The Rediscovery of Shiga Toxin and Its Role in Clinical Disease

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INTRODUCTION

By the end of the 19th century, the fruits of the new science of microbiology began to be realized and one discovery followed another with rapid succession. This year, 1998, celebrates the 100th anniversary of one of these important discoveries by the Japanese microbiologist Kiyoshi Shiga, who identified the etiologic agent of epidemic dysentery in Japan in 1898 (1, 2). Shiga called this organism *Bacillus dysenteriae*, however its genus name was subsequently changed to *Shigella* to honor its discoverer and *S. dysenteriae* type 1 is still often referred to as Shiga's bacillus.

Dr. Shiga, just 26 years of age at the time of his discovery, was the student of Shibasaburo Kitasato, another pioneering Japanese microbiologist and founding director of The Kitasato Institute where we gather for this International Symposium Celebrating the Centennary of the Discovery of Shiga's bacillus. Among his many scientific achievements Dr. Kitasato showed that tetanus and diphtheria toxoids induce antibodies that actively and passively protect animals from their lethal effects (3). These findings set the stage for the development of tetanus and diphtheria toxoid vaccines, which continue to be used to the present day and have contributed to the survival of countless millions of humans over the past century. Dr. Kitasato, trained in the laboratory of Robert Koch and Dr. Shiga, who later studied with Paul Ehrlich in Germany, believed that microbial pathogenesis could often be ascribed to soluble toxins and that anti-toxins and toxoid vaccines were the way to protect the host. It is interesting that 100 years after the discovery of Shiga's bacillus, and his own attempts to develop a toxoid vaccine and passive immunotherapy,
we are returning to this same theme as one of the more promising strategies to induce protection against human illness due to Shiga toxin-producing bacteria.

In this presentation, I will trace the history of Shiga toxin, its rediscovery in 1969, the remarkable progress that has been made in the past 3 decades towards understanding its structure, function, and role in clinical disease, leading us to hope for clinically effective toxoid vaccines and passive immunotherapy in the near future for at least some of the manifestations of Shiga toxin-producing pathogens.

The History of Shiga Toxin

The Early Years, 1898-1947. Although Dr. Shiga and other workers (4) produced evidence that S. dysenteriae type 1 produced a toxic factor in the first few years after Dr. Shiga's publication, the product was highly contaminated with lipopolysaccharide (LPS) endotoxin and it was difficult to distinguish toxic manifestations that were not due to LPS. The first convincing description of symptoms distinct from LPS was that of Conradi in 1903 (5), who is generally credited with the description of the "neurotoxin" effects of parenteral injection of extracts of the organism which caused limb paralysis and death in mice. However, despite the demonstration that the toxic factor induced protective antibody by Bessau in 1911 (6), and considerable work by Dr. Shiga himself, separation of the neurotoxin from endotoxin remained a challenge. Writing in 1936 (7), after nearly 4 decades of work on the topic, Shiga concluded that "dysentery toxin by its nature belongs to the endotoxins and is a constituent of the bacillary body. At its death the toxin is liberated into the surrounding medium... (yet) dysentery toxin produces antitoxin. This contradicts the idea we had hitherto held in regard to an endotoxin." Soon thereafter, however, Boivin and Mesrobeanu published convincing data that Shiga neurotoxin was a protein liberated into the medium following bacteriolysis and was separable from LPS (8). Towards the end of this first half century of work on Shiga toxin Rene Dubos demonstrated that toxin production in vitro was regulated by external environmental factors, specifically iron availability (9), a theme that has returned to a place of prominence in current understanding of the molecular basis of microbial pathogenesis (10).

The Pre-Modern Era, 1948-1967. The next 20 years constitute a distinct phase of continuing research on Shiga toxin as an interesting and potent
Table 1. State of Knowledge 65 Years Following the Discovery of Shiga Toxin

1. Shiga toxin is a protein, distinct endotoxin.
2. Shiga toxin is produced by just one *Shigella* species, *S. dysenteriae* type 1 (Shiga's bacillus).
3. Shiga toxin is a "neurotoxin" in vivo and a "cytotoxin" in tissue culture in vitro.
4. Shiga toxin production in vitro is regulated by iron and anaerobiosis.
5. Shiga toxin has no clear relationship to the pathogenesis of shigellosis.

microbial product without direct relevance to the disease caused by *S. dysenteriae* type 1. In 1953, using the best methods of the day van Heyningen and Gladstone (11) achieved significant purification of Shiga toxin, permitting studies of its biological properties that were previously not possible. For example, several groups in Great Britain explored the basis for the clinical effects of Shiga toxin in animal models, and concluded that "neurotoxic" manifestations were due to toxin-induced hemorrhage in the central nervous system (12, 13). It was not long before the same toxin was tested in animal cell cultures by Vicari et al (14) who reported that neurotoxin was cytotoxic to some but not other cells, indicating some target specificity for the toxin. Despite this steady progress (Table 1), Shiga toxin was relegated to the role of a biological curiosity, no doubt because it was produced by just one member of the genus *Shigella*, because *S. dysenteriae* type 1 was rarely isolated any more from humans with bacillary dysentery, and because "neurotoxin" activity in animals had no obvious relevance to clinical shigellosis.

**The Early Modern Era, 1968-1976.** Thirty years ago, a large epidemic of dysentery began in Guatemala and Mexico, with a sharp increase in the number of dysentery deaths. The outbreak was attributed to epidemic amebiasis, since no bacterial pathogens were found except for amebic cysts in the stool. In 1969, however, Dr. Leonardo Mata working at the Instituto de Nutricion de Centro America y Panama (INCAP) in Guatemala City correctly identified *S. dysenteriae* type 1 as the cause, demonstrating that epidemic bacillary dysentery due to Shiga's bacillus had returned to the world, and with a vengeance (15). With this information, treatment was changed from the toxic and sometimes lethal emetine regimen for amebiasis to antibiotics active against Shiga's bacillus, and the mortality rates plummeted from 20-25%, figures similar to the epidemics in Japan of the 1890's, to under 1%.

In the summer of 1969 I was a post-doctoral infectious diseases fellow...
Table 2. Effect of Crude MK-102 Crude Enterotoxin on Ileal Fluid Secretion

<table>
<thead>
<tr>
<th>Material Tested</th>
<th>Number of Ileal Loops Tested</th>
<th>Fluid Production (ml/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Supernatant</td>
<td>42</td>
<td>1.02±0.12</td>
</tr>
<tr>
<td>Heated Supernatant</td>
<td>9</td>
<td>0.00</td>
</tr>
<tr>
<td>Media Control</td>
<td>13</td>
<td>0.08±0.06</td>
</tr>
</tbody>
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working with Dr. Nevin Scrimshaw at INCAP on the interactions of malnutrition and infection. I took the opportunity to go to the field with Dr. Mata to see these patients, and I noted that many were presenting with watery diarrhea, in contrast to the textbook descriptions of bloody diarrhea and dysentery. Because my own laboratory research concerned the mechanism of cholera enterotoxin, it was an obvious question to ask whether or not a *Shigella* "enterotoxin" might be involved. Together with Mata, I grew a fresh isolate from patient 102, harvested the cell-free spent growth medium, and inoculated these into ligated rabbit ileal loops. Within 36 hours of posing the question we had an answer - yes the cell-free medium from strain MK-102 caused fluid accumulation in the ligated ileal loops, the definition of an "enterotoxin" (Table 2) (16). The "enterotoxin" was partially purified by chromatography on Sephadex G-150, and it co-migrated with mouse lethality, the assay for "neurotoxin". This partially purified enterotoxin also caused an inflammatory enteritis in the rabbit, with apoptosis and death of intestinal epithelial cells leading to mucosal ulcerations (17), the characteristic manifestations of shigellosis. These findings suggested that a cell-free toxin played a role in the disease and I have since spent nearly 30 years working on the biological significance of this discovery.

Whereas the epidemic in Central America and Mexico subsided, epidemic Shiga bacillus dysentery broke out in Bangladesh in 1975 and then spread to India. By that time I had obtained a sample of the 1953 lyophilized purified "neurotoxin" from Dr. Van Heyningen as well as several vials of the anti-dysentery toxin serum produced by Dr. Shiga himself for the League of Nations in the 1920's, which was still stored at the Serumstatins Institute in Denmark. Using these reagents I was able to demonstrate that Van Heyningen's neurotoxin was an enterotoxin in the rabbit ileal loop model, and that antiserum standardized against neurotoxin activity by Dr. Shiga in the 1920's neutralized enterotoxicity, leading to the conclusion that "*Shigella* enterotoxin" was indeed "Shiga toxin rediscovered" (18). More modern protein separation and detection
methods also revealed that the Van Heyningen neurotoxin contained multiple protein bands, while our own toxin preparation, albeit much cleaner, still had multiple bands. Purification of Shiga toxin to homogeneity was later reported by several groups using different methods (19-21), and these “pure” toxins exhibited neurotoxic, cytotoxic and enterotoxic activities. In 1976, together with Dr. Myron Levine's group at the University of Maryland, we reported that human volunteers infected with virulent \textit{S. dysenteriae} type 1 developed toxin neutralizing serum antibodies (22), proving that the toxin must be made in vivo during the infection.

\textbf{The Mid-Modern Era, 1977-1987.} In 1977, the same year my lab reported evidence for a cell surface “\textit{Shigella} toxin” receptor (23), Konowalchuk et al (24) reported that certain strains of \textit{E. coli} produced a cytotoxin for Vero cells, which subsequently became known as Verotoxin (VT). The following year, workers in Bangladesh described hemolytic-uremic syndrome (HUS) following \textit{Shiga} bacillus dysentery (25), as well as its association with endotoxemia and leukemoid reactions, both of which may be related to the pathogenesis of this devastating complication of an already serious disease. Then in 1982, two outbreaks of hemorrhagic colitis occurred in the United States manifested by abdominal cramps and bloody diarrhea, caused by a previously rare serotype of \textit{E. coli}, O157:H7, traced to contaminated fast food restaurant hamburgers (26). O'Brien and colleagues then showed that this strain produced a VT-like cytotoxin and that it was neutralized by antibody to Shiga toxin (27), leading to its designation as Shiga-like toxin (SLT). It soon was appreciated that there were two related serologically distinct toxins, each encoded by a separate transforming phage designated SLT-I (VT-1) and SLT-II (VT-2) (28). The relevance of Shiga toxin itself to pathogenesis of human illness was further supported when Karmali et al reported that sporadic cases of HUS were associated with VT/SLT-producing \textit{E. coli}, as had been shown previously with Shiga toxin producing bacillus dysentery (29). They also reported finding free cytotoxin activity in the stool. My lab subsequently developed a sensitive ELISA for Shiga toxin and we not only reported that free toxin was present in the stool of patients with Shiga bacillus dysentery but we also purified the activity from the stool and found that it had the same subunit structure as authentic Shiga toxin on a Western blot using specific antibodies (30). With HUS now associated with two different organisms having in common the ability to produce Shiga toxin in vivo the potential relevance of toxin to human disease was no longer in doubt - the question was, and remains, how does this happen?
The purification of toxin and the ability to tag it with a $^{125}$I label (31) set the stage for further progress. Toxin was demonstrated to be a heterodimeric protein consisting of an A subunit that inhibited protein synthesis in a cell free system, and a pentamer of 5 B subunits that mediated the binding of toxin to sensitive tissue culture cells and rabbit intestinal microvillus membranes (28). In 1986, while searching for a glycoprotein receptor, my laboratory found that toxin bound only to the lipid front on SDS-polyacrylamide gels. We therefore made lipid extracts of either HeLa cells or rabbit small bowel microvillus membranes, separated these by thin layer chromatography, and studied toxin binding in a solid phase assay. We identified a binding site which migrated as globotriaosylceramide (Gb3), a neutral globoseries glycolipid consisting of a trisaccharide composed of galactose-$\alpha 1\rightarrow4$ galactose-$\beta 1\rightarrow4$ glucose linked $\beta 1$ to ceramide and fatty acids (32). The following year, Lindberg and colleagues reported their independent studies of the previous several years using purified isolated glycolipids on a solid phase also showing that Shiga toxin bound to Gb3 (33).

In order to prove the relevance of Gb3 binding to cytotoxicity, several groups including our own demonstrated that this activity of Shiga toxin was proportional to the amount of Gb3 in the cells employed in the assay (Table 3) (34). By limiting dilution methods, we cloned hypersensitive as well as highly resistant HeLa cells from a standard ATCC HeLa cell line, and again sensitivity to toxin was proportion to the amount of Gb3 compared to the parent cells (35). We also produced Gb3 deficient toxin-resistant HeLa and Vero cells by incubating the cells with an inhibitor of neutral glycolipid synthesis, PDMP. We could restore the latter cells to the sensitive phenotype by means of liposomal fusion methods to insert

<table>
<thead>
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<th>Table 3. Relationship of Cellular Gb3 Content to Sensitivity to Shiga Toxin</th>
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<tbody>
<tr>
<td><strong>Cell Line</strong></td>
</tr>
<tr>
<td>5E7</td>
</tr>
<tr>
<td>3C8</td>
</tr>
<tr>
<td>5C7</td>
</tr>
<tr>
<td>2B7</td>
</tr>
<tr>
<td>Parent 229</td>
</tr>
<tr>
<td>3F3</td>
</tr>
<tr>
<td>Parent CCL-2</td>
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the receptor, Gb3, but not the related glycolipid Gb4 which contains a terminal GalNAc linked $\beta 1\rightarrow 4$ to Gb3. The specificity of this interaction was shown by the ability of Gb4 to restore sensitivity to the related porcine edema disease variant SLT-II toxin, which had been reported to exhibit relative specificity for Gb4 by Lingwood's group (36). Our earlier work (37, 38), and more recent studies by Sandvig et al (39, 40), showed that toxin translocation across the cell membrane was essential for its effects on cellular protein synthesis, that this process was dependent on the presence of the glycolipid toxin receptor, and that the mechanism of translocation had the characteristics of receptor-mediated endocytosis.

In 1987 two groups (my laboratory working together with John Mekalano's group (41), and the laboratory of Alison O'Brien (42)) cloned and sequenced the gene for the SLT-I/VT-1 toxin from E. coli. The predicted sequence of the toxin B subunit was identical to the amino acid sequence we had obtained by standard protein sequencing methods for the B subunit of Shiga toxin from S. dysenteriae type 1 (43). We also found a region of the A subunit with homology to the A subunit of the plant toxin ricin (41), although the significance of this was uncertain at the time. In 1987, a group of Japanese workers including Yoshifumi Takeda, described the mechanism of action of ricin as an RNA N-glycosidase enzyme that removed the ribose from one specific adenine residue in 28S ribosomal RNA, rendering it inactive in protein synthesis (44). It was not long before the same group examined the mechanism of action of Shiga toxin, and demonstrated that it shared the identical enzymatic activity, including the identical rRNA target; in other words, Shiga toxin was procaryotic ricin. A decade ago, then, Shiga toxin from S. dysenteriae type 1 and some of the related toxins from E. coli had been purified, we knew the cell surface receptors and the mechanism of action of these toxins, we had genetic probes for the toxin genes, and it was strongly suspected that these toxins played a role in the pathogenesis of HUS associated with toxin-producing bacteria.

The Late Modern Era, 1988-1997. The past decade has seen even more rapid progress in understanding these toxins and their role in human disease. The identification, purification, cloning and sequencing of several additional immunologically related toxin variants, and the realization that each shared the same mechanism of action as well as stereospecific binding to glycolipids containing the disaccharide galactose- $\alpha 1\rightarrow 4$-galactose, led to the insight that these toxins constituted a family with major sequence homology at the nucleotide and peptide level, the
Table 4. New Nomenclature for the Shiga Family of Toxins

<table>
<thead>
<tr>
<th>Microbial Source</th>
<th>Gene Designation</th>
<th>Toxin Name</th>
<th>Prior Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. dysenteriae type 1</td>
<td>stx</td>
<td>Shiga toxin (Stx)</td>
<td>Shiga toxin</td>
</tr>
<tr>
<td></td>
<td>stx1</td>
<td>Shiga toxin 1 (Stx1)</td>
<td>Shiga-like toxin I Verotoxin 1</td>
</tr>
<tr>
<td></td>
<td>stx2</td>
<td>Shiga toxin 2 (Stx2)</td>
<td>Shiga-like toxin II Verotoxin 2</td>
</tr>
<tr>
<td></td>
<td>stx2c*</td>
<td>Shiga toxin 2c (Stx2c)</td>
<td>Shiga-like toxin IIc Verotoxin 2c</td>
</tr>
<tr>
<td></td>
<td>stx2d*</td>
<td>Shiga toxin 2d (Stx2d)</td>
<td>Shiga-like toxin IId Verotoxin 2d</td>
</tr>
<tr>
<td></td>
<td>stx2e**</td>
<td>Shiga toxin 2e (Stx2e)</td>
<td>Shiga-like toxin Ile Verotoxin 2e</td>
</tr>
</tbody>
</table>

* human Stx2 variant toxins
** porcine edema disease Stx2 variant toxin

prototype of which was Shiga toxin itself (45). On this basis it was proposed that a more rational nomenclature based the name for the gene for Shiga toxin from S. dysenteriae type 1, stx be employed (Table 4) (46). It is even more propitious to implement this change in 1998, the 100th anniversary year of the discovery by Shiga of the prototype organism for the genus Shigella. The family includes Stx1, closely related to Stx itself, and Stx2, which is approximately 57% homologous to Stx1 at the nucleotide and amino acid levels, and several variant toxins immunologically related to Stx2. The latter include Stx2c, a toxin produced by human virulent STEC distinguished by specific amino acid differences from Stx2, preferential binding to certain species of Gb3, and preferential toxicity to Vero rather than HeLa cells, Stx2d, also obtained from human strains but which is activated by intestinal mucus, and Stx2e which has a profound binding preference for Gb4 rather than Gb3, is poorly transported across the intestinal mucosa, and is a critical virulence factor in porcine "edema disease". Stx2, while sharing the critical structure-function attributes of Stx1, including the same enzymatic specificity and exclusive binding to Gb3, is both less avid in its binding to its receptor and more active in several biological assay systems, and it is epidemiologically more closely linked to HUS than is Stx1.

The sequencing of the toxin genes has revealed a number of critical features. First, there is a signal sequence in the Stx and Stx1 operon but not in Stx2 or its variants. Second, Stx and Stx1, but not Stx2 or its variants, has a consensus Fur binding sequence in the promotor region
which mediates iron regulation via the *fur* gene system. Third, the gene for the A subunit is upstream of the B subunit, separated by just 8 nucleotides. Fourth, the A subunit has two cysteines near the C terminus that form an interchain disulfide bond, within which is a tryptic proteolytic site. Toxin activity is increased dramatically by proteolytic nicking, and this appears to take place primarily within an intracellular compartment, and there is good evidence that this event is mediated by the intravesicular protease, furin (47). Removal of the interchain loop by site-directed mutagenesis of one cysteine results in an initial enhancement of toxin activity, perhaps by another proteolytic event, but much less total activity at later times of incubation, apparently due to enhanced degradation of the toxin when the disulfide loop is not present (48).

Further work on the toxin-receptor interaction by Lingwood and his colleagues has shown that the receptor function of Gb3 is not restricted to the carbohydrate ligand. For example, they have found that the chain length and saturation of the fatty acids on the ceramide portion of the receptor may vary while the trisaccharide, which defines the Gb3 specificity, remains constant. However, these changes in the lipid portion exhibits a substantial influence on the cytotoxic action of the toxin by an influence on toxin translocation and intracellular trafficking. Toxin uptake is known to proceed via receptor mediated endocytosis at coated pits. Some studies have suggested that there is a second high-affinity low-capacity receptor (34), however its role, if any, in translocation of toxin is uncertain. It is interesting that insertion of Gb3 into the plasma membrane of Gb3-deficient CHO cells converts these cells to a toxin binding phenotype but does not sensitize them to the toxin, in contrast to insertion of Gb3 in Gb3 deficient HeLa cells (49). It is possible that this second receptor is necessary to translocate toxin bound to the glycolipid “docking” receptor to the cytoplasm. Vesicles containing the toxin are then transported to the Golgi region and in retrograde fashion to the endoplasmic reticulum (50). There the enzymatically active A1 subunit is released, gains access to the cytoplasm, and catalytically and irreversibly inhibits ribosomal protein synthesis by its action on a single specific adenine residue in the 28S ribosomal subunit.

Purification of Stx toxins and subunits in large scale quantity have permitted their crystallization and 3-dimensional constructions of the relationship of the A and B pentamer subunits (51, 52). The crystal structure reveals the A1 subunit positioned above the B pentamer, with the linear A2 chain extending through the central hole of the B pentamer.
The active site, by analogy to the crystal structure of ricin and the identification of key active site residues by site-directed mutagenesis, is a groove into which the substrate fits. The sites of interaction between the B pentamer and the terminal Gal$_\alpha$1→4-Gal disaccharide of Gb3 have also been identified and the receptor site and its adjacent key residues are clearly identified (53). Unfortunately, Stx2 has not yet been successfully crystallized, and while it is assumed that it looks generally similar to Stx and Stx1, the reasons for the major differences in its biological behavior remain unknown.

Another major insight in this past decade has been the identification of endothelial cells (EC) as major targets for Shiga family toxins (54). Until this study, all attention focused on the intestinal epithelial cell, however the realization that toxin production was the common factor between the two types of bacteria epidemiologically linked to the microangiopathic process HUS, that is S. dysenteriae type 1 and STEC, prompted the investigation of toxin effects on endothelium. A number of studies have been performed over the ensuing 10 years, revealing that EC sensitivity to the cytotoxic action of Stx toxins varies with the origin of the EC, their state of activation, and the specific toxin being tested. The macrovascular EC such as human umbilical vein endothelial cells (HUVEC) are quite resistant unless activated by LPS (55) or LPS-induced cytokines such as IL-1$\beta$ and TNF$\alpha$ (56), EC derived from human saphenous veins (SVEC) are moderately sensitive as isolated but are sensitized further by pre-exposure to LPS or cytokines (57), while renal glomerular (58) (GMEC) and human intestinal microvascular EC (HIMEC)(59) are highly sensitive and not altered by LPS or cytokine pre-treatment. Sensitivity among these cells is determined by the level of constitutive expression of Gb3, ranging from very low to very high in the HUVEC and GMEC/HIMEC, respectively, with SVEC somewhere in between. The pathways of regulation appear to be complicated (60), and may be influenced by the conditions of endothelial cell isolation and growth in vitro, and the number of passages of these cells. Much remains to be learned about toxin-EC interactions, particularly with respect to the release of biologically active pro- and anti-coagulants that may affect platelet-EC interactions, the initiation of platelet thrombi and the nidus for a microangiopathic hemolytic process.

At the present time, nearly 30 years after the rediscovery of Shiga toxin, we are in a much better position to evaluate its role in disease pathogenesis. A set of conditions known as Koch's postulates have been
established to evaluate the role of specific microorganisms as the etiologic agents of a disease; in like manner a set of postulates to evaluate the role of toxins in disease pathogenesis have been proposed (Table 5) (61). As applied to the family of Shiga toxins we can affirm first that HUS associated with a diarrheal prodrome is due to Stx-producing organisms (postulate 1), although there are other causes of HUS. Second, we have been able to isolate and purify these toxins. Third, these toxins produce manifestations in experimental animals that resemble the human disease, including thrombotic microangiopathy, even if there is no model that fully reproduces the hallmarks of HUS in humans. Fourth, specific antibody blocks these toxin mediated manifestations in experimental models. These findings suggest the potential utility of developing toxin based immunological control measures for the Shiga toxin producing bacterial infection.

The Immediate Future, 1998-2003  The future is in many ways beginning with this historic meeting at the Kitasato Institute. In the course of this meeting, the clinical, epidemiological and biological features of shigellosis and STEC infections will be thoroughly reviewed, highlighting the prospects for developing new control measures. With respect to the Shiga toxins there are already clinical trials underway to fully examine the potential of adsorption of toxin in the intestinal lumen using a non-absorbable receptor analogue (Synsorb-pk) (62). This strategy aims at blocking toxin uptake to the systemic circulation by irreversibly binding toxin to a silica particle that will be excreted in the stool, however the toxin pool present in the bowel lumen may be of limited importance in pathogenesis, as the small amount of toxin needed for systemic manifestations may be absorbed directly at the site of the intimate attachment of
the organisms to the mucosa. The results of the current trials for prevention and treatment of HUS will become available in the next several years.

A second possibility for the next few years is the application of screening methods and possibly vaccines for the animal reservoirs in developed countries designed to reduce the contamination by STEC in the food supply. This will be difficult to implement, even in these wealthy countries, and will have little impact in the developing countries where STEC infections occur (63) but still appear to be uncommon (64). Even if such measures are successfully implemented, they will not alter the problem associated with endemic and epidemic multi-drug resistant Shiga bacillus dysentery in the third world, because animals are not involved in the transmission of this highly human host-adapted organism (65).

A third possibility is immunotherapy. In his famous address to Harvard University in 1936, Kiyoshi Shiga discussed the possibility of a toxoid based strategy for disease due to S. dysenteriae type 1, recounting his limited success using passive immunization with horse serum during the outbreaks of dysentery in Keiyo, Japan between 1925-1927 (7). He also noted that his attempts to produce a safe toxoid vaccine for human use had not been fruitful and “the hope of using a toxoid for prevention of the disease must be abandoned at present”. Approximately 60 years later, we must reconsider this. Indeed, the ability to purify the protein Shiga family toxins from endotoxin, which proved so difficult for Dr. Shiga, means that we need not worry about LPS side effects which caused so much toxicity in the early studies. The problem we face in producing a safe toxoid is attenuation of the biological activity of the toxin without alteration of its immunogenicity so that it can be safely given to humans to induce neutralizing antibodies. This may be achieved by classical toxoiding methods using protein denaturing agents such as formalin or glutaraldehyde, and such studies are underway (66). In addition, site directed mutagenesis can be employed to target the active site of the toxin to eliminate its biological activity, and such attenuated toxins can be further attenuated by toxoiding. I am certain that within the next 5 years we will have a safe toxoid for human use, and confident that it will be fully compatible with DTaP for immunization of infants to diphtheria, tetanus and pertussis, and will not diminish the response to the three antigens in this vaccine. Thus, the triple vaccine may soon become a quadruple vaccine for universal use. It is our plan as well to use the toxoid vaccine to immunize adults for the purpose of inducing high titer IgG antitoxin antibodies that may be isolated and purified into a
hyperimmune human immunoglobulin anti-Shiga toxin antibody for parenteral use. This product could then be used to quickly and passively immunize patients at high risk for HUS or already showing clinical manifestations of this syndrome. Because it would be of human origin, it would not be likely to cause serum sickness, and the kinetics of clearance of the antibody would be that of human immunoglobulin. A clinical trial would be necessary to determine whether or not it was successful in practice, but I am personally optimistic.

In 1936, Dr. Shiga commented that “I believe I shall retire next winter, and after I retire from bacteriology I suppose that dysentery will disappear” (7). Unfortunately, not only did dysentery not disappear, but Shiga’s bacillus has come back with a vengeance in the past 3 decades. It is now 1998, I am 60 years old, and it is reasonable to believe that I shall retire in the next few years. It is also possible that by the time I retire HUS, the major systemic manifestation of Shiga bacillus dysentery and STEC infections, will disappear as a result of active or passive immunization against the Shiga family toxins, and that the intestinal phase of these infections will be attenuated as well by the same strategy.

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