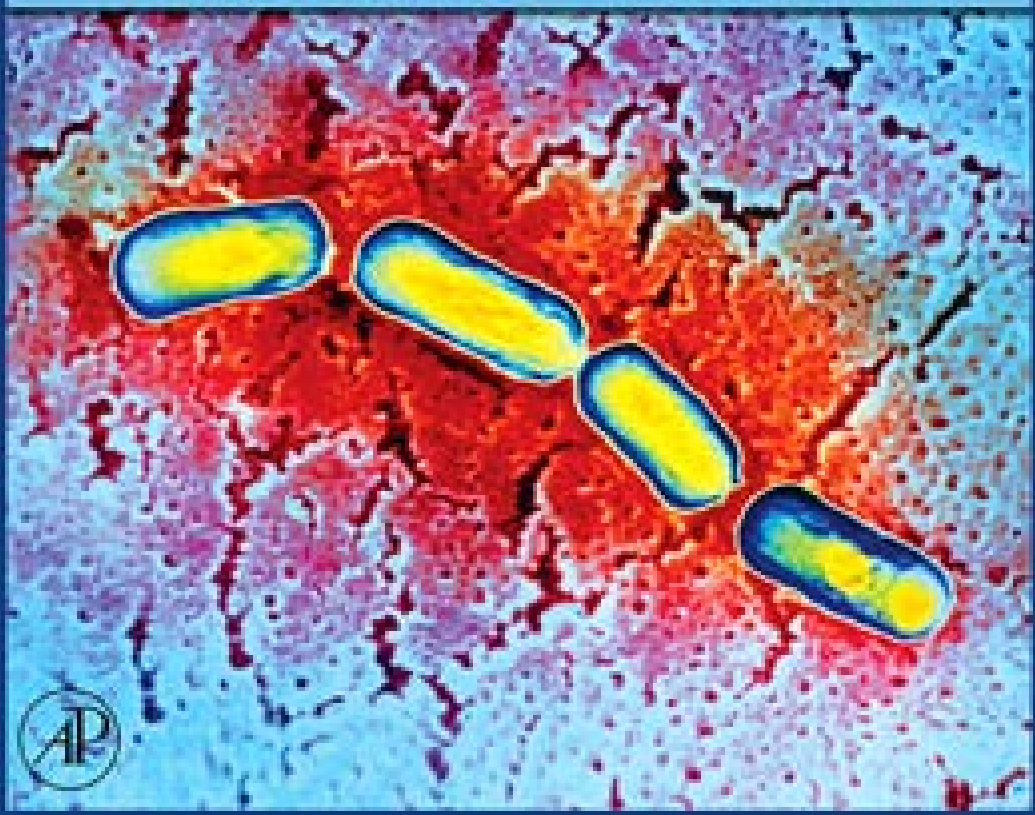
The Comprehensive Sourcebook of BACTERIAL PROTEIN TOXINS

Third Edition

Edited by JOSEPH E. ALOUF and MICHEL R. POPOFF



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and

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In Memoriam

During the making of this third edition of *The Comprehensive Sourcebook of Bacterial Protein Toxins*, Dr. Gianfranco Menestrina (Trento, Italy) unfortunately perished in a motorcycle accident on July 8, 2004. He was 50 years old. This has caused a deep loss and sadness for his family, friends, collaborators, and many of those who had any scientific exchange with him. Gianfranco Menestrina was always interested in membrane biophysics, membrane compounds, and associated regulation of osmotic compartments. He graduated in Physics in 1978 at the University of Trento, and had built his career (more than 95 published articles, many chapters of books, 30 graduated students, and 20 post-doctoral fellows) at the interface of Physics and Biology. In 2002, Gianfranco became the Director of the Section of Biophysics in Trento for the CNR Institute. He was a very active member of different Societies of Biophysics and Toxinology. He was awarded several times and received post-doctoral or visiting Professor Fellows at Universities of Bochum (G), London (UK), Wisconsin (USA), and La Habana (Cuba). His scientific interests swept across membrane-inserted polymers or peptides, with continual interest in pore-forming toxins. He was frequently chosen as a chairman in meetings and organized several meetings with recognized success.

Kind attention to anybody and caution in the language used were Gianfranco's marks. He provided a unanimous impression of an authentic man, of a true, honest, and rigorous scientist. Scientific collaboration with him and his lab was deeply fair, concerted, always sharing the goal of the best science of the moment with the environment provided. Presentation of a scientific problem or argument, an open mind, and explanations of experiments by him were always enriching, satisfying, or favoring scientific exchanges. Reliability, loyalty, friendship, fidelity, distinction, and a gentleman are words kept to the memory of Gianfranco.

Goodbye, Gianfranco; sympathies go to your family, and see you in a better world.

Gilles Prévost and Collaborators
Joseph E. Alouf
Michel R. Popoff

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Preface to Third Edition

An increasingly rapid expansion of our knowledge of the multifaceted aspects of bacterial protein toxins since the end of the 20th century necessitated the publication of this third edition of *The Comprehensive Sourcebook of Bacterial Protein Toxins.*

Among the most important achievement and discovery of the past six years was the explosion of new information in many domains of toxinology, illustrated by the discovery of more than 50 novel toxins (many of them identified through genome screening), extensive bacterial genome mapping, the determination of the nucleotide sequence of encoding genes and corresponding amino acid sequences, genetic regulation of toxin expression, genomic pathogenicity islands, identification of many toxin receptors at the surface of eucaryotic cells, toxin-induced modulation of various components, and effectors of intracellular signal transduction pathways and apoptotic events. Establishment of the three-dimensional crystal structure of more than 20 toxins during the same period allowed deeper knowledge of structure-activity relationships and provided a framework for understanding how toxins recognize receptors, penetrate membranes, and interact with and modify intracellular substrates. Moreover, the study of the structure of newly identified toxins demonstrated that toxins, which modify the cellular signaling pathways, possess the same active sites (functional mimicry) as those of corresponding cell proteins but with a different folding conformation. These new findings allow a better understanding of the mechanism of action of bacterial toxins at the subcellular level. In addition, bacterial toxins are extremely specific and exquisite tools to unravel physiological cell processes.

Recent progress also contributed to a better definition of major issues, namely toxin transcytosis and trafficking in eucaryotic target cells, mechanisms of action at the cellular and molecular levels, genetic and molecular mechanisms of toxin involvement in the virulence and pathogenicity of the relevant bacteria in many human diseases (cholera, anthrax, whooping cough, tetanus, botulism, diptheria, clostridial gas gangrenes, severe diseases caused by superantigenic toxins, *Helicobacter pylori*-associated peptic ulcers and carcinomas, food poisons, etc.). In this respect, the targeting of immune system cells by various toxins led to a better evaluation of both their beneficial effects (for example as immunomodulators in the case of cholera toxin B subunit) and pathophysiolgical effects in certain diseases in connection with the immune system. Finally, the past years witnessed considerable progress on toxin applications in vaccinology, tumor therapy, and new approaches in the treatment of various diseases. Whether some wild-type toxins can be directly used as therapeutic agents, protein engineering permits us to model more specific and efficient molecules or molecules with a novel activity, or to target a restricted subset of cell population. Novel recombinant toxins are already proposed in the treatment of some diseases, as well as new vaccines. One should also not forget the emergence of certain bacterial toxins as biological weapons (era of bioterrorism threats). This issue is considered in chapters dedicated to the relevant toxins (anthrax toxins, botulinal neurotoxins, the Shiga-like plant toxin, ricin) and is also discussed in a specific chapter.

The third edition of the *Sourcebook* is a genuine, timely production comprising 62 chapters (compared to 40 and 20 of the 1999 and 1991 editions, respectively) that are organized into five sections. They are written by a panel of 137 international experts (senior authors and coauthors), who have significantly contributed to the progress within their featured disciplines. About 55% of all contributors are new specialists who did not contribute to the preceding editions of the *Sourcebook*. Authors are from Australia, Belgium, Canada, the Czech Republic, France, Germany, Italy, Japan, New Zealand, Norway, Sweden, Switzerland, the United Kingdom, and the U.S.A.

As editors, we are keen to provide the reader with a sound, authoritative, and interdisciplinary review highlighting the major advances in the field of bacterial protein toxins. Whenever possible, particular emphasis was placed on toxin involvement in the pathogenesis of the diseases caused by the relevant bacteria. At the same time, 14 totally new chapters were created to cover the historical and general aspects of toxinology.

We hope the *Sourcebook* will appeal to a wide readership, including microbiologists and particularly toxinologists, biochemists, cell biologists, clinicians, and medical students who wish to have a better understanding of bacterial toxins in relation to infectious diseases.

We are heavily indebted to all contributors who skillfully documented developments at the cutting edge of their disciplines. Their painstaking efforts are deeply appreciated.

We wish to express our deep thanks to Tessa Picknett, Senior Publisher at Academic Press, Elsevier and her coworkers, Bryony Lott, Victoria Lebedeva (London), and Jeff Freeland (Burlington, USA) for their encouragement and invaluable help through the preparation of this volume.

Joseph E. Alouf and Michel R. Popoff

Preface to Second Edition

It is now eight years since the publication of the first edition of *The Comprehensive Sourcebook of Bacterial Protein Toxins*, and during that period many previously well-recognized toxins now constitute the prototypes of toxin families which share common mechanisms and, most probably, common evolutionary origins (e.g. the RTX toxins, STs and LTs of enterobacteria). Of great significance in this context was the recent recognition of pathogenicity islands which are responsible for the mobility and horizontal spread of toxins and other virulence genes between closely related species. Further remarkable progress has been made in the definition of molecular mechanisms of a wide range of toxins, with increasing numbers having enzymic mechanisms revealed, including ADP-ribosylation and glycosylation of novel targets (e.g. small Ras G-proteins). Among the most exciting discoveries of the 1990s, simply because it explained the potency of botulinum and tetanus toxins which have challenged toxinologists for over 100 years, was the demonstration that they consist of Zn-proteinases of exquisite specificity, cleaving proteins associated with vesicle fusion at presynaptic membranes. We now have the three-dimensional structure of botulinum toxin, which should yield further detailed information on the functional domains of these fascinating molecules.

The host immune system is not only the primary defence against colonization, and sometimes invasion, by toxigenic bacteria but it also constitutes the major target for a growing repertoire of bacterial toxins which can act either directly by cytoxicity towards immune effector cells (e.g. the leucocidins of *Staphylococci* and the leucotoxins of *Pasteurellae*) or by more subtle routes involving deregulation of cytokine production (e.g. the superantigenic toxins of *Staphylococci* and *Streptococci*).

Genome analysis promises to identify many more toxins over the next decade and should provide rich pastures for functional analysis in the next century. These analyses undoubtedly offer new opportunities for use as tools to dissect cellular processes as well as novel potential therapeutic agents.

A volume such as the *Sourcebook*, although attempting to cover the major groups of toxins at a relatively detailed level, inevitably has some gaps, and if these occur in your favorite area of research, then we can only apologize. The content of the book always reflects a compromise between what the editors would like and what they are able to include, yet still meet the deadlines set for the production of the book. We are greatly indebted to all the contributors, especially to those who met the first submission deadline. We wish to record our thanks to Tessa Picknett at Academic Press, who initiated the second edition, and to Lilian Leung who saw the project through to a successful conclusion with persistence and good humour. We also thank Mrs. Patricia Paul for invaluable secretarial work in compiling chapters and correspondence with authors.

Joseph E. Alouf and John H. Freer

Preface to First Edition

Great strides have been made in the depth of our understanding of the structure and mechanisms of action of bacterial toxins over the last decade. The current pace of this advance in knowledge is particularly impressive, and results largely from the power that gene manipulation techniques have offered in experimental biology.

Recent research achievements in the field of bacterial toxins, which consist of about 240 protein toxins as well as a relatively small number of non-protein toxins, reflect the extensive and productive blending of disciplines such as molecular genetics, protein chemistry and crystallography, immunology, neurobiology, pharmacology and biophysics. Furthermore, the exciting developments in many areas of cell biology, and particularly in membrane-associated mechanisms relating to signalling and communication, export and import of proteins and to cytoskeletal functions, have been facilitated because critical steps in these processes constitute the targets for bacterial toxins. Thus, we have toxins available which can be used to probe many fundamental aspects of eukaryotic cell biology.

Disruption of these same central cellular processes *in vivo* can also be the critical event in the pathogenesis of infectious diseases for man or domestic animals. Many such infectious diseases have major social or economic impacts on man, and such considerations have quickened the pace of the search for therapeutic agents. Currently, a number of physically inactivated bacterial or hybrid engineered toxoids are used as immunogens in vaccination programmes, and there is a major international effort to develop new and more effective vaccines based on our deeper understanding of the molecular events in pathogenesis and the host response to infection.

Since the publication of the excellent multi-volume treatise on *Bacterial Toxins* edited by S. Ajl, S. Kadis and T. Montie (Academic Press) in the early 1970s, most of the books published in the past twenty years have covered the subject by presenting individual toxins or groups of toxins in separate chapters. This is not the main approach followed in this book. Our aim is not to give an exhaustive review of the wide spectrum of the protein toxin repertoire but rather to give an "in depth" critical review of the original and the newly expanding body of information accumulated during the past decade or so. The multifaceted aspects of toxin research and the multidisciplinary approaches adopted suggested to us that "state of the art" toxin research might best be presented by putting together in several chapters the common structural and/or functional aspects of toxin "families". Other chapters highlight the various physiological or genetic mechanisms regulating toxin expression and the therapeutic or vaccine applications of genetically engineered toxins.

The 22 chapters of this book have been written by 44 internationally known specialists who have significantly contributed to the progress in the domains covered. It is hoped that this book will appeal to a wide readership, including microbiologists, biochemists, cell biologists and physicians. Also, we hope it will arouse the interest of students and scientists in other disciplines who see the power of these fascinating biological agents, either as exquisitely specific probes of cellular processes or as extremely potent agents of infectious disease.

Finally, we would like to thank all the authors for their contributions, and particularly to those who delivered their manuscripts by the first deadline. We also express our appreciation to the editorial staff at Academic Press for their help and patience throughout the preparation of this book.

J.E. Alouf and J.H. Freer

Introduction

Since the second edition of *The Comprehensive Sourcebook of Bacterial Protein Toxins* was published in 1999, major world events have impacted research on infectious agents, including pathogenic bacteria and their toxins. Most notably, the anthrax-tainted letters delivered via the United States Postal Service in the fall of 2001 sent a shock wave around the world. Although the letters produced only limited morbidity and mortality (22 cases of anthrax, 5 of which were fatal), they caused massive and costly disruption of the United States postal and legislative systems, and exacerbated the terror resulting from the attacks with airliners on the World Trade Center and the Pentagon. As a consequence, there has been a general widespread recognition of the threats from infectious diseases and a major reorientation in priorities for funding biological research. This is exemplified by the establishment of a set of Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases Research across the United States under National Institutes of Health sponsorship. The number of laboratories performing research on the causative agents of anthrax, smallpox, and other diseases on the A, B, and C "select lists" has increased dramatically, of course.

In facing the threats of bioterrorism, emerging infectious diseases, and ancillary problems, such as antibiotic resistance, the international community of scientists is fortunate in being able to draw on a rapidly expanding body of knowledge about pathogenesis. The past several decades have seen a major rise in interest in infectious disease research, due perhaps more than anything else to the development and convergence of methods directed primarily towards answering questions in basic science. The protein toxins produced by bacteria represent an interesting focus to illustrate this point. These toxins have always been of interest from the biological and medical communities, but in recent decades have also attracted biochemists and biophysicists expert in the array of tools developed to examine protein structure and function—x-ray crystallography, fluorescence spectroscopy, directed mutagenesis, mass spectrometry, and so forth. As a result, we now know the atomic level structures of a great many of these proteins, and are rapidly coming to understand their conformational dynamics and the details of their molecular interactions with host molecules (receptors, substrates, etc.). Cell biologists have brought to the table a complementary set of tools—confocal microscopy and fluorescent markers, for example, to track the attachment, entry, and trafficking of toxins. Along the way, the neurobiologists have become interested because some toxins form pores, resembling ion channels, in membranes. And the details of the molecular structures and of molecular and cellular function present questions of mode of action at tissue and organismal levels in a new, more precisely defined form to the physiologists and pathologists. As all of this is occurring, questions pertaining to the array of toxins encoded in bacterial genomes, or in mobile genetic elements, and how their production is controlled, and how they are secreted from the bacteria, are being solved through the application of molecular genetics. This grand evolving body of knowledge presents the scientist seeking to develop new countermeasures to biological threat agents, be they microbes or their toxins, with unprecedented opportunities for dissecting their own individual systems.

As in the previous editions, the third edition of *The Comprehensive Sourcebook of Bacterial Protein Toxins* serves to compile the latest and most relevant information to inform, stimulate, and excite, the researcher, the teacher, and the student.

R. John Collier

INTRODUCTORY SECTION

CHAPTER

1

A 116-year story of bacterial protein toxins (1888–2004): from "diphtheritic poison" to molecular toxinology

Joseph E. Alouf

To the physiologist the poison becomes an instrument which dissociates and analyzes the most delicate phenomenon of living structures and by attending carefully to their mechanism in causing death, he can learn indirectly much about the physiological processes of life.

Claude Bernard, La Science Experimentales, *Paris, 1878*

INTRODUCTION

The concept that pathogenic bacteria might elicit their harmful effects in humans and animals by means of poisons elaborated by these microorganisms in the infected host is almost as old as the notion of pathogenic bacteria itself (William Edward van Heyningen 1950, 1970). The heuristic impact of the concept of bacterial poisons was considerable after the discovery of bacteria and their relation to disease. It was undoubtedly influenced by the knowledge that other biological organisms do produce poisons such as animal venoms and plant alkaloids. Indeed, the idea that the harm caused by infectious disease might be due to microbial poisons was entertained long ago even before the germ theory of disease was established. Both endotoxins and exotoxins were foreseen in 1713 by Vallisnieri, who suggested on one page of his *Riposta* that the "little worms of the most atrocious pests are of themselves of a poisonous nature" (van Heyningen, 1955).

The first attempts to demonstrate experimentally the production of bacterial poisons were undertaken by Edwin Klebs (1834–1913) for staphylococci, Robert Koch (1843–1910) for *Vibrio cholereae*, and Friedrich Loeffler (1852–1915) for diphtheria bacillus, respectively. These attempts failed for methodological reasons that are now understandable.

Klebs (1872) suggested that chemical substances named "sepsins" were responsible for the lesions caused by staphylococci, but no evidence for the existence of such substances was found. The first experimental demonstrations of staphylococcal toxin(s) were reported later by von Leber (1888), De Christmas (1888), van de Velde (1894), and other microbiologists by the early 1900s (see Arbuthnott, 1970).

As concerns cholera, Koch (1884) expressed the view that the disease was a toxicosis, since the causative organism proliferated in the gut without appearing to invade or damage this organ or any of the neighboring tissues. Parenteral injection of *V. cholereae* filtrates did not produce any toxic effects in experimental animals, and the idea of an extracellular poison was abandoned. However, 75 years later, Koch's hypothesis was confirmed when two Indian researchers, De (1959) and Dutta *et al*. (1959) working independently of one another, showed that cell-free preparations from *V. cholerae* caused relevant symptomatology in animal models (adult rabbit ligated loop and infant rabbit, respectively). In 1969, the putative toxin was purified and biochemically characterized by

Finkelstein and Lo Spalluto (1969). Fourteen years later, the cloning and nucleotide sequence of the toxin was determined by Mekalanos *et al*. (1983). Cholera toxin, an 84-kDa oligomeric protein; is the prototype of a wide family of biochemically, immunologically, and pharmacologically related toxins found in human and porcine *E. coli* strains, non 01 *V. cholerae*, *V. mimicus*, *Aeromonas hydrophila*, *Campylobacter jejuni*, *Salmonella enterica serovar typhi*, and *typhimurium* and *Plesiomonas shigelloides* (Sears and Kaper, 1994 and Chapter 19 of this volume).

The same year of Koch's hypothesis, Loeffler (1884) suggested that a soluble poison was the causative agent of diphtheria. This author who had discovered diphtheria bacillus reported that in animals that died after experimental infection, the microorganism remained at the seat of inoculation and was totally absent from the internal organs. Nevertheless, these organs were profoundly damaged ("hemorrhagic edema, effusion into the pleural cavities, catarrhal inflammation of the kidneys, and deep reddening of the suprarenal capsules"). Loeffler concluded that "this clearly indicated that a poison at the seat of inoculation must have circulated in the blood." However the putative poison was not found.

REPERTOIRE OF BACTERIAL PROTEIN AND PEPTIDE TOXINS (1888–2004)

This repertoire comprises so far 339 members including natural variants, serotypes, isoforms, and allelic forms, namely 160 (47%) from Gram-positive bacteria and 179 (52%) from Gram-negative bacteria. Most of them (85%) are extracellular or cell-associated and the rest (15%) are intracellular. As established in the past 30 years, many toxins were found to possess a variety of enzymatic activities (Table 1.1).

DISCOVERY OF THE FIRST BACTERIAL PROTEIN TOXINS: DIPHTHERIA, TETANUS, AND BOTULINAL TOXINS (1888, 1890, 1896)

Diphtheria toxin

In 1888, fortune smiled on Emile Roux (1853–1933) and Alexandre Yersin (1863–1943). At Pasteur Institute, these researchers discovered the first bacterial toxin, namely diphtheria toxin (Roux and Yersin, 1889), six months after the creation by Louis Pasteur (1822–1895) of this "Temple of Science." The "diphtherial poison" was detected in sterile filtrates of diphtheria bacillus (*Corynebacterium diphtheriae*). The filtrates or the material isolated by cadmium chloride precipitation from old alkaline cultures injected into guinea pigs, rabbits, and pigeons mimicked the symptoms and type of death produced by infection with living organisms. Moreover, Roux and Yersin demonstrated that the urine of children taken shortly before death from diphtheria may contain sufficient toxic material to kill guinea pigs with symptoms similar to those elicited by culture filtrates. The earlier prediction of Loeffler was thus verified. For the first time, the mechanism of pathogenicity of a microorganism in humans became clarified and could be explained in terms of a soluble toxic substance released by bacteria named for the first time a *toxin* by the authors (from Greek: τοξιχον, poison) (van Heyningen, 1970). However, according to Carl Lamanna (1990), the term *toxin* was first used in 1886 by E. Ray Lankester in *Science* to name "poisons for animals produced by pathogenic bacteria." Very likely, Roux and Yersin were not aware of Lankester's article.

Rational design and serendipity

The seminal discovery of the "diphtheritic poison" was the fruit of both rational design and serendipity. The culture medium used for bacterial growth was prepared with Paris city tap water, which was known to contain high amounts of calcium. Autoclaving led to the precipitation of calcium phosphate and thereby the lowering of iron concentration in the medium, which empirically favored diphtheria toxinogenesis, as reported ca. 40 years later by Locke and Main (1931), Pope (1932), and Pappenheimer and Johnson (1936). Toxin production was maximal under iron starvation conditions and was severely inhibited under high–iron growth conditions. It is interesting to report how Pappenheimer discovered the "iron effect." He found that cultures growing on a particular medium would produce toxin one day and fail to produce it on the "same" medium the next day. Then his laboratory received a supply of new hard-glass bottles, and in these, toxin was produced without fail. When small amounts of crushed glass from the soft-glass bottles were introduced in the new bottles, toxin production went down. It was found that the inhibition of toxinogenesis was due to the high iron content of the soft glass.

The discovery of the iron effect in the 1930s was a very important event in the history of bacterial toxins (Alouf and Raynaud, 1960). It paved the way for a number of major achievements in toxinology over the past 50 years.

Freeman (1951) discovered that diphtheria toxin is encoded by a bacteriophage gene *tox*+ called β (see

TABLE 1.1 Toxins exhibiting enzyme activity

1. ADP-RIBOSYLATING TOXINS
Transferase and NADase activity
Ref. Han and Tainer (2002); Masignani *et al*. (2004) and Chapter 12 of this volume

2. PHOSPHOLIPASES
Cytolytic phospholipases C: *C. perfringens* zinc-dependent α-toxin, *C. sordellii* γ-toxin, *C. novyi* β and γ-toxins, *C. heamolyticus*, *P. aeruginosa*, *P. aureofaciens*, *A. hydrophila, B. cereus, Acinetobacter calcoaceticus* haemolysins
Vibrio damsela cytolytic phospholipase D
Rickettsia prowazekii haemolytic phospholipase A
S. aureus β-toxin (haemolysin, sphingomyelinase)
Corynebacterium ovis lethal toxin (phospholipase D)
Yersinia pestis murine toxin (phospholipase D)
Ref: Hatheway, C.L (1990); Songer (1997); Jepson and Titball (2000); Hinnebusch *et al*. (2000).

3. ADENYLATE CYCLASES
Bordetella pertussis, *B.parapertussis* and *B. bronchiseptica*, *Bacillus anthracis* bifactorial oedema toxin, *Pseudomonas aeruginosa* exotoxin Y
Ref. Confer and Eaton (1982); Ladant and Ullmann (1999); Hewlett and Gray (2000); Leppla (1988); Lory *et al*. (2004), Yahr *et al*. (1998)

4. METALLOPROTEASES
Tetanus and *botulinum* A, B, C, D, E, F, G (light chain) zinc-dependent neurotoxins
Bacillus anthracis bifactorial lethal toxin, *Bacteroides fragilis* zinc-dependent enterotoxins
Ref. Herreros *et al*. (1999); Rossetto *et al*. (2001); Lalli *et al*. (2003); Franco *et al*. (1997); Pannifer *et al*. (2001); Vitale *et al*. (2000)

5. RNA *N* -GLYCOSIDASES
S. dysenteriae shiga toxin, *E. coli* shiga-like toxins (verotoxins: Stx 1, 1c, 2, 2c, 2d, 2e) and similar toxins from *Aeromonas hydrophila, A. cassia, Enterobacter cloacae, Citrobacter freundii;* ricin and other plant toxins
Ref. Endo (1988); Tesh and O'Brien (1991); O'Loughlin and Robins-Brown (2001)

6. GLUCOSYL TRANSFERASES
C. difficile A, B toxins, *C. sordellii* and *C. novyi* lethal α-toxin
Ref. Aktories (2003); von Eichel-Streiber *et al*. (1996); Selzer *et al*. (1996)

7. DEAMIDASE ACTIVITY
E. coli cytotoxic necrotizing factor-1
Ref. Flatau *et al*. (1997); Schmidt *et al*. (1997)

8. PROTEASE ACTIVITY
S. aureus epidermolytic toxins (exfoliatins) serotypes A, B, C, D; *S. hyicus* exfoliatins at least six serotypes; SHETA, SHETB, Exha, Exhb, Exhc, Exhd
Enteroaggregative *E. coli* pet toxin; *S. pyogenes* cysteine proteinase
Ref. Melish *et al*. (1974); Yamaguchi *et al*., (2002); Ladhani *et al*. (2002); Hanakawa (2004); Ahrens and Andresen (2004); Villaseca *et al*. 2000; Gerlach *et al*. (1994) and Chapter 56 of this volume.

9. DEOXYRIBONUCLEASE ACTIVITY
Cytolethal distending toxins (18 members at least from various Gram-negative bacteria)
Ref. Johnson and Lior (1988a); Pickett and Whitehouse (1999); Dreyfus (2003); Thelestam and Frizan (2004) and Chapter 23 of this volume.

Groman, 1953; Barksdale and Arden, 1974). Then Murphy *et al*. (1978) and Welkos and Holmes (1981) demonstrated that the regulation of toxin expression by iron occurred at the level of *tox* gene transcription under the control of a chromosomal *dtxR* gene encoding a diphtheria toxin repressor (see White *et al*., 1998; Goranson-Siekierke and Holmes, 1999; and Chapter 13 of this volume).

Since the pioneering works on the involvement of iron in the regulation of bacterial toxingenesis, a great number of investigations have been undertaken for other toxins or virulence factors whose synthesis is negatively regulated by iron. This is the case of *Shigella dysenteriae* shiga toxin, *Escherichia coli* Shiga-like toxin-1, and hemolysin, *Vibrio cholerae* hemolysin, *Pseudomonas aeruginosa* exotoxin A, *Clostridium tetani* tetanus toxin, *Helicobacter pylori* VacA cytotoxin, *Plesiomonas shigelloides* CHO cell elongation factor (Hantke *et al*., 2001; Payne, 2003).

"What is the nature of the diphtheric poison? Is it an alkaloid or a diastase?"

Roux and Yersin (1888) addressed these questions and suggested that because of its heat lability, the toxin resembled an enzyme ("diastase") more than an alkaloid. However, eighty years intervened before it was finally proved independently in two laboratories (Honjo *et al*., 1968; and Gill *et al*., 1969) that diphtheria toxin is, in fact, a highly active enzyme of a novel and unique type (Collier, 1975). The toxin inhibits eucaryotic protein synthesis by catalyzing the covalent transfer of the ADP-ribose portion of nicotinamide (NAD) to elongation factor 2, which in the case of diphtheria toxin leads to target cell death. Interestingly, prior to this very important achievement the pioneering experiments of Lennox and Kaplan (1957) and Placido-Souza and Evans (1957) showed that the toxin was lethal for cells in culture from sensitive animals. Then, the elegant experiments of Strauss and Hendee (1959) on HeLa cells provided the first indication that cell death resulted from the inhibition of protein synthesis by diphtheria toxin (see Collier, 1977 for a comprehensive review of this issue).

Diphtheria toxin: eponymous archetype of a vast class of bacterial toxins and virulence factors

The pioneering discovery of the ADP-ribosyltransferase activity of diphtheria toxin opened a new era in

our knowledge of bacterial toxins, their mode of action, and their role in the pathogenesis of various infectious diseases (Barbieri and Burnes, 2003). Indeed, this toxin is the first member and the prototype of a vast family of conserved ADP-ribosyltransferases (ADPRTs) from various bacterial pathogens that display a variety of pharmacologic, physiologic, and toxic activities toward humans and (or) animals. These effectors constitute an immense potential for modern biomedical research.

Discovery of clostridial neurotoxins

Two other major toxins were to follow soon after the discovery of diphtheria toxin: tetanus toxin in 1890 and botulinum toxin in 1896, produced by *Clostridium tetani* and *Clostridium botulinum*, respectively.

Tetanus toxin

Tetanus was recognized since ancient times and described by Hippocrates who first reported the symptoms of a sailor affected by a syndrome characterized by hypercontraction of the skeletal muscles. He termed such a spastic paralysis "tetanus" (from the Greek word τετανυσ, tension).

A "clinical" description of the disease was remarkably illustrated by the Scottish surgeon Sir Charles Bell in his book *The Anatomy and Philosophy of Expression* published in 1832. In 1884, Carle and Rattone showed that tetanus could be transmitted from a human suffering from the disease to a rabbit injected with material from the wound. Implantation of soil samples into mice or rabbits caused symptoms of tetanus. In smears from the wound, *drumstick* forms (*clostridium* in Latin) of *C. tetani* were first observed by Nicolaier (1884), who noted that the microorganism was not distributed in the whole organism, but confined to the wound of entry. Simpson had already observed in 1856 that the symptoms of tetanus were similar (as confirmed later) to those of strychnine poisoning and in 1885 Nicolaier suggested that the microorganism brought about its pathological effects by producing a strychnine-like poison. In 1889, Shibasaburo Kitasato (1852–1931) obtained a pure culture of the bacterium in Robert Koch's laboratory in Berlin.

The toxin was discovered in 1890 in culture supernatents in Denmark by Knud Faber and in Italy by Tizzoni and Cattani (1890). Faber justified Nicholaier's suggestion by showing that it was possible to reproduce the spastic symptoms of tetanus in experimental animals by injecting them with sterile filtrates of cultures of the bacillus (van Heyningen and Mellanby, 1971). The toxin producing these effects was also named *tetanospasmin* (for other references see Rossetto *et al*., 2001; Lalli *et al*., 2003). The toxin was shown by Bruschettini (1897) to move retroaxonally and to act at the spinal-cord level. Active immunization with formaldehyde-treated tetanus toxin was undertaken by Ramon and Descombey (1925).

Tetanus and diphtheria antitoxins: the birth of serotherapy and experimental immunology

In December of the same year, Behring and Kitasato (1890) in Berlin rendered mice and rabbits resistant to the toxic effects of tetanus and diphtheria toxins by inoculating the animals with small doses of toxin preparations attenuated by "Gram liquor" (iodine). The sera of the "immunized" animals not only specifically neutralized the toxins, but still did so when transferred into the bodies of "native" animals challenged with the native toxins. This experiment revealed for the first time the production of neutralizing "Antikörper" (antibodies) and led to the development of serotherapy and to the birth of experimental immunology. In 1884, Emile Roux announced the large-scale production of horse immune sera (antitoxins) for the serotherapy of diphtheria (Roux and Martin, 1884). This was also the case for the preparation of large amounts of antitetanus antitoxins by Behring (1892).

Diphtheria and tetanus toxoids

Another epochal achievement was the independent development of toxoids by Glenny and Sudmersen (1921) in the United Kingdom and Gaston Ramon (1923; Ramon and Descombey, 1925) in France for use in human vaccination. Since then mass immunization with diphtheria and tetanus toxoids has led to a remarkable decrease of the two diseases in developed countries. Unfortunately, around 500,000 annual cases of tetanus (particularly neonatal) are still reported in countries of the Third World for lack of vaccination.

Botulinum toxins

Botulism (*sausage poisoning* in Latin) is the term given to an acute food poisoning caused by the ingestion of spoiled sausages (*botulus)* that has been known as long as 1,000 years ago. The Byzantine Emperor Leo VI (886–911 A.D.) forbade the preparation and eating of blood sausages.

Centuries later (1793) in Württemberg, an outbreak affected 13 persons, six of whom died. The outbreak was attributed to blood sausage consumption; thereby the preparation of this food came under strict government regulation.

In 1820, Justinus Kerner, a poet turned physician and medical officer for the Duchy of Württemberg, reported 200 cases of sausage poisoning.

The etiology of botulism was elucidated in 1895 by the Belgian microbiologist van Ermengen of the University of Ghent in his investigation of a tragic outbreak of botulism in Ellezelles (Belgium) that involved 50 cases among members of a musical society who partook of a meal at a funeral. Three of them died and at least 10 were seriously ill (van Ermengen, 1897).

The outbreak was caused by a salt-cured uncooked ham. Portions of macerated ham fed to mice, guinea pigs, and monkeys caused paralytic signs of the illness observed in the patients and subsequently resulted in death. Filtered extracts of the ham had the same effects as the macerated ham.

Van Ermengem consistently found an anaerobic sporulating bacillus in cultures of the ham, as well as in a culture of the spleen from one of the deceased victims. The organism was named *Bacillus botulinus* and thereafter, *C. botulinum*. Culture filtrates had the same effect as did ham macerates (Sakagushi, 1986; Rossetto *et al*., 2001).

Discovery of different types of botulinum toxins

In 1904, 11 persons died from eating wax-bean salad in Darmstadt (Germany). The antitoxin serum prepared against the Ellezelles strain did not neutralize the toxin from the Darmstadt strain and reciprocally. Thus, the toxins from the two strains were immunologically distinct (Leuchs, 1910) and were later classified into types A and B (Meyer and Gunnison; 1929 in Sakaguchi's chapter). Type B corresponded to the Ellezelles strain. Types C and D were characterized by Bengston (1922) and Theiler (1927). Types E and F were identified by Gunnison (1936) and Dolman and Murakami (1961), respectively. Type G was isolated from a soil sample collected in Argentina (Giménez and Sicarelli, 1970). (See Sakagushi, 1986; Popoff and Marvaud, 1999; Herreros *et al*., 1999 for references.)

Recent developments of the research on clostridial neurotoxins

Great advances in our understanding of the molecular mechanisms of action of these neurotoxins have been made in the past 15 years. Both toxins are structurally related 150-kDa zinc-dependent metalloproteases consisting of three domains, endowed with different functions: neurospecific binding, membrane translocation, and specific proteolysis of three key components of the neuroexocytosis apparatus. After binding to the presynaptic membrane of motoneurons, tetanus neurotoxin (TeNT) is internalized and transported retroaxonally to the spinal cord, where it blocks neurotransmitter release from spinal inhibitory interneurons. In contrast, the seven botulinum neurotoxins (BoNT) act at the periphery and inhibit acetylcholine release from peripheral cholinergic nerve terminals. TeNT and BoNT-B, -D, -F, and -G cleave specifically at single but different peptide bonds, VAMP/synaptobrevin, a membrane protein of small synaptic vesicles. BoNT types -A, -C, and -E cleave SNAP-25 at different sites within the COOH-terminus, whereas BoNT-C also cleaves syntaxin. BoNTs are increasingly used in medicine for the treatment of human diseases characterized by hyperfunction of cholinergic terminals (see Rossetto *et al*., 2001; and Chapters 19 and 20 of this volume). The genes encoding botulinum and tetanus neurotoxins have been widely investigated during the past 25 years as recently reviewed by Raffestin *et al*. (2004). BoNT -A,-B, -E, and -F are encoded by chromosomal genes; BoNT -C and -D are encoded by bacteriophages, while BoNT-G and tetanus neurotoxin genes are localized on large plasmids in *C. argentinense* and *C. tetani*, respectively. Moreover, the regulatory genes involved in the production of clostridial neurotoxins have been recently identified (Raffestin *et al*., 2004). The three-dimensional crystal structure of BoNT-A, -B, and -E and tetanus toxin was determined by the end of the 1990s (Table 1.2).

TOXIN RESEARCH BETWEEN 1900 AND 1975

Since the discovery of the first three major bacterial protein toxins, about 100 new protein toxins produced by a number of Gram-positive and to a lesser extent from Gram-negative bacteria were identified during the first half of the twentieth century. Much effort was particularly focused on anaerobic clostridial species and their toxins as a result of the experience gained during World War I gas gangrene on the battle field. Research on other toxins during the 1900–1965 period was also developed on toxinogenic anaerobes and certain aerobic bacterial pathogens, such as staphylococci, streptococci, *Bacillus anthracis*, and on various membrane-damaging toxins from both Gram-positive and Gram-negative bacteria.

Clostridia

The genus *Clostridium* encompasses over 80 species of Gram-positive, anaerobic spore-forming bacteria. Fifteen account for a wide range of diseases in humans and animals by virtue of their capacity to produce highly potent extracellular protein toxins that are responsible for the pathogenicity of the microorganisms. The toxinogenic species listed below were identified over a period of about 100 years. To date, 60 toxins have been characterized (Hatheway, 1990 and Table 1.3).

TABLE 1.2 Three-dimensional structure of crystallized toxins established to date (2004)

1. *P. aeruginosa* exotoxin A (1986). Allured,V.S. *et al. Proc. Nat. Acad. Sci*. **83**, 1320
2. *E. coli* LT-1 toxin (1991). Sixma, T.K. *et al. Nature*, **351**, 371
3. *Bacillus thuringiensis* δ-toxin (1991). Li, J. *et al. Nature*, **353**, 748
4. Oligomer B of *E. coli* shiga-like toxin (1991). Stein, P.E. *et al. Nature*, **355**, 748
5. Diphtheria toxin (1992). Choe, S. *et al. Nature*, **357**, 216
6. *S. aureus* enterotoxin B (1992). Swaminathan, S. *et al. Nature*, **359**, 801
7. *S. aureus* toxic-shock syndrome toxin-1 (TSST-1). (1993, 1994) Prasad , G.S. *et al. Biochemistry*, **32**, 13761; Acharya, K.R. *et al. Nature*, **367**, 94
   TSST-1 -MHC class II complex (1994). Kim, J. *et al. Science* **266**, 1870
8. *Aeromonas hydrophila* proaerolysin (1994). Parker, M.W. *et al. Nature*, **367**, 292
9. *Pertussis* toxin (1994). Stein, P.E. *et al. Structure* **2**, 45
10. *Shigella dysenteriae* toxin (1994). Fraser, J. *et al. Nature Struct. Biol*. **1**, 59
11. Oligomer B of the choleragenoid form of cholera toxin (1995). Zhang, R.G. *et al. J. Mol. Biol*. **251**, 550
12. Cholera toxin (holotoxin) (1995). Zhang, R.G. *et al. J. Mol. Biol*. **251**, 563
13. *S. aureus* enterotoxin C1 (1995). Hoffmann, M.L. *et al. Infec. Immun*. **62**, 3396
14. *S. aureus* enterotoxin C2 (1995). Papageorgiou, A.C. *et al*. Structure *3*, 769; Kumuran *et al*. (2001) Acta Crystallogr. *D Biol. Crystallogr*. **57**, 1270
15. *S. aureus* enterotoxin A (1995,1996). Schad, E.M. *et al. EMBO J*. **14**, 3292
    Sundström, M. *et al. J. Biol. Chem*. **271**, 32212
16. *S. aureus* enterotoxin C3 (1996). Fields, B.A. *et al. Nature* **384**, 188
17. *S. aureus* enterotoxin D (1996). Sundström, M. *et al. EMBO J*. **15**, 6832
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TABLE 1.3 Clostridium species

MAJOR PATHOGENIC SPECIES	**Toxins**
C. septicum (Pasteur and Joubert, 1877)	4
C. chauvoei (Kitt, 1887)	4
C. tetani (Kitasato, 1889)	2
C. perfringens (Welch and Nuttall, 1892)	17
C. novyi /oedematiens (Novy, 1894)	8
C. botulinum (van Ermengem, 1895)	3
C. histolyticum (Weinberg and Séguin, 1916)	5
C. bifermentans (Weinberg and Séguin, 1919)	3
C. sordellii (Sordelli, 1922)	4
C. haemolyticum (Hall, 1929)	3
C. difficile (Hall and O'Toole, 1935)	3
C. spiroforme (Fitzgerald *et al*., 1965)	2
C. butyricum (Howard *et al*., 1977)	1
C. baratii (Hall *et al*., 1985)	2
C. argentinense (Suen *et al*., 1988)	1
Total	62

Five species are involved in toxin-induced gas gangrenes:
C. perfringens, C. septicum, C. histolyticum, C. novyi, C. sordellii

Four species produce neurotoxins (zinc–dependent proteinases):
C. botulinum, C. tetani, C. baratii, C. argentinense

Nine species produce StreptolysinO-related cholesterol-dependent cytolysins:
C. septicum, C. chauvoei, C. tetani, C. botulinum, C. perfringens, C. novyi /oedematiens, C. histolyticum, C. bifermentans, C. sordellii

References: Bernheimer, A. W. (1944) Alouf and Jolivet-Reynaud (1981) Hatheway (1990); Rood and Cole (1991); Yoshihara *et al*. (1994); Songer (1996); Popoff and Marvaud (1999); Herreros *et al*. (1999); Petit *et al*. (1999); Alouf (2000); Stevens (2000); Rossetto *et al*. (2001); and Chapters 36 and 57 of this volume.

Clostridial gas gangrene

C. perfringens and *C. septicum* are the most representative among the five pathogenic species of the clostridia that cause gas gangrene. The latter causes a form of gangrene in the absence of any external trauma, while the former is the causative agent of traumatic gangrene after deep penetration, invasion, and destruction of healthy muscles (van Heyningen, 1955; Mac Lennan, 1962; and Chapter 56 of this volume).

Clostridium perfringens

This microorganism is historically the main source of one of the major bacterial protein toxins, namely the lethal and hemolytic α-toxin. *C. perfringens* has been known for decades as *Clostridium welchii* in honor of the American pathologist William Henry Welch (1850–1934), who provided the first extensive description of the microorganism. Although this organism was first cultured probably as early as 1891 by Achalme, this author did not make a connnection to gangrene. One year later, Welch and Nuttal (1892) reported the discovery of what they called a strain of *Bacillus aerogenes capsulatus* found in gas-containing blood vessels during post-mortem examination of a corpse of a 38-year-old bricklayer (see Derewenda and Martin, 1998). Gangrene made its most widespread appearance 22 years later during World War I (MacPherson *et al*., 1922). Using strains from the battlefront, Bull and Pritchett (1917) conducted a series of studies that led them to conclude that "the cause of death in the infection is not a blood invasion of the microorganisms but an intoxication with definite and very potent poisons produced in the growth of the bacilli in the tissues of the body." At the end of the 1960s, the bacterium was named *C. perfringens*.

It took two decades of research to conclude that the culture broth contained a varying number of toxins, among them α-toxin, the prominent toxin of this species. As many as 17 exotoxins have been described so far in the literature (McDonel, 1980; Hatheway, 1990; Songer, 1996). A crude *C. perfringens* toxoid vaccine was demonstrated to provide protection as early as 1937 by Penfold and Tolhurst.

Discovery and characterization of C. perfringens α-toxin

Historically, this toxin was one of the major bacterial protein toxins. The toxin was first characterized as a lethal and hemolytic toxin by Nagler (1939), Seiffert (1939), and van Heyningen (1941), who observed its interaction with serum lipids and egg yolk suspensions. An important breakthrough was the seminal discovery by Marjorie Giffen Macfarlane and Knight (1941) that the α-toxin is "a lecithinase which decomposes lecithin into phosphocholine and a diglyceride." This finding constitutes an important landmark in toxinology and for the first time, the mode of action of a protein toxin was elucidated at the molecular level. Other discoveries by Macfarlane were soon to follow (1948, 1950), as she identified the phospholipase activity of *C. oedematiens, C. sordellii*, and *C. haemolyticum. C. perfringens* α-toxin is currently considered as the prototype of the 15 cytolytic bacterial protein toxins so far identified that disrupt eucaryotic cell membranes by hydrolysis of their constitutive phospholipids (Table 1.1). This toxin belongs to the family of the zinc metallo-phospholipases (Titball, 2000; and Chapter 27 of this volume). Its crystal structure has been recently established (Naylor *et al*., 1998).

Group A streptococci (*Streptococcus pyogenes*)

This species produces two types of toxins:

1. The membrane-damaging cytolysins, streptolysins O and S were discovered as early as 1902 by Marmorek and clearly differentiated in the 1920s and 1930s (Neill and Mallory, 1926; Todd 1938). These toxins are described in a comprehensive review (Alouf, 1980) and in Chapters 26, 38, and 42 of this volume.
2. A series of imunocytotropic toxins of the family of superantigens (see Alouf, 1980 section IV; and Chapters 51 and 53 of this volume), the prototype of which is the streptococcal erythrogenic toxin A, which plays an important role in streptococcal pathogenicity and diseases (Cunningham *et al*., 2000; Bisno *et al*., 2003).

Streptococcal erythrogenic toxin

The pioneering work of George F. Dick and Gladys Henry Dick (1924), who provided the proof that streptococci, identified later as group A streptococci (*S. pyogenes)*, are the causative agent of scarlet fever, constitutes an important hallmark in bacterial toxinology and the microbiology of infectious diseases. The Dicks were also the discoverers of the so-called "scarlet fever toxin" (Dick and Dick, 1924 a, b) recently named streptococcal pyrogenic exotoxin (SPE) by certain authors (Schlievert and Gray, 1989; Mc Shan, 1997). Three immunologically different toxin serotypes A, B, and C have been reported (Watson, 1960). Toxin B was later found to be a cysteine proteinase devoid of superantigenic properties (Gerlach *et al.,* 1994). The three toxin serotypes are produced exclusively by group A streptococci, separately, simultaneously, or in various combinations, depending on culture media and strains (Knöll *et al*., 1991).

Streptococcal superantigens

Erythrogenic toxins A and C are the prototypes of the family of structurally related streptococcal superantigens discovered in the past 10 years named streptococcal superantigen (SSA), streptococcal pyrogenic exotoxins G, H, I, J, L, M, and the "streptococcal mitogenic exotoxins" (SMEZ) 2, 3, . . . 24 (Proft *et al*., 2001; Alouf and Müller-Alouf, 2003; Petersson *et al*., 2004; and Chapters 51 and 53 of this volume).

An important discovery relevant to the erythrogenic toxin was the report of Frobisher and Brown as early as 1927 that a filterable agent from scarlet fever strains of group A streptococci induced toxin production by non-scarlatinal strains. This was confirmed by Bingel (1949) and Zabriskie (1964), who found that bacteriophage from streptococcal strain T12gl converted a non-lysogenic, toxin A–negative strain to a lysogenic, toxin A–positive one. Subsequently, Nida and Ferretti (1982) showed that toxic conversion is produced by a number of temperate streptococcal bacteriophages and conversion can be effected in many streptococci. The *speA* gene encoding the erythrogenic toxin A was cloned from bacteriophage T12 (Johnson and Schlievert, 1984) and its nucleotide sequence determined by Weeks and Ferretti (1986) and Johnson *et al*. (1986), respectively.

The demonstration that erythogenic toxin was encoded by a bacteriophagic gene was an important breakthrough in the genetics of bacterial toxins, as was also the case of the discovery of the bacteriophagic origin of the gene encoding diphtheria toxin (Freeman, 1951) below as described.

The conundrum of *Bacillus anthracis* toxin complex

This complex plays a dominant role in the pathogenesis of anthrax disease. It constitutes a particularly interesting and somewhat unusual molecular structure characterized by binary combinations of three secreted proteins: the protective antigen (PA), the lethal factor (LT), a zinc-dependent protease that cleaves six of the seven known mitogen-activated protein kinase kinases (Klimpel *et al*., 1994; Vitale *et al*., 2000), and the edema factor (EF), a calmodulin-dependent adenylate cyclase (Leppla, 1982, 1988). The association of PA and LF constitutes the lethal toxin, while that of PA and EF constitutes the edema toxin. The three proteins are encoded by *pagA*, *lef*, and *cya* genes. These genes are borne by a large (182-kbp) plasmid called pXO1 (Mock and Fouet, 2001; Koehler, 2002).

Historical background

Anthrax was recognized for many centuries as a severe disease of animals and humans. It is believed to have been one of the seven plagues suffered by the Egyptians in the time of Moses and was clearly described in ancient Greece (Lincoln and Fish, 1970).

The disease occurs in two main forms: localized cutaneous infections and septicemic (Lincoln *et al*., 1964, 1970). The most characteristic superficial feature of the cutaneous infections is the black eschar that gives its name to the disease and to the causative microorganism (in Greek, anthrakos ανθρακοσ, *coal*). The study of anthrax and its causative agent *Bacillus anthracis* has attracted the attention of microbiologists since the mid 1850s. In 1850, the French physicians François Rayer (1796–1867) and Casimir Joseph Davaine (1811–1882) detected microscopic rods in the blood of animals with anthrax. These "bodies" were named "bacteridies" by Davaine (1863) who contended that these organisms were the causative agents of the disease. Koch (1876) obtained pure cultures of *B.anthracis* for the first time. Louis Pasteur (1822–1895) isolated the bacterium in broth medium from which he inoculated experimental animals who died from anthrax (Pasteur and Joubert, 1878). He also used *B. anthracis* to develop an effective attenuated live vaccine (Pasteur 1880). Since that time, the production of a toxin was postulated by many authors throughout the first half of the twentieth century, particularly in the 1930s, but no lethal toxin had been demonstrated in either anthrax bacilli or filtrates from laboratory cultures (see Smith, 2002). As early as 1907, Eisenberg reported that the supernatant of a bacterial culture injected into guinea pigs and rabbits quickly killed the animals and evoked this effect as that of a "toxin." A major breakthrough occurred as of 1953 when Harry Smith, J. Kreppie, and their coworkers started intensive research in Porton Down (United Kingdom) and successfully resolved the conundrum of anthrax toxin (Stephen, 1986). The complex steps used by the authors to isolate the toxin from the plasma or exudates from dying mice or guinea pigs previously infected with *B. anthracis* has been reviewed recently by Smith (2002). The three components of the anthrax toxin complex were soon characterized by the British group (Smith *et al*., 1956; Stanley and Smith, 1961), who called them factors I, II, and III, corresponding to the edema factor, protective antigen (PA), and lethal factor (EF) of the American authors (Lincoln and Fish, 1970). Beall *et al*. (1962) independently discovered factor III.

The production and purification of the three components of anthrax toxin has been described in detail by Leppla (1988). Since then, great progress in the purification, genetics, cell biology, biochemistry, and immunological aspects of the toxin has been achieved (see Mock and Fouet, 2001; Turnbull, 2002; Koehler, 2002; Chaudry *et al*., 2002; Mourez *et al*., 2002, Mourez,

2004; and Chapter 8 of this volume). The crystal structures of PA (Petosa *et al*., 1997) and LF (Pannifer *et al*., 2001) have been determined (see Table 1.2).

B. anthracis and bioterrorism

Unfortunately, *B. anthracis* and its spores and toxins recently became agents of bioterrorism after the deliberate release of anthrax in the United States of America in September and October 2001 (Jernigan *et al*., 2001). This criminal use of this toxin completely changed the international perception of the risk of bioterrorism.

MAJOR ACHIEVEMENTS IN TOXIN RESEARCH FROM 1975 TO DATE

The major facets of toxin research of the past 29 years concerned mainly the discovery and characterization of roughly 120 novel toxins among them:

(a) *Helicobacter pylori* vacuolating cytotoxin (see Chapter 24 of this volume).
(b) Several *Pseudomonas aeruginosa* exotoxins: three ADP-ribosyl transferases exotoxin A, Exo S, Exo T, the adenyl cyclase Exo Y, the phospholipase Exo U (Iglewski *et al*., 1977, 1978; Yahr *et al*., 1998; Krall *et al*., 2000; Maresso *et al*., 2004; and Chapter 14 of this volume).
(c) *Staphylococcus aureus* toxic shock syndrome toxin-1 (TSST-1) identified in 1981 by two independent groups from *S. aureus* strains isolated from patients with toxic shock syndrome (TSS) and found to be the cause of this disease (Schlievert *et al*., 1981; Bergdoll *et al*., 1981, McCormick *et al*., 2001; and Chapter 50 of this volume).
(d) Novel *S.aureus* enterotoxins and *S.pyogenes* superantingens.
(e) *Bacteroides fragilis* zinc protease enterotoxin (Van Tassel *et al*., 1992; Sears, 2001; and Chapter 28 of this volume).
(f) The cytolethal distending toxins (Johnson and Lior, 1987a, b; Pickett and Whitehouse, 1999; Thelestam and Frisan, 2004; and see Chapter 23 of this volume).
(g) The binary actin –ADP-ribosylating toxins from various species (see Chapter 12 of this volume).
(h) Novel *C. botulinum, C. baratii, C. butyricum,* and *C. argentinense* neurotoxins (Popoff and Marvaud, 1999; and Chapter 19 of this volume).
(i) *Yersinia pseudotuberculosis* superantigens YPMS for "*Y. pseudotuberculosis*-derived mitogens" (Uchiyama *et al*., 1993; Abe *et al*., 1993), designated YPMa after the discovery of two variants YPMb (Ramamurthy *et al*., 1997), and YPMc (Carnoy et *al*., 2002), respectively. YPMa was shown to behave as a virulence factor (Carnoy *et al*., 2000).
(j) *S. aureus* and *Staphylococcus hyicus* exfoliative toxins (Table 1 and Chapter 56 of this volume).
(k) *Clostridium difficile* toxins A and B (Wren, 1992; Sears and Kaper, 1996).
(l) *E. coli* shiga-like toxins (verotoxins) (see Chapter 17 of this volume).
(m) Enteric toxins from: *Aeromonas species*, *Bacillus cereus, Campylobacter jejuni, E. coli* enteroaggregative, enterohemorragic, enteroinvasive, enteropathogenic, and enterotoxigenic enterotoxins (heat-labile and heat-stable and other toxins), *Plesiomonas shigelloides, Samonella enterica* toxins, *Vibrio cholerae* and other *Vibrio* species toxins (see Sears and Kaper, 1996; Laohachai *et al*., 2003; and relevant chapters of this volume).
(n) Novel ADP-ribosyl transferases.
(o) Novel membrane-damaging toxins (Alouf, 2003).

The cloning and determination of the nucleotide sequence of more than 200 toxin structural genes and a number of regulatory genes

The structural genes are mostly located on bacterial chromosomes (80%), but also on plasmids, bacteriophages, transposons, or pathogenicity islands (PAIs) recently considered as a subtype of genomic islands (GEIs). The discovery of the latter was a considerable breakthrough in the field of bacterial pathogenesis, since these complex genomic segments may carry genes encoding not only protein toxins but also other virulence and pathogenicity factors such as adhesins, secretion, or iron uptake systems (see Hacker and Kaper, 2000; Hacker *et al*., 2004; Chapters 2 and 3 and other relevant chapters of this volume).

Bacteriophage-borne genes encode, among other toxins, diphtheria and cholera toxin, *S. pyogenes* pyrogenic exotoxins A and C, *S. aureus* exfoliative toxin B and enterotoxins A and E, *Clostridium botulinum* neurotoxins C1 and D, and *E. coli* Shiga-like toxins I and II. Plasmid-borne genes encode tetanus neurotoxin and botulinum toxin G, anthrax toxin three-component factors (protective antigen, edema factor, lethal factor), *Enterococcus feacalis* cytolysin, *Shigella dysenteriae* enterotoxin 2, the *E. coli* RTX type enterohemorrhagic (EhxA) toxin, and both heat-labile and heat-stable enterotoxins. The latter

was also shown to be encoded by a transposon gene. On the other hand, the genetic determinants of *E. coli* alpha-hemolysin are generally found on large plasmids in animal isolates of this microorganism or on the chromosome of the strains causing urinary tract infections in humans. The gene encoding *C. perfringens* enterotoxin is located on a transposon integrated between two chromosomal house-keeping genes in human food-poisoning strains, while in animal strains the enterotoxin gene is carried on large plasmids (see relevant chapters of this volume).

Genetic regulation of the expression of bacterial toxins

This field witnessed recently important progress (Stibitz, 2003; Cotter and Jones, 2003; Raffestin *et al.*, 2004; and Chapter 4 of this volume and other relevant chapters). A variety of toxins and enzymes were found to be under the control of two-component regulatory systems consisting of membrane-associated sensor kinases and cytoplasmic response regulators, which are often DNA-binding proteins. This is the case for the BvgA/BvgS system for *Bordetella pertussis*, VirR/VirS for *C. perfringens*, and CovR/CovS for *S. pyogenes*. In the case of *S. aureus*, the production of various toxins and virulence factors is coordinately controlled by at least three genomic regulators, *agr* (accessory gene regulator), *sar* (staphylococcal accessory regulator), and *sae* (*S. aureus* exoprotein expression). Another interesting regulatory system concerns diphtheria toxin (DT), the synthesis of which has been known since the initial investigations of Pappenheimer and Johnson (1936) to take place in low iron concentration. As mentioned before, the structural gene for the toxin is found in a bacteriophage adjacent to the phage *att* site, mapping at the junction between the phage and bacterial chromosomal DNA. The investigations of the groups of Murphy and Holmes showed that DT synthesis is inhibited by a *trans*-acting factor encoded on *C. diphtheriae* chromosome. This led to the discovery of the bacterial *dtxR* gene encoding an 226 amino acid iron responsive repressor protein DtxR. This protein binds to the *tox* operator under conditions of high iron concentration and inhibits transcription of the *tox* gene. As mentioned above the synthesis of many other bacterial toxins and virulence factors is also negatively regulated by iron.

Molecular structure and topology of protein toxins

The determination of the nucleotide sequence of encoding structural genes of bacterial toxins allowed the determination of the amino acid sequences of the encoded toxins, thus leading to the extraordinary development of toxin biochemistry. Most toxins are single-chain polypeptides with molecular sizes ranging from about 2–3 kDa for *E. coli* thermostable enterotoxins up to 300 kDa for *Clostridium difficile* toxins A and B, which are the largest single-chain bacterial protein toxins hitherto identified (Wren, 1992; von Eichel-Streiber *et al.*, 1996).

However, many toxins occur as oligomeric multimolecular complexes comprising two or more noncovalently bonded distinct subunits. Cholera toxin and *E. coli* heat-labile enterotoxins I and II form heterohexamers (A1-B5 complex) composed of one 28 kDa A-subunit (the ADP-ribosylating moiety of the toxin) and five identical 11.8-kDa B-subunits that allow the specific binding of the toxin to ganglioside GM1 at the surface of intestinal target cells. Shiga and *E. coli* Shiga-like toxins also form heterohexamers (A1-B5 complex) composed of a single 32-kDa A-subunit (the N-glycosidase moiety that cleaves a specific adenine residue in the 28 S rRNA component of the eucaryotic ribosomal complex) and five identical 7.7 kDa B-subunits that bind to the terminal galactose residues on globotriasoyl ceramide at the surface of target cells. Pertussis toxin produced by *Bordetella* species, the most complex structure known so far among bacterial toxins, is a multimolecular A1-B5 hexamer composed of five dissimilar subunits. Other A-B type toxins are the bipartite toxins (see Saelinger, 2003; Sandvig, 2003; and Popoff *et al.*, 1989).

Three-dimensional crystal structure

In 1986, Allured and Collier reported the first determination of the three-dimensional (3D) structure of a crystallized bacterial toxin (*P. aeruginosa* exotoxin A). Since then, the 3D structure of more than 40 toxins has been established so far, namely that of diphtheria toxin, pertussis toxin *E. coli*, and *V. cholerae* enterotoxins, Shiga toxin, and various staphylococcal and streptococcal superantigenic toxins, anthrax toxin components, botulinum neurotoxins A, B, and E, etc. (Table 1.2). These important achievements paved the way to fathom in-depth structure-activity relationships.

General paradigms of the mechanisms of bacterial protein toxins' action on eucaryotic cells

At the turn of the 1970s, two revolutionary paradigms heralded the onset of a new era in our understanding of the mechanism of the action of toxins at the subcellular and molecular levels on target human and animal cells. The two paradigms classified the mode of action

of the toxins into two general operational mechanisms: toxins acting ultimately on intracellular targets after crossing the cell membrane and toxins strictly acting at the surface of the cell membrane, respectively.

Protein toxins modifiying intracellular target molecules (the conundrum of the molecular action of A-B type toxins)

Working with diphtheria toxin, R. J. Collier (1975, 1977) was the first to demonstrate that this toxin could enter inside cell cytosol and inactivate an intracellular target molecule leading to the impairment of cell functions. This discovery led to the paradigm that a great number of bacterial (and certain plant) protein toxins consist of two functionally different moieties designated A and B with the A part causing the intracellular damage and the B part serving to bind A to appropriate cell receptors and deliver it to the interior (Popoff, 1998 and Sandvig, 2003). This process involves endocytosis by several complex mechanisms and translocation and trafficking into the cytosol from different intracellular organelles. The A and B moieties can be located on two distinct domains of a single-chain polypeptide (e.g., diphtheria toxin, *P. aeruginosa* exotoxin A) or on two different proteins, example oligomeric toxins (e.g., cholera, pertussis, shiga, and Shiga-like toxins) or binary (bipartite) toxins consisting of two entirely distinct soluble proteins that only associate on the surface of their target cells (see Leppla: in Saelinger, 2003). The A moieties possess various enzymatic activities depending on the nature of the relevant toxins, namely ADP-ribosytransferases, adenylcyclases, metalloproteases, RNA *N*-glycosidases, glycosyl-transferases, and deamidases (see Table 1.1). Recent studies identified many toxin receptors, toxin-induced modulation of various, effectors of intracellular signal transduction pathways, and apoptotic events. The establishment in the past 15 years of the 3D crystal structure of many A-B type toxins (Table 1.2) provided a framework to understand how toxins recognize receptors, penetrate membranes, and interact with and modify intracellular substrates.

Protein toxins acting on cytoplasmic membrane components without intracellular penetration

This process concerns two distinct types of toxins with totally different functional and molecular modes of action.

Receptor-targeted toxins

These toxins bind to appropriate cell receptors with subsequent triggering of intracellular processes via transmembrane signaling followed by harmful effects. Two main classes of toxins exhibit these properties:

1. Superantigenic toxins (see Chapters 49–53).
2. *E. coli* heat-stable ST enterotoxins and related toxins from other Gram-negative bacterial species.

These toxins bind to the guanylate cyclase receptor of target intestinal cells, leading to the activation of cellular guanylate cyclases, which are brush border membrane glycoproteins. The activation provokes an elevation of cyclic GMP that stimulates chloride secretion and/or inhibition of NaCl absorption resulting in net intestinal fluid secretion (diarrhea). A similar effect is observed with the hormone guanylin, which shares 50% homology with the toxin and activates the same receptor.

E. coli ST was first characterized by Kunkel and Robertson (1979) in enterotoxigenic *E. coli* (ETEC). Based on structure-function relationship, STs are divided into two distinct types, namely STa (or STI) and STb (or STII). STa differs from STb by its resistance to proteases, solubility in methanol, and its activity in the suckling mouse model. In enteroaggregative *E. coli* (EAEC), a heat-stable toxin named EAST1 was identified and shown to cause diarrhea in humans. This is often compared to STa. Apart from *E. coli,* other enteric bacteria produce ST toxins. These include *V. cholerae* O1, non O1 or non–O 139, *V. mimicus*, *Yersinia enterocolitica, Y. kristensii, Citrobacter freundii,* and *Klebsiella pneumoniae* (see Sears and Kaper, 1986; Laohachai *et al*., 2003; and Chapters 25 and 48 of this volume).

Membrane-damaging (cytolytic) toxins

Toxin-induced cell damage was historically identified by virtue of the cytolytic action of a great number of toxins on human and animal erythrocytes and to a lesser extent on other cells. These toxins known as *membrane-damaging toxins* (MDTs) or cytolysins produced by both Gram-positive and Gram-negative bacteria possess the ability to damage or disrupt the integrity of the plasma membrane of target cells, reflected by cell swelling and subsequently cell lysis. Many members of the MDTs are important virulence and pathogenicity factors and are sometimes lethal to humans and animals. This family of toxins, initially investigated by the pioneering studies of Alan Bernheimer over three decades since the 1940s (Bernheimer, 1944; Bernheimer and Rudy, 1986) and then by many other groups, constitutes the most important family of bacterial toxins. To date, this family comprises 117 members (35% of the whole repertoire of 337 toxins so far identified). Three types of MDTs can be distinguished, based on their action on cell membranes: (i) enzymically active cytotolysins (phospholipases), which degrade cell-membrane phospholipid bilayer (Table 1.1 and Chapter 27), (ii) tensioactive cytolysins, which solubilize certain cell-membrane components by

a detergent-like action, (iii) pore-forming cytolytic toxins (PFTs), which create channels (pores) through the cytoplasmic membrane.of targetcells. These three types of MDTs encompass 26, 10, and 81 cytolytic proteins, respectively (see Alouf, 2003). The "pore" concept (paradigm) for the last group of MDTs proposed by the end of the 1970s on biochemical and electron microscopic studies initiated by Bernheimer, Freer, Alouf, Bhakdi, and many other authors (see Alouf, 2001; van der Goot, 2003) witnessed a considerable development particularly through the determination of the 3D structures of many cytolysins (Table 1.2) .

The process of pore formation was consistent with the following *modus operandi:* the toxins are released by the bacteria as monomeric water-soluble proteins and bind to cell surface components. Toxin binding facilitates the concentration of the monomers and their transition to form non-covalently associated oligomers, leading to an amphipathic state of the oligomers followed by insertion into the membrane and the formation of protein-lined pores of various sizes, depending on the toxin involved. The various families of pore-forming toxins include particularly the RTX cytolysins (Welch, 2001; Oxhamre and Richter-Dahlfors, 2003), *Aeromonas hydrophila* aerolysin and related toxins (Fivaz *et al*., 2001, and Cole *et al*., 2000) *S. aureus* leucocidins, alpha- and gamma-toxins (Prevost *et al*., 2001), the cholesterol-dependent cytolysins (Alouf, 2001; Billington *et al*., 2001; Tweten *et al*., 2001), the bi-component A/B-type cytolysins from *Serratia, Proteus, Edwardsiella, and Haemophilus* species (Braun and Hertle, 1999), *V. cholerae* and other *V*. species (Shinoda, 1999) (see Chapters 26 and 29 to 47 of this volume).

RATIONAL/CANONICAL CLASSIFICATION OF BACTERIAL TOXINS

The considerable progress of our knowledge of the molecular, structural, and genetic aspects of bacterial protein toxins revealed common functional and/or molecular similarities, which allow a rational/canonical classification of these toxins into families. We briefly describe some major families.

The family of the cytolethal distending toxins (CDTs)

This family (17 members at least) constitutes the most recently discovered group of toxins produced by a variety of Gram-negative bacteria (Thelestam and Fizan, 2001; Dreyfus *et al*., 2003; Nesic *et al*., 2004; and Chapter 23 of this volume). CDTs possess the unique ability to induce DNA double-strand breaks (DSBs) in both proliferating and nonproliferating cells, thereby causing irreversible cell cycle arrest or death of the target cells. CDTs are encoded by three linked genes (*cdtA, cdtB,* and *cdtC*). All three of these gene products are required to constitute the fully active holotoxin, and this is in agreement with the recently determined crystal structure of CDT. The toxin is endocytosed via clathrin-coated pits and requires an intact Golgi complex to exert the cytotoxic activity. Several issues remain to be elucidated regarding CDT biology, such as the detailed function(s) of the cdtA and cdtC subunits, the identity of the cell surface receptor(s) for CDT, the final steps in the cellular internalization pathway, and a molecular understanding of how CDT interacts with DNA. Moreover, the role of CDTs in the pathogenesis of diseases still remains unclear.

The family of RNA *N*-glycosidases, glycosyltransferases, and deamidases: Shiga and Shiga-like toxins, ricin, and other plant toxins

Shiga toxin is an A-B type toxin produced by *Shigella dysenteriae* discovered in 1898 by Kiyoshi Shiga (1870–1957). The toxin is constituted by a ring of identical B-chains non-covalently associated with the A chain. The latter is responsible for the RNA *N*-glycosidase activity, which provokes the cleavage of a purine residue from eucaryotic cell 28S ribosomal RNA, resulting in blockage of protein synthesis and ultimate death of target cell (Endo *et al*., 1988; O'Loughlin and Robins-Browne, 2001; Melton-Celsa and O'Brien, 2003; and Chapter 17 of this volume). Similar structurally and functionally related toxins called *Shiga-like toxins* or *verotoxins* are produced by enterohemorragic *E. coli* (six variants: Stx 1, 1c, 2, 2c, 2d, 2e) and other bacteria such as *Aeromonas hydrophila, A. cavia, Citrobacter freundii,* and *Enterobacter cloacae*. Moreover, very similar toxins from various plants (ricin, abrin, etc.) have been widely investigated (Barbieri *et al*., 1993; Melton-Celsa and O'Brien, 2003).

The family of the bacterial ADP-ribosylating toxins (ADP ribosyl transferases: ADPRTs)

These toxins constitute a class of functionally conserved enzymes produced by a variety of pathogenic Gram-positive and Gram-negative bacterial species, which display toxic and often lethal activity in humans and certain animals. These proteins share the ability to transfer the ADP ribose moiety of β-NAD+ to an eucaryotic target protein. This process impairs essential functions of target cells, thus modifying signal transduction pathways or causing rapid cell death (see

Pallen *et al*., 2001; Barbieri and Burns, 2003; Aktories and Barth, 2004; Masignani *et al*., 2004 for recent references; and Chapter 12 of this volume).

Diphtheria toxin and *Pseudomonas aeruginosa* exotoxin A inhibit protein synthesis by inactivating elongation factor 2 and subsequently cause cell death (Collier 1975, 1977). Other toxins in this family are *P. aeruginosa* cytotoxic exotoxin S, which uncouples Ras signal transduction, pertussis toxin, cholera toxin, and heat-labile enterotoxins LT-I and LT-II from *E. coli,* which interferes with the human host by ADP-ribosylating regulatory heterotrimreric G proteins (Barbieri and Burns, 2003 Masignani *et al*., 2004). The family of binary (bipartite) toxins include the mosquiticidal toxin (MTX) from *B. sphaericus* (Carpusca *et al*., 2004), *Clostridium botulinum* C2 toxin (Ohishi *et al*., 1980 Aktories and Barth, 2004), *C. perfringens* iota toxin (Stiles and Wilkins, 1986) Marvaud *et al*., 2001), *Clostridium spiroforme* toxin (Popoff *et al*., 1989), *C. difficile* ADP-ribosyl transferase (Perelle *et al*., 1997). Other members of the ADPRT family include the low molecular-weight (25–30 kDa) C3 and C3-like enzymes produced by *C. botulinum, C. limosum, B. cereus, S. aureus* respectively, the epidermal differentiation inhibitor (EDIN) from *S. aureus,* and the recently discovered ADPR, SpyA from *Streptococcus pyogenes*, which shares amino acid identity with EDIN and *C. botulinum* C3 (Pallen *et al*., 2001; Coye and Collins, 2004). Recently, the application of *in silico* analyses allowed the identification of more than 20 novel putative members of the ADPRT family and represents a novel challenge in the genomic era (Pallen *et al*., 2001; Masignani *et al*., 2004).

Common molecular features of the ADPRT family

Bacterial ADPRTs have only limited overall amino acid sequence identity. However, the 3D crystal structure and computer modeling studies of those toxins investigated so far indicate that the active site domains of these proteins are related and contain a conserved NAD-binding catalytic domain formed of two perpendicular β-sheet cores and flanked by either one or two α-helices. Moreover, a catalytic glutamate residue is found in the active site of these molecules (Han and Tainer, 2002; Masignani *et al*., 2004).

The family of the glycosylating and deamidating toxins

The family of the glycosylating toxins includes large single-chain proteins *C. difficile* toxins A and B, the lethal and the hemorrhagic toxins from *Clostridium sordellii*, the α-toxin from *C. novyi,* and various toxin isoforms mainly produced by *C. difficile*. These toxins share common features: they have high molecular weights (>250 kDa) and possess glucosyl or N-acetyl glucosaminyltransferase properties. They also share the same functional topology. and are about 40 to 90% identical in their amino acid residues (Aktories, 2003). The toxins inactivate Rho GTPases by glucolysation of a functionally essential threonine (Thr 37) residue. With the exception of *C. novyi* α-toxin, which uses UDP-GlcNAc, the other cytotoxins use UDP-glucose as a substrate. The toxins split the activated nucleotide sugar and transfer only one sugar moeity onto the hydroxyl group of Thr 37 leading to the mono-*O*-glucosylation or mono-*O*-*N*-acetylglucosaminylation of the eucaryotic target protein (von Eichel-Streiber *et al*., 1996; Selzer *et al*., 1996; Popoff *et al*., 1996).

Another process is the deamidation and transglutamination of Rho GTPases by the cytotoxic necrotizing factors (CNFs) 1 and 2 produced by certain *E. coli* strains and the dermonecrotizing toxin (DNT) from *Bordetella pertussis* (Flateau *et al*., 1997; Schmidt *et al*., 1997).

The family of the superantigenic toxins

These toxins exhibit like other bacterial or viral superantigens (SAgs) highly potent, lymphocyte-transforming (mitogenic) activity toward human and/or other mammalian T lymphocytes. Unlike conventional antigens, SAgs bind to certain regions of major histocompatibility complex (MHC) class II molecules of antigen-presenting cells (APCs) outside the classical antigen-binding groove and concomitantly bind under their native form to T cells at the level of certain specific motifs of the variable region of the β chain (Vβ) of T cell receptor (TcR). This interaction triggers the activation (proliferation) of the targeted T lymphocytes and leads to the *in vivo* or *in vitro* release of high amounts of various cytokines and other effectors by immune cells. Each SAg interacts specifically with a characteristic set of Vβ motifs.The repertoire of the superantigenic toxins include: (i) the classical *S. aureus* enterotoxins A, B, C (and antigenic variants) D, E, and the recently discovered enterotoxins G to U, and toxic shock syndrome toxin-1,(ii) the *S. pyogenes* SAgs comprising the classical pyrogenic (erythrogenic) exotoxins A and C and the newly identified pyrogenic toxins G, H, I, J, L, M, as well as SMEZ and SSA, and (iii) the *Yersinia pseudotuberculosis* superantigens.

The structural and genomic aspects of these toxins and their molecular relatedness have been widely investigated (Proft *et al*., 2001; Alouf and Müller-Alouf, 2003; Petersson *et al*., 2004, and Chapters 51 to 55 of this

volume). The 3D crystal structure of many of them have been determined (Table 1.2) as well as that of certain of their complexes with MHC class II molecules and (or) TcR receptors. The production of SAgs by staphylococci and group A streptococci during host infection by these bacteria contributes to a broad spectrum of diseases ranging from mild to severe cutaneous and other tissue infections, to life-threatening septicemia, and toxic shock syndromes (McCormick *et al*., 2001; Llwelyn and Cohen, 2002; Alouf and Müller-Alouf, 2003; Krakauer, 2003; Petersson *et al*., 2004; and Chapters 49 to 53 of this volume).

CONCLUSION

The discovery of the first and major bacterial protein toxins at the end of the nineteenth century paved the way for an outstanding development of a new field not only in microbiology but in many aspects of biological and medical sciences.

The wealth of information obtained during the past 114 years of research in bacterial toxinology has provided a great deal of knowledge regarding the molecular structure, the genetic aspects, and the interaction of these fascinating molecules with the various cellular systems and tissues of the human and animal organisms. The study of the biological effects of bacterial toxins at the subcellular and molecular levels revealed their highly complex implications in a variety of physiological and metabolic processes. The key role of bacterial toxins in the pathogenesis of various acute, chronic, and certain autoimmune diseases afforded new insights in our understanding of infectious diseases. Many questions remain unanswered as yet. Novel toxins will be discovered and new achievements will certainly emerge in the coming years, particularly the design and use of novel therapeutic strategies (drugs, vaccines, adjuvants) in the management of toxin-induced diseases and prophylaxis of susceptible populations. Moreover, several lines of experimental and clinical evidence recently allowed the design of engineered toxins for their potential use against certain tumours and virus-infected cells.

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