

METHODS IN MOLECULAR BIOLOGY™

Volume 380

# Immunological Tolerance

*Methods and Protocols*

*Edited by*

Paul J. Fairchild

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# **Immunological Tolerance**

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# Immunological Tolerance

*Methods and Protocols*

Edited by

**Paul J. Fairchild**

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Oxford, UK*


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# Preface

Recent advances in our understanding of immunological tolerance and the processes that lead to its breakdown have helped to illuminate the etiology of many of the autoimmune diseases that affect up to 1 in 20 members of the population. The prospect of reestablishing a state of self-tolerance in the face of progressive autoimmunity is no longer the distant possibility it once seemed: various strategies have proven successful in animal models of disease, raising hopes for their eventual application in the clinic. Furthermore, the demonstration that tolerance may be extended from self to foreign tissues offers tangible alternatives for the treatment of transplant rejection, which may one day avoid the need for immunosuppression with its attendant risks and long-term side effects. Such an exciting transition phase in the field of immunological tolerance requires rigorous analysis to gauge the effectiveness of novel approaches to its induction. *Immunological Tolerance: Methods and Protocols* seeks to address this need by providing a comprehensive guide to the techniques currently used for culturing and characterizing the cell types responsible for imposing self-tolerance and the experimental models employed to study their function both in vitro and in vivo.

*Immunological Tolerance: Methods and Protocols* has been divided into four sections, each of which is introduced by one or more overview chapters intended to place the material in context and highlight relevant questions that remain to be addressed. Part I focuses on the those cell types whose contribution to the induction of tolerance is unequivocal: while thymic epithelial cells are instrumental to central tolerance and regulatory T cells to dominant tolerance in the periphery, dendritic cells may influence the T cell repertoire in either context, whether imposing negative selection in the thymus or polarizing responding T cells in the periphery towards a regulatory phenotype. This section therefore documents methods for the generation and culture of each of these critical cell types and approaches to their subsequent characterization. Part II describes protocols for the study of tolerance in vitro either by gene expression profiling of the relevant cell types or by recreating the specialized microenvironments in which the necessary cell-cell interactions may occur. While fetal thymus organ cultures and reaggregates of thymic stromal cells have historically illuminated the mechanisms of T cell repertoire selection, the use of three-dimensional collagen matrices represents a recent development which has begun to address the inadequacy of conventional in vitro approaches to the study of immune cell dynamics.

Part III explores issues related to the study of tolerance *in vivo* by describing animal models of autoimmunity, inflammatory disease and transplantation while documenting recent techniques for monitoring the outcome of therapeutic intervention. Finally, Part IV outlines novel and established strategies for the induction of tolerance experimentally through mixed chimerism, the adoptive transfer of regulatory T cells or the administration of biologicals such as monoclonal antibodies or exosomes derived from tolerogenic dendritic cells. Needless to say, many of the protocols in these sections involve procedures on live animals: since the regulatory framework surrounding such experiments varies considerably between countries, it is important to ensure that local ethical committee approval and the necessary licenses have been obtained before implementing the protocols described. With this proviso in mind, may I wish all readers success in applying the insights described in this volume within their chosen field of study.

I am, of course, most grateful for the efforts and dedication of all the authors who have contributed, not only the protocols they have developed or modified for the study of tolerance, but also for their first-hand experiences of immunology, gained from many years at the bench. I am also deeply indebted to my mentors, both past and present, who have instilled in me their enthusiasm and passion for the study of tolerance: to Jonathan Austyn, who first introduced me to the fascination and foibles of dendritic cells; to David Wraith, with whom I spent several fond years in Cambridge, grappling with autoimmunity, and to Herman Waldmann, whose immeasurable contribution to the field of tolerance I can only dream of emulating.

Finally, my heart-felt thanks go to Jackie, my wife, and Richard, my son, for their unfaltering love and support throughout the preparation of this volume, even when their own immunological tolerance had undoubtedly been pushed to the limit!

*Paul J. Fairchild*

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# Contents

Preface .....	v
Contributors .....	xi
1 Frontiers of Immunological Tolerance <i>Giorgio Raimondi, Hèth R. Turnquist, and Angus W. Thomson</i> .....	1
<b>PART I CELL TYPES CONTRIBUTING TO IMMUNOLOGICAL TOLERANCE</b>	
2 Balancing Tolerance and Immunity: <i>The Role of Dendritic Cell and T Cell Subsets</i> <i>Elena Shklovskaya and Barbara Fazekas de St. Groth</i> .....	25
3 Differentiation of Dendritic Cell Subsets from Mouse Bone Marrow <i>Ludovica Bruno</i> .....	47
4 Genetic Modification of Dendritic Cells Through the Directed Differentiation of Embryonic Stem Cells <i>Paul J. Fairchild, Kathleen F. Nolan, and Herman Waldmann</i> .....	59
5 Generation of Immunocompetent T Cells from Embryonic Stem Cells <i>Renée F. de Pooter and Juan Carlos Zúñiga-Pflücker</i> .....	73
6 Isolation, Expansion, and Characterization of Human Natural and Adaptive Regulatory T Cells <i>Silvia Gregori, Rosa Bacchetta, Laura Passerini,        Megan K. Levings, and Maria Grazia Roncarolo</i> .....	83
7 Derivation, Culture, and Characterization of Thymic Epithelial Cell Lines <i>Michiyuki Kasai and Toshiaki Mizuochi</i> .....	107
<b>PART II THE STUDY OF IMMUNOLOGICAL TOLERANCE IN VITRO</b>	
8 Thymus Organogenesis and Development of the Thymic Stroma <i>Craig S. Nowell, Alison M. Farley,        and C. Clare Blackburn</i> .....	125
9 Generation of a Tissue-Engineered Thymic Organoid <i>Fabrizio Vianello and Mark C. Poznansky</i> .....	163
10 Studying T-Cell Repertoire Selection Using Fetal Thymus Organ Cultures <i>Philip G. Ashton-Rickardt</i> .....	171



- 11 Investigating Central Tolerance with Reaggregate  
Thymus Organ Cultures  
**Graham Anderson and Eric J. Jenkinson ..... 185**
- 12 Estimating Thymic Function Through Quantification  
of T-Cell Receptor Excision Circles  
**Marie-Lise Dion, Rafick-Pierre Sékaly, and Rémi Cheynier ..... 197**
- 13 Gene Expression Profiling of Dendritic Cells by Microarray  
**Maria Foti, Paola Ricciardi-Castagnoli, and Francesca Granucci ... 215**
- 14 SAGE Analysis of Cell Types Involved in Tolerance Induction  
**Kathleen F. Nolan, Stephen P. Cobbold, and Herman Waldmann . 225**
- 15 Analyzing the Physicodynamics of Immune Cells  
in a Three-Dimensional Collagen Matrix  
**Peter Reichardt, Frank Gunzer, and Matthias Gunzer ..... 253**

### **PART III THE STUDY OF IMMUNOLOGICAL TOLERANCE IN VIVO**

- 16 Etiology of Autoimmune Disease:  
*How T Cells Escape Self-Tolerance*  
**Eli Sercarz and Claudia Raja-Gabaglia ..... 271**
- 17 Animal Models of Spontaneous Autoimmune Disease:  
*Type 1 Diabetes in the Nonobese Diabetic Mouse*  
**Nadia Giaratana, Giuseppe Penna, and Luciano Adorini ..... 285**
- 18 Antigen-Based Therapy and Immune Regulation  
in Experimental Autoimmune Encephalomyelitis  
**Mandy J. McGeachy, Richard O'Connor, Leigh A. Stephens,  
and Stephen M. Anderton ..... 313**
- 19 Induction and Regulation of Inflammatory Bowel Disease  
in Immunodeficient Mice by Distinct CD4<sup>+</sup> T-Cell Subsets  
**Kevin J. Maloy ..... 327**
- 20 In Vivo Models for the Study of Transplantation Tolerance  
**Hannah Stewart, Rommel Ravanan, and Richard Smith ..... 337**
- 21 Ectopic Transplantation of Tissues Under the Kidney Capsule  
**Nathan J. Robertson, Paul J. Fairchild,  
and Herman Waldmann ..... 347**
- 22 Intravital Two-Photon Imaging of T-Cell Priming  
and Tolerance in the Lymph Node  
**Susanna Celli and Philippe Bousso ..... 355**
- 23 Tracing Tolerance and Immunity In Vivo by CFSE-Labeling  
of Administered Cells  
**Elizabeth Ingulli ..... 365**

**PART IV METHODS FOR INDUCING AND BREAKING IMMUNOLOGICAL TOLERANCE**

24 Thymic Involution: *Implications for Self-Tolerance*  
**Frances T. Hakim and Ronald E. Gress** ..... 377

25 Inducing Mixed Chimerism and Transplantation  
 Tolerance Through Allogeneic Bone Marrow  
 Transplantation with Costimulation Blockade  
**Ines Pree and Thomas Wekerle** ..... 391

26 Induction of Dominant Tolerance  
 Using Monoclonal Antibodies  
**Ana Água-Doce and Luis Graça** ..... 405

27 Induction of Tolerance by Adoptive Transfer of Treg Cells  
**Kanji Nagahama, Eiji Nishimura, and Shimon Sakaguchi** ..... 431

28 Modulation of the Immune Response  
 Using Dendritic Cell–Derived Exosomes  
**Nicole R. Bianco, Seon-Hee Kim, Adrian E. Morelli,  
 and Paul D. Robbins** ..... 443

29 Breaking Self-Tolerance to Tumor-Associated Antigens  
 by In Vivo Manipulation of Dendritic Cells  
**Ines Mende and Edgar G. Engleman** ..... 457

Index ..... 469



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