evolution cannot be ruled out as an explanation of this homology, it is attractive to speculate that the two toxic A chains evolved from a common ancestor. Given the amino acid homology, it is not surprising that both toxins have the same biochemical mode of action, cleaving the *N*-glycosidic bond of adenine at nucleotide position 4324 in the 28*S* rRNA of the 60*S* ribosomal subunit [20].

In the structural model the Shiga toxin B chain represents the B domain, mediating binding of the holotoxin to susceptible cells. Isolated, renatured B subunit—but not A subunit—binds to HeLa cells. In addition, the B subunit competitively inhibits both the binding and the cytotoxicity of holotoxin to HeLa cells [33]. Shiga toxin binds to Gal-α1→4Gal-containing glycolipids, and the isolated B subunit competitively inhibits the binding of toxin to these glycolipids [32]. The primary amino acid sequence of the Shiga toxin B subunit consists of 69 amino acids with one internal disulfide bond [36]. Comparison of amino acid homologies has revealed distant homology with cholera toxin B subunit and another Gal-α1→4Gal-binding protein, the adhesin protein of the P pilus of uropathogenic *E. coli* [37].

When thinking about a toxin molecule, one generally tends to consider that the binding domain is merely the cell delivery mechanism whereas the business portion of the molecule—that determining biologic activity—is the catalytically active domain. Shiga toxin is a molecule that underscores the importance of the binding domain in determining biologic activity. The A domain activity of this toxin, inhibition of protein synthesis, is similar to that of diphtheria toxin; unlike diphtheria toxin, however, Shiga toxin causes fluid accumulation in the rabbit intestine. In light of the mechanism of action of the two classic enterotoxins, cholera and *E. coli* LT, investigators have examined whether Shiga toxin is able to elevate intracellular levels of cyclic AMP. The conclusions of these studies are that Shiga toxin does not act like cholera or *E. coli* LT. Any elevation in intracellular levels of cyclic AMP occurs well after fluid accumulation begins [38]. An alternative explanation based on the biochemical action of Shiga toxin is that it inhibits protein synthesis in intestinal epithelial cells and secondarily alters functions involved in electrolyte transport. The data of Keenan et al. [39] are consistent with this explanation; these authors have shown by high-resolution light and scanning and transmission electron microscopy that purified Shiga toxin selectively damages absorptive villus epithelial cells in rabbit ileum, leaving secretory crypt cells and goblet cells intact. Our laboratory has obtained physiologic data demonstrating that this is the case [40, 41]. These data indicate that the enterotoxic activity of Shiga toxin is the result of the selective targeting of rabbit intestinal absorptive cells and is mediated by the B subunit of the toxin and the presence of a functional receptor on these cells [42].

Downloaded from http://cid.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 13, 2016

## Conclusions

Structurally, Shiga toxin is not unlike cholera toxin. Both toxins consist of two polypeptide chains: a larger A subunit (mol wt, ~30,000) and a smaller B subunit (mol wt, 11,600 for cholera toxin and 7,700 for Shiga toxin). The subunit stoichiometry of both is 1A:5B. The A subunits of both toxins have a site that, after proteolytic nicking by trypsin and reduction of a disulfide bond, divides the polypeptide into two fragments. The larger fragment, $A_1$, possesses the catalytic activity of each toxin. The functional role of the B subunit of both toxins is to mediate binding to cell surface glycolipid receptors. Although structurally similar, the two toxins are quite distinct. Polyclonal or monoclonal antibodies that cross-react have not been reported, and the Gal-$\alpha$1→4Gal binding specificity and the rRNA *N*-glycosidase activity of Shiga toxin are totally different from the binding specificity and biochemical mode of action of cholera toxin.

At present, then, we define the Shiga-like family of toxins by their rRNA *N*-glycosidase activity on adenine 4324 of 28*S* rRNA and by their ability to bind to Gal-$\alpha$1→4Gal-containing glycolipids. The recent finding of *E. coli* Shiga-like toxin IIv, which has *N*-glycosidase activity but apparently does not bind to the same set of receptors, may eventually result in a refinement of the definition of the Shiga-like family [43]. Nevertheless, the basic structure and function of the subunits of Shiga toxin should be considered a model for the Shiga-like family of toxins.

### References


1. Conradi H. Ueber lösliche durch aseptische Autolyse erhaltene Giftstoffe von Ruhr- und Typhusbazillen. Dtsch Med Wochenschr **1903**; 29:26–8
2. Vicari G, Olitzki AL, Olitzki Z. The action of the thermolabile toxin of *Shigella dysenteriae* on cells cultivated *in vitro*. Br J Exp Pathol **1960**;41:179–89
3. Keusch GT, Grady GF, Mata LJ, McIver J. The pathogenesis of *Shigella* diarrhea. I. Enterotoxin production by *Shigella dysenteriae* 1. J Clin Invest **1972**;51:1212–8
4. Brown JE, Griffin DE, Rothman SW, Doctor BP. Purification and biological characterization of Shiga toxin for *Shigella dysenteriae* 1. Infect Immun **1982**;36:996–1005
5. Donohue-Rolfe A, Keusch GT, Edson C, Thorley-Lawson D, Jacewicz M. Pathogenesis of shigella diarrhea. IX. Simplified high yield purification of shigella toxin and characterization of subunit composition and function by the use of subunit-specific monoclonal and polyclonal antibodies. J Exp Med **1984**;160:1767–81
6. Eiklid K, Olsnes S. Animal toxicity of *Shigella dysenteriae* cytotoxin: evidence that the neurotoxic, enterotoxic, and cytotoxic activities are due to one toxin. J Immunol **1983**;130:380–4
7. O'Brien AD, LaVeck GD, Griffin DE, Thompson MR. Characterization of *Shigella dysenteriae* 1 (Shiga) toxin purified by anti–Shiga toxin affinity chromatography. Infect Immun **1980**;30:170–9
8. Konowalchuk J, Dickie N, Stavric S, Speirs JI. Comparative studies of five heat-labile toxic products of *Escherichia coli*. Infect Immun **1978**;22:644–8
9. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med **1983**;308:681–5
10. O'Brien AD, Lively TA, Chen ME, Rothman SW, Formal SB. *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a *Shigella dysenteriae* 1 (Shiga) like cytotoxin [letter]. Lancet **1983**;1:702
11. O'Brien AD, Newland JW, Miller SF, Holmes RK, Smith HW, Formal SB. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science **1984**; 226:694–6
12. Scotland SM, Smith HR, Rowe B. Two distinct toxins active on Vero cells from *Escherichia coli* O157 [letter]. Lancet **1985**;2:885–6
13. Strockbine NA, Marques LRM, Newland JW, Smith HW, Holmes RK, O'Brien AD. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun **1986**;53:135–40
14. Strockbine NA, Jackson MP, Sung LM, Holmes RK, O'Brien AD. Cloning and sequencing of the genes for Shiga toxin from *Shigella dysenteriae* type 1. J Bacteriol **1988**;170:1116–22
15. Takao T, Tanabe T, Hong YM, Shimonishi Y, Kurazono H, Yutsudo T, Sasakawa C, Yoshikawa M, Takeda Y. Identity of molecular structure of Shiga-like toxin I (VT1) from *Escherichia coli* O157:H7 with that of Shiga toxin. Microb Pathog **1988**;5:57–69
16. Jackson MP, Neil RJ, O'Brien AD, Holmes RK, Newland JW. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. FEMS Microbiol Lett **1987**;44:109–14
17. O'Brien AD, LaVeck GD. Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect Immun **1983**;40:675–83
18. Waddell T, Head S, Petric M, Cohen A, Lingwood C. Globotriosyl ceramide is specifically recognized by the *Escherichia coli* verocytotoxin 2. Biochem Biophys Res Commun **1988**;152:674–9
19. Cohen A, Hannigan GE, Williams BRG, Lingwood CA. Roles of globotriosyl- and galabiosylceramide in verotoxin binding and high affinity interferon receptor. J Biol Chem **1987**;262:17088–91
20. Endo Y, Tsurugi K, Yutsudo T, Takeda Y, Ogasawara T, Igarashi K. Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA *N*-glycosidase activity of the toxins. Eur J Biochem **1988**;171:45–50
21. O'Brien AD, LaVeck GD, Thompson MR, Formal SB. Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. J Infect Dis **1982**;146:763–9
22. O'Brien AD, Chen ME, Holmes RK, Kaper J, Levine MM. Environmental and human isolates of *Vibriocholerae* and *Vibrio parahaemolyticus* produce a *Shigella dysenteriae* 1 (Shiga)–like cytotoxin. Lancet **1984**;1:77–8
23. Olsnes S, Eiklid K. Isolation and characterization of *Shigella shigae* cytotoxin. J Biol Chem **1980**;255:284–9
24. Brown JE, Rothman SW, Doctor BP. Inhibition of protein synthesis in intact HeLa cells by *Shigella dysenteriae* 1 toxin. Infect Immun **1980**;29:98–107
25. Keusch GT, Donohue-Rolfe A, Jacewicz M, Kane AV. Shiga toxin: production and purification. In: Harshman S, ed. Methods in enzymology. Vol. 165. Microbial toxins: tools in enzymology. San Diego: Academic Press, **1988**:152–62, 399–401
26. Dubos RJ, Geiger JW. Preparation and properties of Shiga toxin and toxoid. J Exp Med **1946**;84:143–56
27. van Heyningen WE, Gladstone GP. The neurotoxin of *Shigella shigae*. 1. Production, purification and properties of the toxin. Br J Exp Pathol **1953**;34:202–16
28. Acheson D, Kane A, Keusch G, Donohue-Rolfe A. One step analogue affinity chromatography: purification of Shiga toxin from *S. dysen-*


Downloaded from http://cid.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 13, 2016

*teriae* 1 [abstract B 86]. In: Abstracts of the 89th annual meeting of the American Society for Microbiology. Washington, DC: American Society for Microbiology, **1989**
29. Landsteiner K, Levine P. Further observations on individual differences of human blood. Proc Soc Exp Biol Med **1927**;24:941–2
30. Marcus DM, Kundu SK, Suzuki A. The P blood group system: recent progress in immunochemistry and genetics. Semin Hematol **1981**;18: 63–71
31. Morgan WTJ, Watkins WM. Blood group $P_1$ substance: (I) chemical properties. In: Proceedings of the 9th Congress of the International Society for Blood Transfusion. Basel, Switzerland: Karger, **1964**:225–9
32. Jacewicz M, Clausen H, Nudelman E, Donohue-Rolfe A, Keusch GT. Pathogenesis of shigella diarrhea. XI. Isolation of a shigella toxin-binding glycolipid from rabbit jejunum and HeLa cells and its identification as globotriaosylceramide. J Exp Med **1986**;163:1391–404
33. Donohue-Rolfe A, Jacewicz M, Keusch GT. Isolation and characterization of functional Shiga toxin subunits and renatured holotoxin. Mol Microbiol **1989**;3:1231–6
34. Reisbig R, Olsnes S, Eiklid K. The cytotoxic activity of *Shigella* toxin. Evidence for catalytic inactivation of the 60 S ribosomal subunit. J Biol Chem **1981**;256:8739–44
35. Calderwood SB, Auclair F, Donohue-Rolfe A, Keusch GT, Mekalanos JJ. Nucleotide sequence of the Shiga-like toxin genes of *Escherichia coli*. Proc Natl Acad Sci U S A **1987**;84:4364–8
36. Seidah NG, Donohue-Rolfe A, Lazure C, Auclair F, Keusch GT, Chrétien M. Complete amino acid sequence of *Shigella* toxin B-chain. A novel polypeptide containing 69 amino acids and one disulfide bridge. J Biol Chem **1986**;261:13928–31
37. Lund B, Lindberg F, Marklund BI, Normack S. The PapG protein is the $\alpha$-D-galactopyranosyl-(1→4)-$\beta$-D-galactopyranose-binding adhesin of uropathogenic *Escherichia coli*. Proc Natl Acad Sci U S A **1987**;84:5898–902
38. Keusch GT, Donohue-Rolfe A, Jacewicz M. *Shigella* toxin(s): description and role in diarrhea and dysentery. Pharmacol Ther **1981**;15: 403–38
39. Keenan KP, Sharpnack DD, Collins H, Formal SB, O'Brien AD. Morphologic evaluation of the effects of Shiga toxin and *E. coli* Shiga-like toxin on the rabbit intestine. Am J Pathol **1986**;125:69–80
40. Kandel G, Donohue-Rolfe A, Jacewicz M, Donowitz M, Keusch GT. Shigella toxin (ST) selectively targets villus but not crypt cells in rabbit jejunum, providing an explanation for its enterotoxic activity [abstract]. Gastroenterology **1987**;92:1459
41. Keusch GT, Jacewicz M, Mobassaleh M, Donohue-Rolfe A. Shiga toxin: intestinal cell receptors and pathophysiology of enterotoxic effects. Rev Infect Dis **1991**;13(Suppl 4):S304–10
42. Mobassaleh M, Donohue-Rolfe A, Jacewicz M, Grand RJ, Keusch GT. Pathogenesis of shigella diarrhea: evidence for a developmentally regulated glycolipid receptor for shigella toxin involved in the fluid secretory response of rabbit small intestine. J Infect Dis **1988**;157:1023–31
43. Weinstein DL, Jackson MP, Samuel JE, Holmes RK, O'Brien AD. Cloning and sequencing of a Shiga-like toxin type II variant from an *Escherichia coli* strain responsible for edema disease of swine. J Bacteriol **1988**;170:4223–30

Downloaded from http://cid.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 13, 2016