

Bacterial Protein Toxins

Editors

Klaus Aktories

Ingo Just



Springer

Contents

CHAPTER 1

Uptake of Protein Toxins Acting Inside Cells

S. OLSNES, J. WESCHE, and P.Ø. FALNES. With 3 Figures	1
A. Introduction and Brief Description of Relevant Toxins	1
B. Binding to Cell-Surface Receptors	4
C. Endocytosis	5
D. Retrograde Vesicular Transport	7
I. Transport to the Golgi Apparatus	7
II. Transport to the Endoplasmic Reticulum	7
E. Translocation to the Cytosol	8
I. From the Surface	8
II. From Endosomes	9
III. From the ER	9
F. Stability of Toxins in the Cytosol	11
G. Translocation of Fusion Proteins	12
References	14

CHAPTER 2

Common Features of ADP-Ribosyltransferases

V. MASIGNANI, M. PIZZA, and R. RAPPOLI. With 5 Figures	21
A. Introduction	21
B. The Well-Characterized Toxins	21
C. A Common Structure for the Catalytic Site	26
I. Region 1	27
II. Region 3	29
III. Region 2	30
D. Other Bacterial Toxins with ADP-Ribosylating Activity	33
E. Eukaryotic Mono-ADP-Ribosyltransferases	35
F. Practical Applications	37
References	39

CHAPTER 3

Diphtheria Toxin and the Diphtheria-Toxin Receptor	
T. UMATA, K.D. SHARMA, and E. MEKADA. With 4 Figures	45
A. Introduction	45
B. Diphtheria Toxin	45
I. Synthesis of Diphtheria Toxin	45
II. Toxicity of Diphtheria Toxin	46
III. Structure and Function of Diphtheria Toxin	48
1. The Catalytic Domain	48
2. The T Domain	48
3. The R Domain	49
IV. Sensitivity to Diphtheria Toxin	50
C. The Diphtheria-Toxin Receptor	51
I. Identification of the Diphtheria-Toxin-Receptor Protein	51
II. Cloning of the Diphtheria-Toxin-Receptor Gene	52
III. The Structure and Function of the Diphtheria-Toxin Receptor	53
IV. Molecules Associated with the Diphtheria-Toxin Receptor	55
1. DRAP27/CD9	55
2. Heparin-Like Molecules	57
V. Receptor and Toxin Entry Process	58
VI. Physiological Role of the Diphtheria-Toxin Receptor	59
1. EGF-Family Growth Factor	59
2. Juxtacrine Growth Regulator	59
3. Conversion of the Membrane-anchored Form to the Soluble Form	60
References	61

CHAPTER 4

<i>Pseudomonas aeruginosa</i> Exotoxin A: Structure/Function, Production, and Intoxication of Eukaryotic Cells	
S.E.H. WEST. With 3 Figures	67
A. Introduction	67
I. Basic Structure	68
II. Role of ETA in Disease	70
B. Production of ETA by the Bacterial Cell	71
I. Characterization of the <i>toxA</i> Structural Gene	71
II. Environmental and Temporal Signals Affecting ETA Production	71
III. Regulation of ETA Production	72
IV. Secretion from the Bacterial Cell	75

C. Intoxication of Eukaryotic Cells	76
I. Binding to a Specific Receptor on the Eukaryotic Cell Surface and Internalization by Receptor-Mediated Endocytosis	77
II. Activation by Proteolytic Cleavage and/or a Conformational Change	79
III. Removal of a Terminal Lysine Residue and Translocation into the Cytosol	80
IV. ADP-Ribosylation of Elongation Factor 2	80
References	82

CHAPTER 5

Diphtheria-Toxin-Based Fusion-Protein Toxins Targeted to the Interleukin-2 Receptor: Unique Probes for Cell Biology and a New Therapeutic Agent for the Treatment of Lymphoma

J.R. MURPHY and J.C. VANDERSPEK	91
A. Introduction	91
B. Diphtheria-Toxin-Based Cytokine Fusion Proteins	91
C. DAB389IL-2 as a Novel Biological Probe for Cell Biology	95
D. Pre-Clinical Characterization of DAB486IL-2 and DAB389IL-2	97
E. Clinical Evaluation of DAB486IL-2 and DAB389IL-2	98
I. Rheumatoid Arthritis	99
II. Psoriasis	100
III. Non-Hodgkin's Lymphoma	101
IV. Cutaneous T-Cell Lymphoma	102
References	104

CHAPTER 6

Structure and Function of Cholera Toxin and Related Enterotoxins

F. VAN DEN AKKER, E. MERRITT, and W.G.J. HOL. With 4 Figures	109
A. Introduction	109
B. Three-Dimensional Structures of Holotoxins	110
C. Toxin Assembly and Secretion	112
I. Design of Assembly Antagonists	113
D. Cell-Surface-Receptor Recognition	113
I. Design of Receptor Antagonists	115
E. Toxin Internalization	115
F. Enzymatic Mechanism	117
I. Substrates, Artificial Substrates, and Inhibitors	117
II. NAD-Binding Site	119

G. The LT-II Family	120
H. Perspectives	125
References	125

CHAPTER 7

Mechanism of Cholera Toxin Action: ADP-Ribosylation Factors as Stimulators of Cholera Toxin-Catalyzed ADP-Ribosylation and Effectors in Intracellular Vesicular Trafficking Events

W.A. PATTON, N. VITALE, J. MOSS, and M. VAUGHAN. With 4 Figures	133
A. Introduction	133
B. Cholera Toxin	135
I. Structure	135
II. Biochemistry	135
III. Toxin Internalization	136
C. ADP-Ribosylation Factors	137
I. Discovery of ARFs	137
II. Biochemical Characterization of ARFs	138
III. ARF Structure	139
1. The Primary Structures of ARFs	139
2. The Tertiary Structures of ARFs	140
IV. Other ARF Family Members	141
1. ARF-Related Proteins	141
2. ARF-Domain Protein 1	143
V. Molecules that Regulate ARF Function: GEPs and GAPs	145
1. ARF Guanine Nucleotide-Exchange Proteins	145
2. ARF GTPase-Activating Proteins	147
VI. Other ARF-Interacting Molecules	149
1. Phospholipase D	149
2. Arfaptins	150
VII. ARF in Cells	150
1. ARFs' Role in Vesicular Trafficking Events	150
2. Subcellular Localization of ARF	151
D. Summary	152
References	152

CHAPTER 8

Pertussis Toxin: Structure–Function Relationship

C. LOCHT, R. ANTOINE, A. VEITHEN, and D. RAZE. With 5 Figures	167
A. Introduction	167
B. The Receptor-Binding Activity of PTX	168

C. Membrane Translocation of PTX	170
D. The Enzymatic Activity of S1	175
E. The Enzyme Mechanism of S1-Catalyzed ADP-Ribosylation	176
F. The Catalytic Residues of PTX	178
G. Substrate Binding by PTX	180
H. Conclusions	182
References	182

CHAPTER 9

Pertussis Toxin as a Pharmacological Tool

B. NÜRNBERG. With 3 Figures	187
A. Introduction	187
B. Molecular Aspects of PT Activity on G Proteins	189
I. General Considerations	189
II. PT-Sensitive G Proteins	190
1. Mechanism of PT Action	190
2. G-Protein Specificity	191
III. PT as a Tool with which to Study G-Protein-Subunit Composition	193
C. Functional Consequences of PT Activity	195
I. PT-Affecting Receptor-G-Protein-Effector Coupling	195
II. PT-Affecting Receptor-Independent Activation of G Proteins	197
III. Use of PT in Studying Cellular Signal Transduction	198
Appendix: Experimental Protocols for Using PT	199
I. Source of PT and Preparation of Solutions	199
II. Treatment of Mammalian Cell Cultures with PT	199
III. Activation of PT for in Vitro ADP-Ribosylation	199
IV. ADP-Ribosylation of Cell-Membrane Proteins by PT	200
V. ADP-Ribosylation of Isolated Proteins by PT	200
VI. Preparation of Samples for Sodium Dodecyl Sulfate Polyacrylamide-Gel Electrophoresis	200
VII. Cleavage of ADP-Ribose from G α Subunits	201
References	201

CHAPTER 10

***Clostridium Botulinum* C3 Exoenzyme and C3-Like Transferases**

K. AKTORIES, H. BARTH, and I. JUST. With 7 Figures	207
A. Introduction	207
B. Origin and Purification of C3 Exoenzymes	207
I. Origin of C3 Exoenzymes	207
II. Purification of C3 Exoenzymes	208

C. Genetics of C3 and C3-Like Exoenzymes	209
D. Structure-Function Analysis of C3 Exoenzymes	210
E. ADP-Ribosyltransferase Activity	212
I. Basic Properties	212
II. Regulation by Detergents and Divalent Cations	212
III. Rho Proteins as Substrates for C3	213
IV. Functional Consequences of the ADP-Ribosylation of Rho	215
F. Application of C3-Like Exoenzymes as Tools	218
G. Cellular Effects of C3 Exoenzymes	219
I. Effects of C3 on Cell Morphology and Actin Structure	219
II. Effects of C3 on Cell-Cell Contacts	222
III. Effects of C3 on Endocytosis and Phagocytosis	223
IV. Effects of C3 on Cell Signalling not Directly Involving the Actin Cytoskeleton	223
1. Phospholipase D and PIP5 Kinase	223
2. Signalling to the Nucleus and Gene Transcription	224
H. Concluding Remarks	224
References	225

CHAPTER 11

Pseudomonas aeruginosa Exoenzyme S, a Bifunctional Cytotoxin Secreted by a Type-III Pathway

J.T. BARBIERI and D.W. FRANK. With 6 Figures	235
A. Introduction	235
B. Initial Biochemical Characterization of ExoS	236
C. Genetic Analysis of the Structural Genes Encoding ExoS	236
D. ExoS Requires FAS to Express ADP-Ribosyltransferase Activity	237
E. Molecular Properties of ExoS	237
F. Secretion of ExoS via a Type-III Secretion Pathway	239
G. Regulation of <i>exoS</i> Regulon Expression	240
H. The Carboxyl Terminus of ExoS Comprises the ADP-Ribosyltransferase Domain	240
I. Functional Mapping of ExoS	240
II. ExoS is a Biglutamic-Acid Transferase	240
III. ExoS can ADP-Ribosylate Numerous Proteins	242
IV. ExoS ADP-Ribosylates Ras at Multiple Arginine Residues	243
I. ExoS is a Bifunctional Cytotoxin	244
I. Cytotoxic Properties of ExoS	245
II. The Amino Terminus of ExoS Stimulates Rho-Dependent Depolymerization of Actin	245

III. The Carboxyl Terminus of ExoS is an ADP-Ribosyltransferase that is Cytotoxic to Cultured Cells	246
J. Mechanism for the Inhibition of Ras-Mediated Signal Transduction by ExoS	246
K. Functional and Sequence Relationship Between ExoS and the Vertebrate ADP-Ribosyltransferases	247
L. Conclusion	248
References	248

CHAPTER 12

Structure and Function of Actin–Adenosine-Diphosphate-Ribosylating Toxins

I. OHISHI. With 2 Figures	253
A. Introduction	253
B. <i>Clostridium Botulinum C₂</i> Toxin	253
I. Actin–ADP-Ribosylating Toxin of <i>C. Botulinum</i>	
Types C and D	253
II. Molecular Structure of Botulinum C ₂ Toxin	254
III. Molecular Functions of Two Components of C ₂ Toxin	256
IV. ADP-Ribosylation of Actin by C ₂ Toxin	257
1. ADP-Ribosylation of Intracellular Actin of Cultured Cells	258
2. ADP-Ribosylation of Purified Actin	260
C. <i>C. Perfringens</i> Iota Toxin	262
I. Actin–ADP-Ribosylating Toxin of <i>C. Perfringens</i> Type E	262
II. Molecular Structure and Function	263
D. <i>C. Spiroforme</i> Toxin	265
I. Actin–ADP-Ribosylating Toxin of <i>C. Spiroforme</i>	265
II. Molecular Structure and Function	265
E. <i>C. Difficile</i> Toxin	266
I. Actin–ADP-Ribosylating Toxin of <i>C. Difficile</i>	266
II. Molecular Structure and Function	266
F. Concluding Remarks	268
References	269

CHAPTER 13

Molecular Biology of Actin-ADP-Ribosylating Toxins

M.R. POPOFF. With 9 Figures	275
A. Introduction	275
B. Bacteria Producing Actin-ADP-Ribosylating Toxins	276
I. <i>C. Botulinum</i>	276
II. <i>C. Perfringens</i>	276

III. <i>C. Spiroforme</i>	277
IV. <i>C. Difficile</i>	277
C. Families of Actin-ADP-Ribosylating Toxins	277
I. C2-Toxin Family	278
II. Iota-Toxin Family	279
III. Relatedness Between C2-Toxin and Iota-Toxin Families	279
D. Actin-ADP-Ribosylating-Toxin Genes and Predicted Molecules	282
I. Iota-Toxin Genes and Iota-Toxin Proteins	282
II. <i>C. Spiroforme</i> Toxin and CDT Genes	283
III. <i>C. Spiroforme</i> Toxin and CDT Proteins	283
IV. C2-Toxin Genes and C2 Proteins	284
E. Relatedness of Actin-ADP-Ribosylating Toxins with Other Toxins	284
I. Relatedness with ADP-Ribosylating Toxins	284
II. Relatedness with <i>Bacillus anthracis</i> Toxins	288
1. Sequence Homology	288
2. Immunological Relatedness	289
3. Functional Comparison	289
III. Relatedness with Other Binary Toxins	290
1. <i>Bacillus</i> Binary Toxins	290
2. Leukocidins and γ -Lysins	291
F. Genetics of the Actin-ADP-Ribosylating Toxins	292
I. Genomic Localization	292
II. Gene Transfer	293
G. Gene Expression	294
I. Genes of Enzymatic and Binding-Component Genes are Organized in an Operon	294
II. Gene Regulation	295
H. Identification of Actin-ADP-Ribosylating-Toxin-Producing Clostridia by Genetic Methods	297
I. Functional Domains	297
I. Enzymatic-Component Domains	297
1. Enzymatic Site	297
2. Enzymatic-Component Domain which Interacts with the Binding Component	299
3. Actin-Binding Site	300
II. Binding-Component Domains	300
K. Concluding Remarks	302
References	302

CHAPTER 14

Molecular Mechanisms of Action of the Large Clostridial Cytotoxins

I. JUST, F. HOFMANN, and K. AKTORIES. With 6 Figures	307
A. Introduction	307
B. Structure of the Toxins	308
C. Cell Entry	309
D. Molecular Mode of Action	315
I. Elucidation of the Molecular Mechanism of Action	315
II. Enzymatic Activity	316
1. Co-Substrates	316
2. Catalytic Domain and Requirements for Catalysis	318
3. Recognition of the Protein Substrates	320
III. Cellular Targets of the Cytotoxins	321
1. Rho and Ras Proteins as Substrates	321
2. Site of Modification	321
3. Cellular Functions of Rho Proteins	322
IV. Functional Consequences of Glucosylation	324
1. Consequences on the GTPase Cycle	324
2. Biological Consequences	325
E. Concluding Remarks	327
References	327

CHAPTER 15

Molecular Biology of Large Clostridial Toxins

J.S. MONCRIEF, D.M. LYERLY, and T.D. WILKINS. With 2 Figures	333
A. Introduction	333
B. Purification and Characterization of Large Clostridial Toxins	335
I. Toxin Production	335
II. Purification and Physicochemical Properties	336
1. <i>C. Difficile</i> Toxins	336
2. <i>C. Sordellii</i> and <i>C. Novyi</i> Toxins	337
III. Biological Properties	337
1. <i>C. Difficile</i> Toxins	337
2. <i>C. Sordellii</i> and <i>C. Novyi</i> Toxins	339
3. Receptors	340
C. Mechanism of Action	341
D. Molecular Genetics of the Toxins	342
I. <i>C. Difficile</i> Toxin A and B Genes	342
II. <i>C. Difficile</i> Toxigenic Element	344
III. Atypical Strains of <i>C. Difficile</i>	344
IV. <i>C. Sordellii</i> and <i>C. Novyi</i> Genes	345
V. Sequence Identity and Conserved Features of the Toxins	346

1. N-Terminal Glucosyltransferase Domain	346
2. Repeating Units	347
3. Additional Conserved Features	348
VI. Gene Transfer in <i>C. difficile</i>	349
E. Regulation of <i>C. difficile</i> Toxins	349
F. Conclusions	351
References	351

CHAPTER 16

The Cytotoxic Necrotizing Factor 1 from *Escherichia coli*

P. BOQUET and C. FIORENTINI. With 5 Figures	361
A. Introduction	361
B. The CNF1 Gene and the Prevalence of CNF1-Producing Strains among Uropathogenic <i>E. coli</i>	362
I. The CNF1 Gene	362
II. Prevalence of CNF1-Producing Strains among Uropathogenic <i>E. coli</i>	363
C. Production, Purification and Cellular Effects of <i>E. coli</i> CNF1	364
I. Production and Purification of <i>E. coli</i> CNF1	364
II. Cellular Effects of <i>E. coli</i> CNF1	365
D. CNF1 Molecular Mechanism of Action	365
I. Intracellular Enzymatic Activity of CNF1	365
II. Consequences of CNF1 Activity on Rho GTP-Binding Proteins	368
E. Structure-Function Relationships of CNF1 and the Family of Dermonecrotic Toxins	373
I. The C-Terminal Part of CNF1 Contains its Enzymatic Activity	373
II. The N-Terminal Part of CNF1 Contains its Cell-Binding Activity	375
F. Possible Roles for CNF1 as a Virulence Factor	375
I. CNF1 and Induction of Phagocytosis	375
II. CNF1 and Cell Apoptosis	377
III. CNF1: Epithelial Cell Permeability and PMN Trans-Epithelial Migration	378
G. Conclusions	379
References	379

CHAPTER 17**Shiga Toxins of *Shigella dysenteriae* and *Escherichia coli***

A.R. MELTON-CELSA and A.D. O'BRIEN. With 1 Figure	385
A. Profile of the Shiga-Toxin Family	385
I. Nomenclature and History	385
II. The Stx Family	386
1. Traits that Make the Members Part of a Family	386
2. Characteristics that Distinguish Stx Family Members ..	387
III. Role of Stxs in <i>S. Dysenteriae</i> Type 1 and STEC Disease	388
1. Pathogenesis of Infection Caused by Organisms that Produce Stxs	388
2. Associations Between Stxs and Development of HC and/or the HUS	388
3. Findings with Animal and Tissue Culture Models that Support a Primary Role for Stxs in Virulence of Shiga's Bacillus and STEC	389
B. Stx Genetics and Expression	389
I. Location, Organization, and Nucleotide and Deduced Amino Acid Sequences of Stx Family Member Operons ..	389
II. Regulation of Toxin Production	391
III. Toxin Purification	391
C. Structure-Function Analyses of Stx Family Members	392
I. Structure of Stx	392
II. Genetic Analyses of Stx Function	393
D. Intracellular Trafficking of the Shiga Toxins	396
E. Virulence/Toxicity Differences among the Stxs	397
F. Immune Response to Stxs, Passive Anti-Stx Therapy, and Vaccine Development	397
I. Anti-Toxin Responses during STEC Infection	397
II. Passive Therapy with Anti-Stx Antibodies	398
III. Vaccine Development	398
G. Summary	399
References	399

CHAPTER 18**Clostridial Neurotoxins**

H. BIGALKE and L.F. SHOER. With 8 Figures	407
A. Introduction	407
B. Tetanus and Botulism in Man and Animals	409
I. Modes of Poisoning	409
II. Clinical Manifestations	410
III. Pathophysiology	410

C. Structure of Clostridial Neurotoxins	412
I. Genetic Determination	412
II. Structure of Proteins	413
D. Toxicokinetics of Clostridial Neurotoxins	417
I. Receptor Binding and Internalization	417
II. Translocation from Endosomes into the Cytosol and Priming	419
III. Sorting, Routing and Axonal Transport	421
E. Toxicodynamics of Clostridial Neurotoxins	422
I. Mode of Action of Clostridial Neuroproteases	422
II. Function of Substrates	425
F. Clostridial Neurotoxins serve as Tools in Cell Biology and as Therapeutic Agents	427
References	431

CHAPTER 19

Anthrax Toxin

S.H. LEPPA. With 1 Figure	445
A. Introduction	445
B. Toxin Genes	446
I. Gene Location and Organization	446
II. DNA Sequences and Transcriptional Regulation	446
C. Toxin-Component Proteins	447
I. Toxin Production, Purification, and Properties	447
II. PA Structure and Function	448
III. LF Structure and Function	452
IV. EF Structure and Function	454
V. PA Family Members	455
D. Cellular Uptake and Internalization	456
I. Cellular Receptor for PA	456
II. Proteolytic Activation of PA	456
III. LF and EF Binding to PA63	457
IV. Endocytic Uptake	458
V. Channel Formation	459
VI. Translocation and Cytosolic Trafficking	461
E. Intracellular Actions	462
I. EF Adenylate Cyclase	462
II. LF Metalloprotease	463
F. Therapeutic Applications of LF Fusion Proteins	464
G. Summary and Future Prospects	465
References	465

CHAPTER 20

Adenylyl-Cyclase Toxin from *Bordetella pertussis*

E.L. HEWLETT and M.C. GRAY. With 1 Figure	473
A. Introduction and Background	473
B. Gene and Protein Structure	474
C. Biological Activities of AC Toxin	475
I. Enzymatic Activity	475
II. Cell-Invasive Activity	476
III. Pore Formation and Hemolysis	477
IV. Summary	479
D. Possible Role/s for AC Toxin in Pathogenesis	480
E. AC toxin as a Protective Antigen	481
F. Uses of AC Toxin as a Novel Research Reagent	482
G. Future Directions	482
References	483

CHAPTER 21

***Helicobacter pylori* Vacuolating Cytotoxin**

W. FISCHER and R. HAAS. With 4 Figures	489
A. Introduction	489
B. Identification and Purification of the <i>H. pylori</i> Vacuolating Cytotoxin	490
C. Gene Structure and Mechanism of Secretion	491
I. Cloning and Molecular Characterisation of <i>vacA</i> Encoding the Vacuolating Cytotoxin	491
II. Autotransporter Organisation of the VacA Precursor	493
III. Mosaic Gene Structure of <i>vacA</i> Alleles in the <i>H. pylori</i> Population	493
IV. Consequences of the <i>vacA</i> Mosaic Gene Structure	494
V. Presence of <i>vacA</i> Homologues in the <i>H. pylori</i> Genome	494
D. Regulation of <i>vacA</i> Gene Expression	495
E. Extracellular Structure and Activation of the Vacuolating Cytotoxin	496
I. Processing and Quaternary Structure	496
II. Activation by Acid	496
F. Effects of VacA on Eucaryotic Cells	497
I. Binding to Target Cells and Mechanism of Uptake	497
II. Vacuole Formation	499
III. Other Effects of VacA	501
G. Clinical Relevance of the Vacuolating Cytotoxin	502
H. VacA as a Vaccine Candidate	503
I. Concluding Remarks	503
References	504

CHAPTER 22

Staphylococcal α Toxin

S. BHAKDI, I. WALEV, M. PALMER, and A. VALEVA. With 8 Figures	509
A. Occurrence and Biological Significance	509
B. Purification and Properties of Monomeric Toxin	509
C. Mechanism of Action	510
I. Binding	510
II. Oligomerization	511
III. Pore Formation	512
D. Structure of Oligomeric Pores	512
I. Structure of the Heptameric Pore Formed in Detergent Solution	512
II. Structure of the Membrane-Bound Oligomer	514
E. Biological Effects	517
I. Cytocidal Action	517
II. Secondary Cellular Reactions	517
1. Reactions Provoked by Transmembrane Flux of Monovalent Ions	517
2. Ca^{2+} -Dependent Reactions	518
3. Long-Range Effects of α Toxin	520
4. Synergism Between α Toxin and Other Toxins	520
F. Resistance and Repair Mechanism	520
G. Use of α Toxin in Cell Biology	522
H. Medical Relevance of α Toxin	523
References	524

CHAPTER 23

Bacterial Phospholipases

R.W. TITBALL and J.I. ROOD. With 5 Figures	529
A. Introduction	529
B. Related Groups of Phospholipases	529
I. Zinc Metallophospholipase Cs	530
II. Gram-Negative PLCs	535
III. Phosphatidylinositol PLCs	535
IV. Phospholipase Ds	536
C. Functional and Biological Properties of Phospholipases	536
D. Modulation of Eukaryotic Cell Metabolism	538
I. Hydrolysis of Membrane Phospholipids	538
II. Hydrolysis of Membrane Phospholipids Modulates Cell Metabolism	541
E. Regulation	542
I. Regulation of the <i>C. perfringens plc</i> gene	542

II. Environmental Control of PLC Production in <i>P. aeruginosa</i>	542
III. Regulation of the <i>Listeria</i> Phospholipases by PrfA	543
F. Role in Disease	544
I. Gas Gangrene	544
II. <i>P. aeruginosa</i> Infections	546
III. The Pathogenesis of Listeriosis	547
IV. Caseous Lymphadenitis in Ruminants	548
G. Conclusions	548
References	549

CHAPTER 24

Pore-Forming Toxins as Cell-Biological and Pharmacological Tools	
G. AHNERT-HILGER, I. PAHNER, and M. HÖLTJE. With 5 Figures	557
A. Permeabilized Cells: an Approach to the Study of Intracellular Processes	557
B. α -Toxin and SLO as Tools with which to Study Functional Aspects of Intracellular Organelles	559
I. Biological Activity and Cell Permeability	559
1. Protocol 1: Permeabilization of Attached Cells by α -Toxin or SLO	560
a) Alternate Protocol 1	560
2. Protocol 2: Permeabilization of Cells in Suspension	560
a) Commentary for Protocols 1 and 2	561
3. Protocol 3: Assay to Compare Biological Activity of Various Pore-Forming Toxins Using Rabbit Erythrocytes	561
a) Commentary for Protocol 3	562
4. Protocol 4: Trypan-Blue Exclusion Test	562
a) Commentary for Protocol 4	563
II. Introduction of Membrane Impermeable Proteins	563
1. Protocol 5: Introduction of Membrane-Impermeable Proteins. Immunofluorescence for Synaptophysin	565
a) Commentary for Protocol 5	565
2. Protocol 6: Introduction of Membrane-Impermeable Proteins. TeNT/LC	566
a) Commentary for Protocol 6	567
III. Assay for Exocytosis in Permeabilized Cells	567
1. Protocol 7: Measuring Exocytosis in Permeabilized Suspension Cells	568
2. Protocol 8: Measuring Exocytosis in Permeabilized Attached Cells	570
a) Commentary for Basic Protocols 7 and 8	570

IV. Regulation of Vesicular Transmitter Transporters in Permeabilized Cells	570
1. Protocol 9: Regulation of Vesicular Transmitter Transporters in Permeabilized Cells	571
a) Commentary for Basic Protocol 9	572
C. Chemicals Used in the Protocols	572
D. Concluding Remarks	573
References	573

CHAPTER 25

Heat-Stable Enterotoxin of <i>Escherichia Coli</i>	
T. HIRAYAMA and A. WADA. With 3 Figures	577
A. Introduction	577
B. Heat-Stable Enterotoxin STa	578
I. Structure and Biological Properties of STa	578
II. Receptor for STa	580
C. STa-like Heat-Stable Enterotoxin	585
D. Heat-Stable Enterotoxin STb	585
I. Structure of STb	585
II. Biological Function of STb	586
E. Concluding Remarks	588
References	588

CHAPTER 26

Superantigenic Toxins	
B. FLEISCHER. With 1 Figure	595
A. Summary	595
B. Introduction	595
C. PETs of <i>S. Aureus</i> and <i>S. Pyogenes</i>	596
I. Genes and Molecules	596
II. Molecular Mechanism of Action	600
1. Binding to MHC class-II Molecules	600
2. Non-MHC Receptors	601
3. Interaction with the TCR	602
III. Biological Significance of PETs	604
1. Role of PETs as Virulence Factors	604
2. Role in Pathogenesis	604
3. Association with Human Autoimmune Disease	605
4. The Enterotoxic Activity	606
D. Other Superantigens (or Pseudosuperantigens) of Gram-Positive Coccii?	607

I. The ETs of <i>S. Aureus</i>	607
II. M Proteins and SPE B of <i>S. Pyogenes</i>	607
III. The Mitogenic Factor of <i>S. Pyogenes</i>	608
E. The <i>M. Arthritidis</i> Superantigen	610
F. The <i>Y. Pseudotuberculosis</i> Mitogen	610
G. Concluding Remarks	611
References	611

CHAPTER 27

Structure and Activity of Endotoxins

S. HAUSCHILDT, W. BRABETZ, A.B. SCHROMM, L. HAMANN, P. ZABEL, E.T. RIETSCHEL, and S. MÜLLER-LOENNIES. With 10 Figures	619
A. Introduction	619
B. The Chemical Structure of LPS	624
I. Structural Characteristics of the O-Specific Chain	627
II. Structural Characteristics of the LPS Core.....	627
III. Structural Characteristics of Lipid A	629
C. Biosynthesis of LPS	632
I. Biosynthesis of Lipid A	632
II. Biosynthesis of the Core Region	632
III. Biosynthesis of the O-Specific Chain	633
D. Structure-Activity Relationships of LPS and Lipid A	634
E. Cellular and Humoral Responses to LPS in Mammals	636
F. Strategies for the Treatment of Gram-Negative Infections	644
I. Antibacterial Agents	645
II. Antagonists of Endotoxic Effects	647
III. Neutralizing Antibodies Against Endotoxin	649
G. Final Remarks	651
References	652

CHAPTER 28

Translocated Toxins and Modulins of *Yersinia*

M. AEPFELBACHER, R. ZUMBIHL, K. RUCKDESCHEL, B. ROUOT, and J. HEESEMANN. With 5 Figures	669
A. Introduction	669
B. <i>Yersinia</i> Protein Type-III Secretion/Translocation System	670
I. Virulence Plasmid pYV	670
II. Regulation of Yop Expression, Secretion and Translocation	671
C. Translocated Toxins and Modulins of <i>Yersinia</i> (Effector Yops) ...	674

I. YopH, a Highly Active Tyrosine Phosphatase	674
II. YopE, an Actin-Disrupting Cytotoxin	677
III. YopP, Modulator of Multiple Signal Pathways Leading to Apoptosis and Cytokine Suppression	678
IV. YopT, Another Actin-Disrupting Cytotoxin	681
V. YopM – So Far, no Evidence for an Intracellular Function	681
VI. YpkA, a Putative Serine/Threonine Kinase Affecting Cell Shape	682
D. Perspectives	683
References	685
Subject Index	691