

*Methods in Enzymology*

*Volume 236*

*Bacterial Pathogenesis*

*Part B*

*Interaction of Pathogenic Bacteria with Host Cells*

EDITED BY

*Virginia L. Clark*

*Patrik M. Bavoil*

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY  
SCHOOL OF MEDICINE AND DENTISTRY  
UNIVERSITY OF ROCHESTER  
ROCHESTER, NEW YORK



ACADEMIC PRESS

San Diego New York Boston London Sydney Tokyo Toronto

## Table of Contents

CONTRIBUTORS TO VOLUME 236 . . . . .	ix
PREFACE . . . . .	xiii
VOLUMES IN SERIES . . . . .	xv

### Section I. Bacterial Effects on Immune Cells

1. Induction of Cytokines in Mononuclear and Vascular Cells by Endotoxin and Other Bacterial Products	HARALD LOPPNOW, HELMUT BRADE, ERNST THEODOR RIETSCHEL, AND HANS-DIETER FLAD	3
2. Effect of Bacterial Products on Colony-Stimulating Factor Production	MELINDA S. DETRICK, ROBERT KREISBERG, AND ROBERT N. MOORE	11
3. Effect of Bacterial Products on Tumor Necrosis Factor Production: Quantitation in Biologic Fluids or Tissues	ZELL A. MCGEE AND CHRISTOPHER M. CLÉMENS	23
4. Induction of Prostaglandin Release from Macrophages by Bacterial Endotoxin	KEN-ICHI TANAMOTO	31
5. Effect of Bacterial Products on Interferon Production	GERALD SONNENFELD	42
6. Regulation of Osteoclastic Activity in Infection	J. EDWARD PUZAS, DAVID G. HICKS, SUSAN D. REYNOLDS, AND REGIS J. O'KEEFE	47
7. Effect of Bacterial Products on Neutrophil Chemotaxis	JOHN R. KALMAR AND THOMAS E. VAN DYKE	58

### Section II. Phagocytosis and Killing Assays

8. Measurement of Nonopsonic Phagocytic Killing by Human and Mouse Phagocytes	RICHARD F. REST AND DAVID P. SPEERT	91
9. Measurement of Opsonic Phagocytosis by Human Polymorphonuclear Neutrophils	JOHN R. KALMAR	108

10. Measurement of Human Neutrophil Respiratory Burst Activity during Phagocytosis of Bacteria	RICHARD F. REST	119
11. Complement-Mediated Bacterial Killing Assays	DANIEL P. MCQUILLEN, SUNITA GULATI, AND PETER A. RICE	137
12. Measurement of Phagosome-Lysosome Fusion and Phagosomal pH	THOMAS H. STEINBERG AND JOEL A. SWANSON	147
13. Neutrophil Defensins: Purification, Characterization, and Antimicrobial Testing	SYLVIA S. L. HARWIG, TOMAS GANZ, AND ROBERT I. LEHRER	160
14. Purification and Assay of Bactericidal/Permeability-Increasing Protein	JERROLD WEISS	173
15. Regulation of Bacterial Gene Expression in Response to Oxidative Stress	GISELA STORZ AND MICHEL B. TOLEDANO	196

### Section III. Adherence

16. Bacterial Lectinlike Adhesins: Determination and Specificity	JANINA GOLDHAR	211
17. Possible Interaction between Animal Lectins and Bacterial Carbohydrates	ROBERT E. MANDRELL, MICHAEL A. APICELLA, R. LINDSTEDT, AND HACKEN LEFFLER	231
18. Coaggregation between Bacterial Species	SUSAN A. KINDER AND STANLEY C. HOLT	254
19. Purification of Somatic Pili	GARY K. SCHOOLNIK	271
20. Genetic, Biochemical, and Structural Studies of Biogenesis of Adhesive Pili in Bacteria	META J. KUEHN, FRANÇOISE JACOB-DUBUISSON, KAREN DODSON, LYNN SLONIM, ROBERT STRIKER, AND SCOTT J. HULTGREN	282
21. Isolation and Identification of Eukaryotic Receptors Promoting Bacterial Internalization	GUY TRAN VAN NHIEU AND RALPH R. ISBERG	307
22. Identification of Fibronectin as a Receptor for Bacterial Cytoadherence	JOHN F. ALDERETE, ROSSANA ARROYO, AND MICHAEL W. LEHKER	318
23. Interactions of Bacteria with Leukocyte Integrins	EVA ROZDZINSKI AND ELAINE TUOMANEN	333
24. Solid-Phase Binding of Microorganisms to Glycolipids and Phospholipids	CAROLYN D. DEAL AND HOWARD C. KRIVAN	346

- |  |   |     |
|--|---|-----|
| 25. Characterization of Microbial Host Receptors | PER FALK,<br>THOMAS BORÉN,<br>AND STAFFAN NORMARK | 353 |
|--|---|-----|

#### Section IV. Invasion and Intracellular Survival in Eukaryotic Cells

- |   |  |     |
|---|--|-----|
| 26. Culture and Isolation of <i>Chlamydia trachomatis</i>   | JULIUS SCHACHTER AND<br>PRISCILLA B. WYRICK                            | 377 |
| 27. Culture of <i>Treponema pallidum</i>  | DAVID L. COX   | 390 |
| 28. Measurement of Invasion by Gentamicin Resistance  | ERIC A. ELSINGHORST  | 405 |
| 29. Measurements of Invasion by Antibody Labeling and Electron Microscopy                                     | JOSEPH P. M. VAN PUTTEN,<br>JAN F. L. WEEL,<br>AND HEIKE U. C. GRASSMÉ | 420 |
| 30. Polarized Epithelial Monolayers: Model Systems to Study Bacterial Interactions with Host Epithelial Cells | M. GRACIELA PUCCIARELLI<br>AND B. BRETT FINLAY                         | 438 |
| 31. Interjunctional Invasion of Endothelial Cell Monolayers   | DAVID A. HAAKE<br>AND MICHAEL A. LOVETT                                | 447 |
| 32. Intracellular Growth of Bacteria  | SIAN JONES AND<br>DANIEL A. PORTNOY                                    | 463 |
| 33. Inhibitors of Cytoskeletal Function and Signal Transduction to Study Bacterial Invasion                   | ILAN ROSENSHINE,<br>SHARON RUSCHKOWSKI,<br>AND B. BRETT FINLAY         | 467 |
| 34. Methods to Visualize Actin Polymerization Associated with Bacterial Invasion                              | LEWIS G. TILNEY<br>AND MARY S. TILNEY                                  | 476 |
| 35. Measurement of Free Intracellular Calcium Levels in Epithelial Cells as Consequence of Bacterial Invasion | JOHN L. PACE AND<br>JORGE E. GALÁN                                     | 482 |

#### Section V. Identification of Genes Involved in Invasion

- |  |   |     |
|--|---|-----|
| 36. <i>Shigella flexneri</i> : Isolation of Noninvasive Mutants of Gram-Negative Pathogens | ROBERT MÉNARD AND<br>PHILIPPE J. SANSONETTI | 493 |
| 37. Isolation of <i>Salmonella</i> Mutants Defective for Intracellular Survival            | FRANCES BOWE<br>AND FRED HEFFRON            | 509 |
| 38. Small Plaque Mutants   | SIAN JONES AND<br>DANIEL A. PORTNOY         | 526 |
| 39. Isolation of Hyperinvasive Mutants of <i>Salmonella</i>                                | CATHERINE A. LEE<br>AND STANLEY FALKOW      | 531 |

---

40. Molecular Cloning of Invasion Genes from <i>Yersinia</i> and <i>Salmonella</i>	VIRGINIA L. MILLER AND BARBARA J. STONE	546
41. Molecular Cloning and Expression of Internalin in <i>Listeria</i>	JEAN-LOUIS GAILLARD, S. DRAMSI, PATRICK BERCHE, AND PASCALE COSSART	551
42. Use of <i>Staphylococcus aureus</i> Coated with Invasin Derivatives to Assay Invasin Function	SUSANNAH RANKIN, GUY TRAN VAN NHIEU, AND RALPH R. ISBERG	566
AUTHOR INDEX . . . . .		579
SUBJECT INDEX . . . . .		617