

# Contents

- 1 Basic Immunobiology . . . . . 1**
  - George A. Parker and Tracey L. Papenfuss . . . . . 1
  - 1.1 Introduction . . . . . 1
  - 1.2 General Purpose, Function and Characteristics of the Immune System . . . . . 2
    - 1.2.1 Phylogeny of the Immune System . . . . . 2
    - 1.2.2 Overview of Organs and Tissues of the Immune System . . . 4
    - 1.2.3 Primary Immune System Organs . . . . . 4
    - 1.2.4 Secondary Immune System Organs . . . . . 5
    - 1.2.5 Tertiary Lymphoid Tissue . . . . . 6
    - 1.2.6 Non-immune Organs with Non-specific and Specific Immunologic Functions . . . . . 8
  - 1.3 Innate Immune System Overview . . . . . 9
    - 1.3.1 Signaling Pathways of the Innate Immune System . . . . . 10
    - 1.3.2 Natural Antibodies . . . . . 12
    - 1.3.3 Complement . . . . . 13
    - 1.3.4 Cells of the Innate Immune System . . . . . 15
    - 1.3.5 Antigen Processing and Presentation . . . . . 22
      - 1.3.5.1 MHC Class I . . . . . 23
      - 1.3.5.2 MHC Class II . . . . . 24
  - 1.4 Adaptive Immune System Overview . . . . . 26
    - 1.4.1 CD4<sup>+</sup> T Cell Activation and Function . . . . . 27
    - 1.4.2 CD4<sup>+</sup> Helper T Cell Populations . . . . . 30
    - 1.4.3 CD8<sup>+</sup> T Cell Activation and Function . . . . . 32
    - 1.4.4 B Cells: Activation, Antibody Production and Generation of Humoral Responses . . . . . 34
  - 1.5 Regulation of the Immune Response . . . . . 37
    - 1.5.1 Cytokines . . . . . 37
    - 1.5.2 Chemokines . . . . . 41
    - 1.5.3 Interferon Type I . . . . . 43
    - 1.5.4 Extracellular Vesicles/Exosomes and Immunity . . . . . 44

|          |                                                                               |           |
|----------|-------------------------------------------------------------------------------|-----------|
| 1.6      | Refinement of Immune Responses. . . . .                                       | 45        |
| 1.6.1    | Affinity Maturation . . . . .                                                 | 45        |
| 1.6.2    | Generation of Immunological Memory . . . . .                                  | 45        |
| 1.6.2.1  | Memory B Cells . . . . .                                                      | 46        |
| 1.6.2.2  | Memory T Cells . . . . .                                                      | 47        |
| 1.7      | Immune Responses to Infectious Diseases . . . . .                             | 48        |
| 1.8      | Adverse or Nonspecific Immune Activation and Immune<br>Dysregulation. . . . . | 51        |
| 1.8.1    | Accidental or Incidental Immunologic Activation. . . . .                      | 51        |
| 1.8.2    | Heterologous Immunity . . . . .                                               | 52        |
| 1.8.3    | Hypersensitivity Reactions Type I–V . . . . .                                 | 53        |
| 1.8.4    | Immune Dysregulation in Cancer . . . . .                                      | 53        |
| 1.8.5    | Immunosenescence . . . . .                                                    | 55        |
| 1.8.6    | Cytokine Storm . . . . .                                                      | 56        |
| 1.8.7    | Eicosanoid Storm. . . . .                                                     | 58        |
| 1.9      | Major Ancillary Concepts and Processes. . . . .                               | 59        |
| 1.9.1    | Leukocyte Emigration and Chemotaxis. . . . .                                  | 59        |
| 1.9.2    | Programmed Cell Death and Immunity . . . . .                                  | 61        |
| 1.9.3    | Autophagy and Immunity . . . . .                                              | 63        |
| 1.9.4    | Microbiome and Immunity . . . . .                                             | 67        |
|          | References. . . . .                                                           | 70        |
| <b>2</b> | <b>Cells of the Immune System. . . . .</b>                                    | <b>95</b> |
|          | George A. Parker                                                              |           |
| 2.1      | Introduction . . . . .                                                        | 96        |
| 2.2      | Primary Immune System Cells . . . . .                                         | 96        |
| 2.2.1    | Cells of the Innate Immune System. . . . .                                    | 96        |
| 2.2.1.1  | Monocytes and Macrophages . . . . .                                           | 98        |
| 2.2.1.2  | Neutrophils . . . . .                                                         | 103       |
| 2.2.1.3  | Eosinophils, Basophils & Mast Cells . . . . .                                 | 109       |
| 2.2.1.4  | NK & NKT Cells . . . . .                                                      | 121       |
| 2.2.1.5  | Innate Lymphoid Cells (MAIT Cells<br>and iNKT Cells) . . . . .                | 122       |
| 2.2.1.6  | Myeloid Suppressor Cells. . . . .                                             | 123       |
| 2.2.2    | Cells of the Adaptive Immune System . . . . .                                 | 124       |
| 2.2.2.1  | T Lymphocytes . . . . .                                                       | 125       |
| 2.2.2.2  | B Lymphocytes and Plasma Cells. . . . .                                       | 134       |
| 2.2.2.3  | Dendritic Cells . . . . .                                                     | 137       |
| 2.2.2.4  | Stromal Cells. . . . .                                                        | 143       |
| 2.3      | Cells of Tissues with Secondary Immune Functions. . . . .                     | 151       |
| 2.3.1    | Liver. . . . .                                                                | 151       |
| 2.3.1.1  | Kupffer Cells. . . . .                                                        | 152       |
| 2.3.1.2  | Liver-adapted NK Cells ('pit cells')<br>and NKT Cells . . . . .               | 153       |
| 2.3.1.3  | Sinusoidal Endothelial Cells. . . . .                                         | 154       |

|          |                                                                                           |            |
|----------|-------------------------------------------------------------------------------------------|------------|
| 2.3.1.4  | Hepatocytes . . . . .                                                                     | 156        |
| 2.3.1.5  | Intrahepatic Dendritic Cells . . . . .                                                    | 157        |
| 2.3.2    | Intestinal Cells . . . . .                                                                | 158        |
| 2.3.2.1  | Mucosal Epithelial Cells . . . . .                                                        | 158        |
| 2.3.2.2  | Tuft Cells . . . . .                                                                      | 158        |
| 2.3.2.3  | Paneth Cells . . . . .                                                                    | 158        |
| 2.3.2.4  | NK Cells/Large Granule Leukocytes . . . . .                                               | 160        |
|          | References . . . . .                                                                      | 162        |
| <b>3</b> | <b>Signaling and Effector Molecules in Immunity . . . . .</b>                             | <b>203</b> |
|          | Michael C. Milone                                                                         |            |
| 3.1      | Introduction . . . . .                                                                    | 203        |
| 3.1.1    | Innate Immune Recognition . . . . .                                                       | 204        |
| 3.1.1.1  | Pattern Recognition Receptors (PRRs) . . . . .                                            | 204        |
| 3.1.1.2  | Toll-Like Receptors (TLRs) . . . . .                                                      | 205        |
| 3.1.1.3  | PRRs Beyond the TLRs . . . . .                                                            | 207        |
| 3.1.1.4  | The “Inflammasome” as a Unique<br>Signaling Complex Driving Inflammation . . . . .        | 208        |
| 3.1.1.5  | NF- $\kappa$ B: A Master Regulator of Inflammation . . . . .                              | 210        |
| 3.1.2    | Adaptive Immune Recognition . . . . .                                                     | 211        |
| 3.1.2.1  | T Cell Receptor (TCR) . . . . .                                                           | 211        |
| 3.1.2.2  | B Cell Receptor (BCR) and Antibodies . . . . .                                            | 213        |
| 3.1.3    | Immune Recognition that Blurs the Lines Between<br>Innate and Adaptive Immunity . . . . . | 214        |
| 3.1.3.1  | Fc Receptors . . . . .                                                                    | 214        |
| 3.1.3.2  | NK Cell-Associated Receptors . . . . .                                                    | 215        |
| 3.1.3.3  | KIR and Ly-49 Families of Receptors . . . . .                                             | 216        |
| 3.1.3.4  | CD94: NKG2A/C Receptors . . . . .                                                         | 217        |
| 3.1.3.5  | NKG2D: A Receptor for Altered Self . . . . .                                              | 218        |
| 3.1.3.6  | Natural Cytotoxicity Receptors (NCRs) . . . . .                                           | 219        |
| 3.1.4    | Immune Effector Molecules . . . . .                                                       | 219        |
| 3.1.4.1  | Defensins and Other Antimicrobial Peptides . . . . .                                      | 219        |
| 3.1.4.2  | Complement . . . . .                                                                      | 220        |
| 3.1.4.3  | Cytokines . . . . .                                                                       | 223        |
|          | References . . . . .                                                                      | 235        |
| <b>4</b> | <b>Development of Immune System Organs . . . . .</b>                                      | <b>245</b> |
|          | George A. Parker                                                                          |            |
| 4.1      | Introduction . . . . .                                                                    | 248        |
| 4.2      | Embryogenesis and Organogenesis of the Immune<br>System Organs . . . . .                  | 248        |
| 4.2.1    | Bone Marrow and Liver . . . . .                                                           | 249        |
| 4.2.2    | Thymus . . . . .                                                                          | 251        |
| 4.2.3    | Spleen . . . . .                                                                          | 255        |

|          |                                                                                               |            |
|----------|-----------------------------------------------------------------------------------------------|------------|
| 4.2.4    | Lymph Nodes . . . . .                                                                         | 258        |
| 4.2.5    | Mucosa-Associated Lymphoid Tissue (MALT). . . . .                                             | 262        |
| 4.3      | Postnatal Development of Immune System Organs. . . . .                                        | 264        |
| 4.3.1    | Bone Marrow and Liver. . . . .                                                                | 264        |
| 4.3.2    | Thymus . . . . .                                                                              | 266        |
| 4.3.3    | Spleen. . . . .                                                                               | 270        |
| 4.3.4    | Lymph Nodes. . . . .                                                                          | 273        |
| 4.3.5    | Mucosa-Associated Lymphoid Tissue (MALT). . . . .                                             | 277        |
| 4.3.5.1  | Gut-Associated Lymphoid Tissue (GALT). . . . .                                                | 278        |
| 4.3.5.2  | Bronchus-Associated Lymphoid Tissue (BALT) . . . . .                                          | 285        |
| 4.3.5.3  | Nasopharynx-Associated Lymphoid Tissue (NALT) . . . . .                                       | 286        |
| 4.4      | Tertiary Lymphoid Tissue and Milky Spots. . . . .                                             | 288        |
|          | References. . . . .                                                                           | 288        |
| <b>5</b> | <b>Aging of Immune System Organs . . . . .</b>                                                | <b>295</b> |
|          | George A. Parker                                                                              |            |
| 5.1      | Introduction . . . . .                                                                        | 296        |
| 5.2      | General Features of Immunosenescence . . . . .                                                | 297        |
| 5.3      | Effects of Aging on Hematopoietic Stem Cells and Committed Lymphoid Progenitor Cells. . . . . | 297        |
| 5.4      | Effects of Aging on the Innate Immune System . . . . .                                        | 298        |
| 5.4.1    | Effects of Aging on Neutrophils . . . . .                                                     | 300        |
| 5.4.2    | Effects of Aging on Monocytes and Macrophages . . . . .                                       | 302        |
| 5.4.3    | Effects of Aging on Dendritic Cells. . . . .                                                  | 303        |
| 5.4.4    | Effects of Aging on NK Cells . . . . .                                                        | 305        |
| 5.4.5    | Effects of Aging on NKT Cells . . . . .                                                       | 305        |
| 5.4.6    | Effects of Aging on Mast Cells and Eosinophils. . . . .                                       | 306        |
| 5.5      | Effects of Aging on the Adaptive Immune System . . . . .                                      | 307        |
| 5.5.1    | Effects of Aging on T Cells . . . . .                                                         | 307        |
| 5.5.2    | Effects of Aging on B Cells. . . . .                                                          | 311        |
| 5.5.3    | Effects of Aging on the Germinal Center Reaction. . . . .                                     | 312        |
| 5.6      | Effects of Aging on Cytokines. . . . .                                                        | 313        |
| 5.7      | Clinical Effects of Immunosenescence . . . . .                                                | 313        |
| 5.8      | Histomorphology of Immunosenescence. . . . .                                                  | 314        |
| 5.8.1    | Histomorphological Effects of Aging on the Bone Marrow . . . . .                              | 315        |
| 5.8.2    | Histomorphological Effects of Aging on the Thymus. . . . .                                    | 316        |
| 5.8.3    | Histomorphological Effects of Aging on the Spleen. . . . .                                    | 321        |
| 5.8.4    | Histomorphological Effects of Aging on Lymph Nodes. . . . .                                   | 325        |
| 5.9      | Summary Points. . . . .                                                                       | 329        |
|          | References. . . . .                                                                           | 330        |

|          |                                                                                       |            |
|----------|---------------------------------------------------------------------------------------|------------|
| <b>6</b> | <b>Flow Cytometry and Immunophenotyping in Drug Development</b>                       | <b>343</b> |
|          | Tracey L. Papenfuss                                                                   |            |
| 6.1      | Introduction                                                                          | 344        |
| 6.2      | Uses of Flow Cytometry in Toxicology                                                  | 348        |
| 6.2.1    | Early Discovery                                                                       | 348        |
| 6.2.2    | Safety Assessment and Toxicology                                                      | 349        |
| 6.2.3    | Clinical Testing and Monitoring                                                       | 349        |
| 6.3      | Considerations in the Implementation of Flow Cytometric Analysis                      | 351        |
| 6.3.1    | General Considerations                                                                | 351        |
| 6.3.2    | Instrumentation                                                                       | 356        |
| 6.3.3    | Sample Collection and Processing for Immediate Flow Cytometric Analysis               | 357        |
| 6.3.4    | Advanced and Enhanced Sample Preparation                                              | 358        |
| 6.3.5    | Sample Preservation and Storage for Delayed Flow Cytometric Analysis                  | 359        |
| 6.3.6    | Antibodies                                                                            | 360        |
| 6.3.7    | Data Acquisition, Compensation, Controls and Thresholds                               | 361        |
| 6.3.8    | Data Analysis and Reporting                                                           | 362        |
| 6.3.9    | Validation                                                                            | 363        |
| 6.4      | Integration of Flow Cytometry with Other Investigative Techniques in Drug Development | 364        |
| 6.5      | Conclusions                                                                           | 364        |
|          | References                                                                            | 365        |
| <b>7</b> | <b>Pathology Evaluation for Detection of Immunomodulation</b>                         | <b>371</b> |
|          | George A. Parker and Paul W. Snyder                                                   |            |
| 7.1      | Introduction                                                                          | 373        |
| 7.2      | Necropsy and Histotechnology                                                          | 374        |
| 7.2.1    | Fixation of Tissue Specimens for Routine Histopathological Evaluation                 | 376        |
| 7.2.2    | Histotechnique Considerations for Immune System Organs                                | 376        |
| 7.3      | Organ Weight Evaluation                                                               | 379        |
| 7.4      | Histopathology                                                                        | 379        |
| 7.4.1    | Morphological Alterations in Organs of the Immune System                              | 380        |
| 7.4.1.1  | Germinal Center Reaction                                                              | 384        |
| 7.4.2    | Secondary Evidence of Immune System Dysfunction                                       | 390        |
| 7.4.3    | Stress Response Versus Immunomodulation                                               | 391        |
| 7.4.4    | Adverse Versus Non-adverse Determinations                                             | 396        |
| 7.5      | Special Circumstances in the Pathology Evaluation                                     | 398        |
| 7.5.1    | Studies Conducted in Juvenile Animals                                                 | 398        |
| 7.5.2    | Studies Involving Biologics                                                           | 399        |

|          |                                                                                   |            |
|----------|-----------------------------------------------------------------------------------|------------|
| 7.5.3    | Immunomodulatory Experimental Procedures and Vehicles . . . . .                   | 402        |
| 7.5.3.1  | Infusion Studies . . . . .                                                        | 402        |
| 7.5.3.2  | Saline Infusion or Injection . . . . .                                            | 404        |
| 7.5.3.3  | Polyethylene Glycol-Conjugated Test Articles . . . . .                            | 404        |
| 7.5.4    | Immunomodulation Due to Concurrent Disease . . . . .                              | 405        |
| 7.5.4.1  | Auricular Chondritis . . . . .                                                    | 405        |
| 7.5.4.2  | Inflammatory Foot Lesions in Rats . . . . .                                       | 406        |
| 7.5.5    | Syphacea Infestation . . . . .                                                    | 407        |
| 7.5.6    | CAR Bacillus Infection . . . . .                                                  | 409        |
| 7.5.7    | Murine Mycoplasmosis . . . . .                                                    | 410        |
| 7.5.8    | Reactivated Viral Infections in Nonhuman Primates . . . . .                       | 412        |
| 7.5.9    | Candidiasis as a Complication of Immunomodulation . . . . .                       | 415        |
| 7.5.10   | Demodex-Related Skin Lesions . . . . .                                            | 420        |
| 7.5.11   | Oesophagostomiasis in Nonhuman Primates . . . . .                                 | 421        |
| 7.5.12   | <i>Pneumonyssus</i> Mites and Lung Pigment in Nonhuman Primates . . . . .         | 421        |
| 7.5.13   | Cercopithecine Herpesvirus Type-1 (Herpes B virus) in Nonhuman Primates . . . . . | 422        |
| 7.5.14   | Atypical Infections . . . . .                                                     | 422        |
| 7.5.15   | Non-specific Inflammatory Lesions . . . . .                                       | 423        |
| 7.5.16   | Amyloid Deposition in Tissues . . . . .                                           | 424        |
| 7.5.17   | Pseudoamyloid . . . . .                                                           | 425        |
| 7.5.18   | Miscellaneous Histologic Changes . . . . .                                        | 426        |
| 7.6      | 'Whole Animal' Approach to Immunopathology . . . . .                              | 428        |
| 7.7      | Summary Points . . . . .                                                          | 431        |
|          | References . . . . .                                                              | 432        |
| <b>8</b> | <b>Enhanced Immunohistopathology . . . . .</b>                                    | <b>443</b> |
|          | Susan A. Elmore                                                                   |            |
| 8.1      | Introduction and Historical Perspective . . . . .                                 | 443        |
| 8.2      | Enhanced Histopathology Defined . . . . .                                         | 444        |
| 8.3      | Methodology and Organ-Specific Examples of Enhanced Histopathology . . . . .      | 445        |
| 8.3.1    | Evaluation of the Thymus . . . . .                                                | 447        |
| 8.3.2    | Evaluation of the Bone Marrow . . . . .                                           | 448        |
| 8.3.3    | Evaluation of the Spleen . . . . .                                                | 450        |
| 8.3.4    | Evaluation of the Lymph Nodes . . . . .                                           | 450        |
| 8.3.5    | Evaluation of the Mucosa-Associated Lymphoid Tissue . . . . .                     | 451        |
| 8.4      | Points to Consider and Caveats . . . . .                                          | 452        |
|          | References . . . . .                                                              | 455        |

|                                                                                                           |     |
|-----------------------------------------------------------------------------------------------------------|-----|
| <b>9 Clinical Pathology Assays in Immunopathology</b> .....                                               | 457 |
| Josely F. Figueiredo, Kirstin F. Barnhart, and Niraj Tripathi                                             |     |
| 9.1 Introduction .....                                                                                    | 457 |
| 9.2 Clinical Pathology Testing .....                                                                      | 458 |
| 9.2.1 Hematology .....                                                                                    | 458 |
| 9.2.1.1 Leukocytes .....                                                                                  | 459 |
| 9.2.1.2 Red Blood Cells .....                                                                             | 462 |
| 9.2.1.3 Platelets .....                                                                                   | 463 |
| 9.2.2 Cytological Evaluation of Bone Marrow .....                                                         | 463 |
| 9.2.3 Clinical Chemistry .....                                                                            | 465 |
| 9.2.3.1 Globulins .....                                                                                   | 465 |
| 9.2.3.2 Acute Phase Proteins .....                                                                        | 468 |
| 9.2.4 Urinalysis .....                                                                                    | 469 |
| 9.2.5 Coagulation .....                                                                                   | 470 |
| 9.2.6 Hormones .....                                                                                      | 472 |
| 9.2.7 Autoantibodies .....                                                                                | 473 |
| 9.2.7.1 Anti-platelet Antibodies .....                                                                    | 474 |
| 9.2.7.2 Anti-erythrocyte Antibodies .....                                                                 | 475 |
| 9.2.7.3 Systemic Autoantibodies .....                                                                     | 477 |
| 9.2.8 Complement .....                                                                                    | 478 |
| 9.2.9 Cytokines .....                                                                                     | 480 |
| References .....                                                                                          | 481 |
| <b>10 Application of Immunohistochemistry in Toxicologic Pathology of the Hematolymphoid System</b> ..... | 489 |
| Jerold E. Rehg and Jerrold M. Ward                                                                        |     |
| 10.1 Introduction .....                                                                                   | 489 |
| 10.2 Technical Considerations .....                                                                       | 490 |
| 10.2.1 Tissue Preparation and Fixation .....                                                              | 490 |
| 10.2.2 Fixative and Fixation Time .....                                                                   | 496 |
| 10.2.3 Decalcification .....                                                                              | 496 |
| 10.2.4 Immunohistochemistry Fundamentals .....                                                            | 496 |
| 10.2.5 Specific Staining .....                                                                            | 497 |
| 10.2.6 Controls .....                                                                                     | 497 |
| 10.2.7 Antibodies and Antigens for FFPE Tissues .....                                                     | 498 |
| 10.3 Panleukocyte Antigen .....                                                                           | 498 |
| 10.4 B-Cell and Plasma Cell Lineage Antigens .....                                                        | 498 |
| 10.4.1 B-Cell Antigens .....                                                                              | 498 |
| 10.4.2 Immunoglobulins .....                                                                              | 501 |
| 10.4.3 Plasma Cell Antigens .....                                                                         | 501 |
| 10.5 T-, NK-T and NK Cell Lineage Antigens .....                                                          | 502 |
| 10.6 Other Lymphoid Antigens .....                                                                        | 508 |
| 10.7 Myeloid Lineage Antigens .....                                                                       | 511 |
| 10.7.1 Undifferentiated Myeloid Antigens .....                                                            | 511 |
| 10.7.2 Common Granulocyte and Monocyte Antigens .....                                                     | 512 |

|           |                                                                                                                                                                 |            |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| 10.7.3    | Erythroid Cell Antigens . . . . .                                                                                                                               | 513        |
| 10.7.4    | Megakaryocyte Antigens . . . . .                                                                                                                                | 514        |
| 10.7.5    | Eosinophil Antigens . . . . .                                                                                                                                   | 515        |
| 10.7.6    | Mast Cell and Basophil Antigens. . . . .                                                                                                                        | 515        |
| 10.7.7    | Tissue Histiocyte (Macrophage) Antigens . . . . .                                                                                                               | 518        |
| 10.7.8    | Dendritic Lineage Antigens . . . . .                                                                                                                            | 523        |
| 10.8      | Proliferation and Apoptotic Antigens . . . . .                                                                                                                  | 524        |
| 10.8.1    | Proliferation Antigens . . . . .                                                                                                                                | 524        |
| 10.8.2    | Apoptosis Antigens . . . . .                                                                                                                                    | 526        |
| 10.8.3    | Cross-Species Immunoreactivity of Antibodies. . . . .                                                                                                           | 526        |
| 10.8.4    | Rat, Monkey, Dog, and Pig Lymphoid<br>Immunohistochemistry. . . . .                                                                                             | 526        |
| 10.9      | Examples of the Use of IHC for Evaluating the<br>Hematolymphoid System. . . . .                                                                                 | 528        |
| 10.9.1    | Assessment of the Normal Expression Patterns<br>of Hematolymphoid Cells in Lymphoid Tissue<br>and Bone Marrow. . . . .                                          | 528        |
| 10.9.2    | Development of Abnormal Patterns of Antigen<br>Expression in Lymphoid Cells and Tissues after<br>Exposure to Toxins and Toxicants and<br>in Neoplasia . . . . . | 530        |
| 10.9.3    | Loss of an Antigen Commonly Associated<br>with a Cell . . . . .                                                                                                 | 533        |
| 10.9.4    | Alterations in the Number or Distribution<br>of Hematolymphoid Cells in a Tissue . . . . .                                                                      | 534        |
| 10.9.5    | Changes in the Expression of Antigens Associated<br>with a Cell . . . . .                                                                                       | 536        |
| 10.9.6    | Identification of Hematolymphoid Cells Associated<br>with an Inflammatory Lesion . . . . .                                                                      | 536        |
| 10.9.7    | Cell Proliferation Markers . . . . .                                                                                                                            | 540        |
| 10.9.8    | Diagnosis of Hematolymphoid Disorders . . . . .                                                                                                                 | 542        |
| 10.9.9    | Diagnosis of Myeloid Proliferations . . . . .                                                                                                                   | 543        |
| 10.9.10   | Diagnosis of Lymphoma . . . . .                                                                                                                                 | 548        |
| 10.9.11   | IHC Criteria for B-Cell Malignancy . . . . .                                                                                                                    | 548        |
| 10.9.12   | IHC Criteria for T-Cell Malignancy. . . . .                                                                                                                     | 549        |
|           | References. . . . .                                                                                                                                             | 551        |
| <b>11</b> | <b>Morphometry and Stereology in Immunopathology . . . . .</b>                                                                                                  | <b>563</b> |
|           | Danielle L. Brown and Cynthia L. Swanson                                                                                                                        |            |
| 11.1      | Introduction . . . . .                                                                                                                                          | 563        |
| 11.2      | Histomorphometry in Immunopathology . . . . .                                                                                                                   | 564        |
| 11.3      | Limitations of Histomorphometry . . . . .                                                                                                                       | 568        |
| 11.4      | Stereology in Immunopathology . . . . .                                                                                                                         | 570        |
| 11.4.1    | Tissue Sampling for Stereology. . . . .                                                                                                                         | 570        |



|           |                                                                            |            |
|-----------|----------------------------------------------------------------------------|------------|
| 11.4.2    | Sampling Fields of View and Applying Stereological Probes . . . . .        | 575        |
| 11.4.3    | Applications of Stereology in Immunopathology . . . . .                    | 577        |
|           | References . . . . .                                                       | 582        |
| <b>12</b> | <b>Molecular Techniques in Immunopathology . . . . .</b>                   | <b>585</b> |
|           | Bevin Zimmerman, Jason Aligo, and Daniel Weinstock                         |            |
| 12.1      | Introduction . . . . .                                                     | 585        |
| 12.2      | Sample Preparation for Molecular Analysis . . . . .                        | 587        |
| 12.2.1    | Procurement and Fixation . . . . .                                         | 587        |
| 12.2.2    | Processing and Storage . . . . .                                           | 588        |
| 12.3      | Polymerase Chain Reaction (PCR) . . . . .                                  | 589        |
| 12.4      | In Situ Hybridization . . . . .                                            | 590        |
| 12.4.1    | Probe Design and Label Selection . . . . .                                 | 591        |
| 12.4.1.1  | Probe Generation—Traditional Approaches . . . . .                          | 591        |
| 12.4.1.2  | Radiolabeled Probes . . . . .                                              | 592        |
| 12.4.1.3  | Hapten-Conjugated Probes . . . . .                                         | 592        |
| 12.4.1.4  | Fluorescent Labeled Probes . . . . .                                       | 593        |
| 12.4.1.5  | Branched DNA Probe Technology . . . . .                                    | 594        |
| 12.4.2    | Validation Strategies and Selection of Controls . . . . .                  | 595        |
| 12.5      | Next Generation Sequencing . . . . .                                       | 596        |
| 12.6      | Examples of Molecular Techniques Added Value in Drug Development . . . . . | 596        |
| 12.7      | Utility of Molecular Techniques as Companion Diagnostics . . . . .         | 597        |
| 12.8      | Summary . . . . .                                                          | 598        |
|           | References . . . . .                                                       | 598        |
| <b>13</b> | <b>Current Issues in Developmental Immunotoxicity . . . . .</b>            | <b>601</b> |
|           | Jamie C. DeWitt and Deborah E. Keil                                        |            |
| 13.1      | What Is Developmental Immunotoxicity (DIT)? . . . . .                      | 601        |
| 13.1.1    | DIT Defined . . . . .                                                      | 601        |
| 13.1.2    | Current Methods of Evaluating DIT . . . . .                                | 602        |
| 13.2      | Critical Windows of Immune System Development . . . . .                    | 604        |
| 13.2.1    | Overview of the Developing Immune System . . . . .                         | 604        |
| 13.2.1.1  | Early-Life Risk . . . . .                                                  | 604        |
| 13.2.1.2  | Vulnerability of the Developing Immune System . . . . .                    | 605        |
| 13.2.1.3  | Critical Stages of Immunological Maturation . . . . .                      | 605        |
| 13.2.1.4  | Epigenetics and Its Role in Immunological Development . . . . .            | 606        |
| 13.2.1.5  | One Xenobiotic, Multiple Mechanisms of Action . . . . .                    | 607        |

|           |                                                                                                                                         |            |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------|------------|
| 13.2.2    | Examples from Established Developmental Immunotoxicants . . . . .                                                                       | 608        |
| 13.3      | Lifetime Risks After DIT. . . . .                                                                                                       | 609        |
| 13.3.1    | Diseases with Known DIT Risk Factors . . . . .                                                                                          | 609        |
| 13.3.2    | Disease Patterns Thought to be Associated with DIT. . . . .                                                                             | 609        |
| 13.3.3    | Monitoring the Impact of Early-Life Environmental Factors on the Immune System for Prevention of Disease Strategies in Old Age. . . . . | 610        |
| 13.4      | Why Is There a Need for DIT Testing of Chemical Substances? . . . . .                                                                   | 611        |
| 13.5      | Toxicology in the Twenty-First Century and DIT . . . . .                                                                                | 611        |
| 13.5.1    | Tox21 Defined . . . . .                                                                                                                 | 611        |
| 13.5.2    | Tox21 and Developmental Endpoints . . . . .                                                                                             | 612        |
| 13.5.3    | Tox21 and TSCA in the US . . . . .                                                                                                      | 613        |
| 13.6      | Alternative Methods of Detecting DIT . . . . .                                                                                          | 614        |
| 13.7      | What the Future Holds. . . . .                                                                                                          | 614        |
|           | References. . . . .                                                                                                                     | 615        |
| <b>14</b> | <b>The Murine Local Lymph Node Assay . . . . .</b>                                                                                      | <b>619</b> |
|           | Ton van Huygevoort                                                                                                                      |            |
| 14.1      | Introduction: Allergic Contact Dermatitis . . . . .                                                                                     | 620        |
| 14.2      | Animal Models . . . . .                                                                                                                 | 621        |
| 14.3      | Delayed Type IV Hypersensitivity Response . . . . .                                                                                     | 623        |
| 14.4      | The Local Lymph Node Assay (LLNA) Principle. . . . .                                                                                    | 625        |
| 14.5      | Alternative Methods for the Assessment of Cell Proliferation . . . . .                                                                  | 630        |
| 14.6      | Critical Performance Factors . . . . .                                                                                                  | 631        |
| 14.7      | LLNA Negative and Positive Control Data . . . . .                                                                                       | 632        |
| 14.8      | Limitations to the LLNA . . . . .                                                                                                       | 633        |
| 14.9      | Advantages to the LLNA. . . . .                                                                                                         | 634        |
| 14.10     | Non-Animal Models . . . . .                                                                                                             | 635        |
|           | References. . . . .                                                                                                                     | 636        |
| <b>15</b> | <b>Host Resistance Assays for Efficacy and Immunotoxicology Safety Evaluations . . . . .</b>                                            | <b>639</b> |
|           | Gary R. Burleson, Stefanie C. M. Burleson, and Florence G. Burleson                                                                     |            |
| 15.1      | Approaches to Evaluate Immunotoxicity . . . . .                                                                                         | 640        |
| 15.1.1    | Standard Toxicity Studies (STS) . . . . .                                                                                               | 640        |
| 15.1.2    | Functional Assays . . . . .                                                                                                             | 640        |
| 15.1.3    | Host Resistance Assays (Animal Disease Models): The “Gold Standard” . . . . .                                                           | 642        |
| 15.2      | Comprehensive Host Resistance Assay . . . . .                                                                                           | 643        |
| 15.2.1    | Influenza Virus Host Resistance Assay . . . . .                                                                                         | 643        |

|          |                                                                                      |            |
|----------|--------------------------------------------------------------------------------------|------------|
| 15.3     | Targeted Host Resistance Assays . . . . .                                            | 644        |
| 15.3.1   | Bacterial Host Resistance Assays. . . . .                                            | 644        |
| 15.3.1.1 | Evaluation of Neutrophil and/or<br>Macrophage Function . . . . .                     | 644        |
| 15.3.1.2 | Evaluation of Anti-Inflammatory<br>Therapeutics . . . . .                            | 646        |
| 15.3.1.3 | Evaluation of Therapeutics Targeting<br>TNF- $\alpha$ . . . . .                      | 646        |
| 15.3.1.4 | Marginal Zone B (MZB) Cell<br>Evaluation . . . . .                                   | 646        |
| 15.3.2   | Viral Reactivation Host Resistance Assay . . . . .                                   | 647        |
| 15.3.2.1 | Murine Cytomegalovirus (MCMV)<br>Latent Viral Model . . . . .                        | 648        |
| 15.3.2.2 | Murine Gamma Herpesvirus 68<br>(MVH-68) Latent Viral Reactivation<br>Model . . . . . | 648        |
| 15.3.3   | Fungal Host Resistance . . . . .                                                     | 648        |
|          | References. . . . .                                                                  | 648        |
|          | <b>Index. . . . .</b>                                                                | <b>651</b> |

Immunopathology in Toxicology and Drug Development  
Volume 1, Immunobiology, Investigative Techniques,  
and Special Studies

Parker, G. (Ed.)

2017, XXII, 670 p. 255 illus., 250 illus. in color.,

Hardcover

ISBN: 978-3-319-47375-8

A product of Humana Press