Preface to the Handbook

Natural toxins are unique toxins which possess some common properties, whether they are obtained from plants, microorganisms, or animals. One common characteristic is that they exert a pronounced effect on the metabolism and biological functions of the intoxicated animals with just a minute quantity. Since ancient times human beings have pondered the physiological effects of various toxins and venoms. How do these natural poisons work? Despite possessing some common nature, each toxin, however, has its unique mode of action and its own characteristic structure.

Drugs are compounds that have specific beneficial effects with a minute quantity. Usually, natural toxins also have very specific effects. Therefore, it is not surprising that many natural toxins are potentially good drugs.

Heretofore, the study of each field of toxins has been taking an independent pathway. Scientists in a specific toxin field are often unaware of the activity in other toxin fields. It is thus desirable to have a primary source of information on all natural toxins so that scientists in a specific discipline of toxin research can easily obtain useful information from other toxin researchers.

The editor expresses sincere thanks to Maurits Dekker for initiating this project.

Anthony T. Tu

Preface to Volume 8

The past decade has seen remarkable growth in our understanding of the structure and function of bacterial toxins as well as the recognition of some hitherto unknown members of this remarkable family. The contributors to this volume have attempted to provide, in relatively concise chapters, detailed and up-to-date information on many aspects of the structure and function of some of these fascinating toxic proteins.

Diphtheria toxin occasioned the first description of a mono-ADP-ribosyltransferase reaction, although it was considerably later that the ADP-ribose acceptor in elongation factor 2 was identified as diphthamide, a histidine that has undergone complex posttranslational modification. The molecule that serves as the diphtheria toxin receptor on the cell surface is still not completely characterized, although at least one part of it appears to be a heparin-binding EGF-like protein. Like many of the potent protein toxins, diphtheria toxin has two distinct domains. The A, or active, moiety acts catalytically within the cell, whereas the B, or binding, portion interacts with a specific receptor molecule on the cell surface and facilitates the entry of A. In some toxins, the A and B functions are carried out by separate proteins; in diphtheria toxin they are in different parts of the same polypeptide.

The ADP-ribosyltransferase activity of cholera toxin was first described in 1976; ganglioside G_{M1} was soon identified as the cell surface receptor and $G_{\alpha s}$, the stimulatory guanine nucleotide-binding protein of adenylyl cyclase, as the cellular substrate. It took somewhat longer, however, to identify the specific arginine residue that is modified. Many similarities among cholera toxin and the several *E. coli* heat-labile enterotoxins have been noted. Some of the most exciting current work in this area is elucidating the molecular mechanisms of assembly of these toxins and three-dimensional structures based on X-ray crystallography.

Pertussis toxin, an ADP-ribosyltransferase that modifies a cysteine near the carboxy terminus of several G protein α subunits, is only one of several *Bordetella pertussis* virulence factors, which include the pertussis adenylyl cyclase as well as the tracheal cytotoxin (TCT). The latter has recently been identified as a muramyl peptide that is

released during bacteria growth and is toxic to ciliated epithelial cells. It has been suggested that TCT stimulates macrophages to release IL-1, which induces production of nitric oxide (NO) with resulting inhibition of ribonucleotide reductase and ATP synthesis.

Several clostridial toxins are also ADP-ribosyltransferases. The substrate for C2, for example, is non-muscle actin in the monomeric form, which cannot polymerize after modification. A related clostridial transferase, C3, ADP-ribosylates a ~20-kDa GTP-binding protein termed rho. This toxin and similar C3-like toxins apparently lack a B component, and for many studies they have been artificially introduced into cells. Leukocidins, which are 30- to 40-kDa ADP-ribosyltransferases, have been purified and crystallized from *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Their cytocidal effects appear to involve cell protein kinase C as well as phospholipases A and C and calcium influx, although molecular mechanisms, including the proteins that are ADP-ribosylated, remain to be precisely defined.

Like *Bordetella pertussis*, *Bacillus anthracis* releases an adenylyl cyclase that increases host cell cAMP and thereby interferes with phagocytosis. The cyclase requires another protein termed PA (protective antigen) for activity, as does another product of *B. anthracis* called lethal factor (LF), which together with PA causes release of TNF and IL-1 from macrophages, with resulting cytotoxicity.

Several bacterial toxins increase cell cAMP content, because the toxin itself either catalyzes cAMP synthesis from cell ATP or, like cholera toxin, activates the endogenous adenylyl cyclase or, like pertussis toxin, blocks its inhibition. *E. coli* heat-stabile toxin, on the other hand, increases cGMP by acting at the surface of the intestinal cell to increase guanylyl cyclase activity. The toxin structure and effect on cGMP were known for almost a decade before the receptor-cyclase with which it interacts and then guanylin, the physiological peptide ligand for that receptor, were described.

Other toxins produced by certain strains of *E. coli* include Shiga and Shiga-like (Vero) toxins. The A portion (like ricin) has RNA-*N*-glycosidase activity; the B moiety binds to and defines the specificity of interaction with a globoside in the plasma membrane. In addition to its well-known ADP-ribosyltransferase, *Vibrio cholerae* secretes two other interesting toxins. One, named accessary cholera enterotoxin (Ace), causes fluid accumulation in the ileal loop model and ion secretion in vitro. Its deduced structure has similarities to known ion-transport ATPases. The other, termed Zot for zona occludens toxin, binds to the cell surface and reversibly alters actin microfilaments, thus loosening intercellular tight junctions.

The two *Clostridium difficile* toxins that cause pseudomembranous colitis apparently alter tight junctions by acting irreversibly on the cytoskeleton and cause other effects indirectly as a result of activation of host immune responses. Similarly, the devastating effects of endotoxin, which is a ubiquitous component of gram-negative organisms, result from activation of the host immune system, which then releases proinflammatory as well as anti-inflammatory mediators. Endotoxins differ somewhat in structure, but a glycophospholipid called lipid A, which reproduces all of their effects, is found in all, hence the interest in these molecules as targets for therapy or prevention of endotoxic shock.

The dire effects of tetanus toxin have been known for a very long time. It is only relatively recently, however, that its mechanisms of cell entry and processing have become known. Several neurotoxins produced by *Clostridium botulinum* resemble this product of *Clostridium tetani* in structure and function. In 1992, it was found that tetanus toxin, a metallo-protease, cleaves synaptobrevin, a critical synaptic vesicle protein, and thereby blocks neurotransmitter release. The ability of the toxin to undergo retrograde axonal

transport suggested its use to deliver other molecules (e.g., enzymes, antibodies) to sites in the central nervous system. In the past few years, a similar botulinum A toxin has found diverse applications in the treatment of muscle spasm or dystonia by intramuscular injection, although it is unclear just how localized the toxin remains during the period of therapeutic effectiveness. Other potential biomedical applications of toxins include the use of liposomes as carriers in vaccines and toxin-fusion proteins for delivery of a toxin to specific cells.

This volume offers, we hope, a summary of much (not all, of course) of the new information that is rapidly accumulating concerning several bacterial toxins that act in diverse and intriguing ways to subvert cellular function (metabolism/signaling). In many instances, unraveling a mechanism of toxin action has provided new information concerning cell physiology or biochemistry, as well as, not infrequently, new toxin tools for experimental studies or clinical therapy.

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