

The Biology of Dendritic Cells and HIV Infection

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Edited by

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Cover illustration: Illustration of dendritic cells and T cells. The education of killer T cells by dendritic (Langerhans) cells occurs in a lymph node. These dendrites have processed antigenic information obtained from an autologous vaccine made with proteins purified from the patient's own cancer cells. Killer T cells then target all cells tagged with the antigen.

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*We dedicate this book in memory
of the late Prof. Giovanni Battista Rossi,*

who was for many years chairman of the Department of Virology of the Italian National Institute of Health until he passed away (February 20, 1994). Prof. Rossi, Giovanni to his friends, was the promoter and scientific coordinator of the Italian National Project on AIDS (1987–1993) and the organizer of the VII International Conference on AIDS (Florence, June 1991). All these activities had earned him the admiration of the international scientific community. Giovanni was both a mentor and a friend. We commenced our scientific careers in his laboratory working on the differentiation of Friend virus–transformed mouse leukemia cells and on the antiviral and antitumoral effects of interferons. He inspired us to start research projects on AIDS, with the vision that the knowledge of cytokines could be instrumental in the understanding of complex aspects of the pathogenesis and immunology of HIV infection. Giovanni at the time knew little about dendritic cells, because it took several years to understand the crucial importance of these cells in the regulation of the immune response after the pioneering work of Ralph Steinman and Zanvil Cohn in 1973. However, having personally known his great curiosity and interest in cell differentiation, we are certain that he would have been captivated by the recent knowledge of the biology of dendritic cells and its potential importance in the development of novel strategies of immunotherapy of cancer and HIV infection. Giovanni's major interest was in the research of cell differentiation, HIV, and cancer. It is now becoming evident that differentiation of dendritic cells can play an important role in pathogenesis and control of both cancer and HIV infection. It is a pity that Giovanni had not had the chance to witness the recent exciting progress in dendritic cell biology! Dedicating this book to him is in strong recognition not only of his commitment to AIDS research but also to his interdisciplinary vision of biomedical research.

Preface

This book represents a special effort to bring together the most recent information on the biology of dendritic cells (DCs) focusing on the role of these cells in the pathogenesis and immunity of HIV-1 infection. The recent progress in immunology has revealed the key importance of DCs in the regulation of the immune response to infections as well as to certain malignant, allergic, and autoimmune diseases. In particular, research on DCs has recently emerged as a fundamental aspect for the comprehension of the mechanisms underlying the pathogenesis of viral diseases, as well as for the progress on the development of prophylactic and therapeutic vaccines. Recent findings have led to substantial conceptual advances in our understanding of the role of DCs in HIV infection and clarified that DCs are important targets and reservoirs of HIV, playing a crucial role in several aspects of viral pathogenesis. Interestingly, HIV can exploit many of the cellular processes responsible for the generation and regulation of the adaptive immune responses to gain access to its main target cells, namely the CD4⁺ T lymphocytes. Hence, the central role of DCs in stimulating T-cell activation not only provides a route for viral transmission but also represents a vulnerable point where HIV-1 can interfere with the initiation of T cell-mediated immunity. Recent studies have revealed that several HIV proteins can profoundly influence the phenotype and functions of DCs even in the absence of a productive infection, often resulting in an abnormal immune response. This knowledge has resulted in the identification of some major mechanisms involved in the pathogenesis of HIV-1 infection. In addition, the recent advances in DC biology have opened perspectives in the research on new adjuvants and novel strategies for the *in vivo* targeting of antigens to DCs, which are instrumental in the development of HIV vaccines.

This book is of special interest to researchers in the fields of basic and applied immunology, virology, cell biology, and vaccine development and to practitioners and students. It offers the reader 14 chapters written by international experts on DCs and the pathogenesis and immunology of HIV infection. The text is divided into three parts. Part I includes a detailed overview of the biology, ontogeny, function, and therapeutic potential of human DC subsets, which is intended to give readers an introduction to the field of DCs and to their possible clinical

exploitation, as well as provide a framework for a fuller understanding of the content of subsequent chapters. Part II focuses on some general aspects of the immunopathogenesis of HIV infection and provides a comprehensive overview of cells and factors involved in the innate and adaptive immune response to HIV-1. These general concepts are of relevance for a deeper understanding of the functional consequences of HIV-1 interaction with DCs. This then becomes the specific topic of Part III, which also focuses on the mechanisms leading to immune evasion and dysfunction and on the importance of DCs as targets and tools for the immunotherapy of HIV infection. We have now begun to understand the complex interactions between HIV and DCs in the pathogenesis of AIDS and have learned lessons about how to prepare potentially effective DCs for cancer vaccines. We assume that in the future, DC-based therapeutic vaccines can represent a topic of increasing interest for the immunotherapy of HIV-1–infected patients. Although DC-based vaccines will certainly not solve the drastic needs of HIV-infected individuals in the developing countries, the progress of the research in this field will help us to identify novel and practical strategies for the *in vivo* targeting of HIV antigens to DCs. We thank all the authors for their generous participation in this initiative, which permitted us to provide the reader with a comprehensive overview on the biology of DCs and their role in the pathogenesis and immunity of HIV-1 infection.

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Contents

Preface	vii
Acknowledgments	ix
Contributors	xxi
Part I General Aspects of Dendritic Cell Biology, Functions, and Clinical Application	1
Chapter 1 Dendritic Cell Biology: Subset Heterogeneity and Functional Plasticity	3
<i>Vassili Soumelis, Yong-Jun Liu, and Michel Gilliet</i>	
1.1 Introduction	3
1.2 Origin and Development of Human DC Subsets	4
1.3 Anatomic, Phenotypic, and Functional Features of DC Subsets	7
1.3.1 Thymic DCs	7
1.3.2 Blood DCs.....	8
1.3.3 Skin and Mucosal DCs.....	9
1.3.4 DCs in Solid Organs	9
1.3.5 DCs in Secondary Lymphoid Organs	10
1.4 Innate Functions of DC Subsets	11
1.4.1 Invariant Receptors on DCs	11
1.4.2 Antigen Uptake and Processing by DC Subsets	14
1.4.3 Cytokine and Chemokine Production by DCs	15
1.4.4 Cross-talk Between DCs and Other Innate Immunity Cell Types	16
1.4.5 DC Maturation and Migration to the Secondary Lymphoid Organs.....	17
1.5 Role of DC Subsets in Adaptive Immunity.....	18
1.5.1 MDCs in Adaptive Immune Responses.....	18
1.5.2 pDCs in Adaptive Immune Responses	20
1.5.3 Molecular Basis for DC-mediated Th1/Th2 Polarization.....	22
1.6 DCs and Tolerance	23

1.7 Concluding Remarks	25
References	25
Chapter 2 Dendritic Cells and Their Role in Linking Innate and Adaptive Immune Responses.....	45
<i>Mary F. Lipscomb, Julie A. Wilder, and Barbara J. Masten</i>	
2.1 Introduction	45
2.2 DC Origins, Subsets, and Differentiation	46
2.3 Plasticity and Trafficking Pathways.....	51
2.4 DC–T Cell Interactions	55
2.4.1 Antigen Uptake	56
2.4.2 Antigen Processing.....	56
2.4.2.1 MHC Class II Presentation	56
2.4.2.2 MHC Class I Presentation.....	58
2.4.3 Costimulation and Suppression Molecules	59
2.4.4 Events at the DC–T Cell Interface	60
2.5 DCs Link Innate and Acquired Immunity.....	61
2.5.1 Influence of the Microenvironment on DC Phenotype and Function.....	61
2.5.1.1 Exogenous Signals	64
2.5.1.2 Endogenous Signals	64
2.5.2 DCs in T-Cell Activation and Differentiation.....	66
2.5.3 Regulation of Pulmonary Immunity by Lung DCs	68
2.5.4 DCs and B-Cell Function	69
2.6 Concluding Remarks	69
References	70
Chapter 3 Dendritic Cell and Pathogen Interactions in the Subversion of Protective Immunity.	85
<i>John E. Connolly, Damien Chaussabel, and Jacques Banchereau</i>	
3.1 Introduction	85
3.2 Dendritic Cell Biology	86
3.2.1 Dendritic Cells Are Composed of Subsets	86
3.2.1.1 Distinct DC Subsets Are Endowed with Distinct Functional Properties	86
3.2.2 Dendritic Cell Ontogeny	88
3.2.2.1 DC Progenitors and Precursors	88
3.2.3 Antigen Capture, Processing, and Presentation	88
3.2.3.1 Presentation via MHC Class I	90
3.2.3.2 Presentation via MHC Class II	91
3.2.3.3 Presentation via CD1 Family of MHC Molecules	91
3.2.4 Dendritic Cell Maturation.....	92

3.2.4.1 Maturation Signals	92
3.2.5 DC Migration	94
3.2.6 Dendritic Cells and Tolerance.....	96
3.2.6.1 Central Tolerance	96
3.2.6.2 Peripheral Tolerance Through DCs	96
3.2.6.3 Inhibitory Receptors	97
3.2.7 Dendritic Cell Death	98
3.3 Subversion of Dendritic Cell Function by Pathogens	99
3.3.1 Pathogens Evade Dendritic Cell Antigen Processing	99
3.3.1.1 Evasion of DC Uptake	99
3.3.1.2 Inhibition of Classical Class I Processing and Presentation	100
3.3.1.3 Upregulation of Inhibitory Nonclassical Class I Molecules	102
3.3.1.4 Inhibition of Class II Processing and Presentation	102
3.3.1.5 Inhibition of CD1 Processing and Presentation	104
3.3.2 Selective Targeting of DC Subsets by Pathogens.....	104
3.3.3 Pathogens Target DC Precursors	105
3.3.4 Inhibition of DC Maturation by Pathogens.....	106
3.3.4.1 Inhibition of DC Activation	106
3.3.4.2 Pathogen Modulation of DC Maturation	107
3.3.5 Pathogen-mediated Inhibition of DC Migration	108
3.3.6 Viral Modulation of DC Tolerance.....	109
3.3.7 Pathogen-mediated Dendritic Cell Death	109
3.4 Concluding Remarks	110
References	110

Chapter 4 Dendritic Cells as Keepers of Peripheral Tolerance 129

Sabine Ring, Alexander H. Enk, and Karsten Mahnke

4.1 Introduction	129
4.2 The Concept of “Steady State” versus “Activated” DC.....	130
4.3 Factors That Affect Tolerogenic DCs	136
4.3.1 TNF- α and Semimature DCs	136
4.3.2 Vitamin D ₃ Affects DC Maturation	139
4.3.3 IL-10 Modulates DCs for Tolerance Induction.....	143
4.4 Molecular Mechanisms Involved in Tolerance Induction	144
4.4.1 Costimulatory Molecules.....	144
4.4.2 RelB Translocation Is Crucial for DC Maturation.....	146
4.4.3 The Role of Indoleamine 2,3-dioxygenase (IDO) in Tolerance Induction	148
4.5 Are There Specialized Subsets of Tolerogenic DCs?	151
4.5.1 Surface Marker Expression.....	151
4.5.2 Cytokine Expression	153
4.6 “Designer DC”: Tailored for Tolerance Induction.....	154

4.7 Concluding Remarks	158
References	159
Chapter 5 Adjuvants, Dendritic Cells, and Cytokines: Strategies for Enhancing Vaccine Efficacy	171
<i>Paola Rizza, Imerio Capone, and Filippo Belardelli</i>	
5.1 Introduction	171
5.2 Brief Historical Background on Adjuvants	172
5.3 The Need of New Adjuvants for Vaccine Development and crucial importance of Adjuvant–Dendritic Cell Interactions for the Polarization of Immune Response	175
5.4 New Classification of Vaccine Adjuvants	179
5.5 Ligands for TLRs and Their Potential Role as Adjuvants	183
5.5.1 PAMP-dependent Targeting of DCs	183
5.5.2 CpG Oligonucleotides	184
5.5.3 PAMP-independent Activation of DCs	186
5.5.4 Small-Molecule Immune Potentiators: Imidazoquinolines	187
5.6 Interaction of Cytokines with DCs: Importance for Vaccine Development	187
5.7 Concluding Remarks	194
References	195
Chapter 6 <i>Ex Vivo</i>–Generated Dendritic Cells for Clinical Trials versus <i>In Vivo</i> Targeting to Dendritic Cells: Critical Issues	203
<i>Joannes F.M. Jacobs, Cândida F. Pereira, Paul J. Tacken, I. Jolanda M. de Vries, Cornelus J.A. Punt, Gosse J. Adema, and Carl G. Figdor</i>	
6.1 Introduction	203
6.2 DC Culture, from Bench to Clinical-Grade Product	205
6.2.1 Introduction	205
6.2.2 Precursor Isolation	205
6.2.3 Differentiation into DC Phenotype	206
6.2.4 Maturation	207
6.2.4.1 Maturation Signals	207
6.2.4.2 Effect of Maturation on the Function of DCs	208
6.2.5 The Immune Target	209
6.2.5.1 MHC Class I and Class II Loading	209
6.2.5.2 Antigen Source	210
6.2.5.2.1 Loading DCs with Peptides	210
6.2.5.2.2 Loading DCs with Proteins	211

6.2.5.2.3 Loading DCs with Whole Target	212
6.2.5.2.4 Loading DCs with RNA or DNA	212
6.2.5.3 Methods for Loading DCs with Antigen	213
6.2.5.4 Loading of DCs with Antigens: Summary	214
6.2.6 Storage	214
6.3 DC Quality Check	214
6.3.1 Introduction	214
6.3.2 DC Phenotype and Purity	215
6.3.3 Function	215
6.4 Vaccine Administration	216
6.5 Immunomonitoring	216
6.6 Targeting DCs <i>In Vivo</i>	218
6.6.1 Introduction	218
6.6.2 Maturing DCs <i>In Vivo</i>	219
6.6.3 Targeting Antigens to the MHC Class I and II Pathway	219
6.6.4 Drug Delivery Systems	220
6.6.4.1 Live Vectors to Deliver DNA to DCs	221
6.6.4.2 Microparticles to Deliver DNA, Protein, or Peptides to DCs	221
6.6.4.3 Receptor Ligands to Deliver Protein or Peptides to DCs	223
6.6.5 Targeting DC Surface Receptors	224
6.6.5.1 Mac-1	225
6.6.5.2 Gb3	225
6.6.5.3 CD40	225
6.6.5.4 Fc Receptors	226
6.6.5.5 C-type Lectin Receptors	227
6.7 Concluding Remarks	229
References	229

Part II General Aspects of the Pathogenesis and Immune Response to HIV-1 Infection 243

Chapter 7 Immunopathogenesis of HIV Infection 245 *Elisa Vicenzi, Massimo Alfano, Silvia Ghezzi, and Guido Poli*

7.1 Introduction	245
7.2 The Life Cycle of HIV-1 in CD4 ⁺ T Cells and Mononuclear Phagocytes	246
7.3 CCR5- versus CXCR4-dependent HIV-1 Infections	252
7.4 Intrinsic Resistance to HIV Infection	259
7.5 HIV Cytopathicity	261
7.6 HIV Replication in Lymphoid Organs and Central Nervous System	265
7.7 Host Determinants of HIV Propagation: Cytokines and Chemokines	268

7.8 Concluding Remarks	273
References	274
Chapter 8 Innate Cellular Immune Responses in HIV Infection	297
<i>Barbara Schmidt, Nicolai A. Kittan, Sabrina Haupt, and Jay A. Levy</i>	
8.1 Characteristics of Innate Immunity	297
8.2 Cells and Soluble Factors Involved in Innate Immunity Against HIV	299
8.3 Plasmacytoid and Myeloid Dendritic Cells and Their Association with HIV Infection	301
8.3.1 Overview	301
8.3.2 PDC Characteristics	302
8.3.3 Relationship of Dendritic Cells to the HIV Clinical State	303
8.3.4 HIV Interaction with Dendritic Cells and IFN Production	306
8.4. The Role of Noncytotoxic CD8 ⁺ T Cells in Anti-HIV Responses	307
8.4.1 Overview	307
8.4.2 The CD8 ⁺ T-Cell Antiviral Factor (CAF)	309
8.4.3 Mechanism of Action	310
8.4.4 Characteristics of CAF	310
8.4.5 Relationship of CNAR to Clinical State	311
8.5 Other Innate Immune Cells	312
8.5.1 Natural Killer Cells	312
8.5.2 NK-T Cells	313
8.5.3 $\gamma\delta$ T Cells	315
8.6 Concluding Remarks	316
References	317
Chapter 9 Adaptative Immune Responses in HIV-1 Infection	333
<i>Mara Biasin and Mario Clerici</i>	
9.1 Introduction to the Characteristics of Specific Immunity	333
9.2 Functional Immune Disregulation in the Different Phases of HIV-1 Infection	334
9.3 Cellular Immune Response to HIV-1	336
9.3.1 Differentiation and Functions of CTLs	337
9.3.2 CTLs in HIV-1 Infection	339
9.3.3 Viral Escape from CTLs	341
9.3.4 Antiviral Soluble Factors	344
9.3.5 Alteration of T-Helper Functions in HIV-1 Infection	346
9.3.6 Immunologic Profile of People Living in Different Areas of the World	350

9.4 Humoral Immune Responses in HIV-1 Infection	351
9.4.1 Differentiation and Functions of B Cells	352
9.4.2 Antibody Responses in HIV-1 Infection.....	354
9.4.3 Viral Escape from Antibodies.....	356
9.5 Immune Responses in HIV-Exposed Seronegative Individuals	357
9.6 Immune Responses in Long-Term Nonprogressors	359
9.7 Concluding Remarks	361
References	362

**Part III Dendritic Cells and HIV Interactions and Their
Role in Pathogenesis and Immunity379**

**Chapter 10 Binding and Uptake of HIV by Dendritic Cells
and Transfer to T Lymphocytes: Implications
for Pathogenesis.....381**

*Anthony L. Cunningham, John Wilkinson, Stuart Turville, and
Melissa Pope*

10.1 Introduction	381
10.2 Transmission of HIV	381
10.3 HIV Infection of Female Genital Tract.....	382
10.4 The Role of Dendritic Cells in HIV Infection.....	384
10.4.1 Immature versus Mature DCs.....	385
10.4.2 HIV Receptors on DCs.....	386
10.4.2.1 C-type Lectin Receptors.....	386
10.4.2.2 Diversity of CLR Expression on DCs	388
10.5 Transmission of HIV to T Cells by DCs.....	390
10.5.1 Effect of Maturation	393
10.5.2 HIV, CLRs, and Nonepithelial DCs.....	394
10.5.3 Control of HIV Spread and Vaginal Microbicides.....	394
10.5.4 Targeting the Virus.....	394
10.5.5 Targeting the Cell.....	395
10.6 Models for Examining HIV Infection and Testing of Vaginal Microbicides in the Genital Mucosa	396
10.6.1 Monocyte-derived Dendritic Cells and Monocyte-derived Langerhans Cells.....	396
10.6.2 <i>Ex Vivo</i> Cervical Explants	397
10.6.3 Macaque Models	397
10.7 Concluding Remarks	398
References	399

Chapter 11 Loss, Infection, and Dysfunction of Dendritic Cells in HIV Infection	405
<i>Steven Patterson, Heather Donaghy, and Peter Kelleher</i>	
11.1 Overview	405
11.2 Introduction to Myeloid and Plasmacytoid DCs	406
11.2.1 Myeloid DCs.....	406
11.2.1.1 Phenotype and Location	406
11.2.1.2 Maturation	407
11.2.1.3 Development.....	408
11.2.1.4 Function	408
11.2.2 Plasmacytoid DCs.....	409
11.2.2.1 Location and Development	409
11.2.2.2 Maturation and Function	410
11.3 Practical Issues in DC Research.....	411
11.4 Differentiation of Blood Myeloid DCs	412
11.5 DCs and Transmission of HIV	415
11.6 Loss of DCs in HIV Infection	416
11.6.1 Tissue Langerhans Cells	416
11.6.2 Blood DCs	417
11.6.3 Anti-retroviral Drugs and DC Numbers	419
11.7 Infection of DCs by HIV	420
11.7.1 <i>In Vitro</i> Studies.....	420
11.7.1.1 HIV Receptor Expression on DCs.....	420
11.7.2 <i>In Vitro</i> Infection Studies.....	422
11.7.2.1 Blood Plasmacytoid DCs	422
11.7.2.2 Blood Myeloid DCs.....	424
11.7.2.3 Monocyte-derived DCs	424
11.7.2.4 Langerhans Cells	426
11.7.3 DC Infection <i>In Vivo</i>	426
11.7.3.1 Blood DCs	426
11.7.3.2 Langerhans Cells	427
11.8 Dysfunction of DCs in HIV Infection	427
11.8.1 Blood Myeloid DCs	428
11.8.2 Monocyte-derived DCs	430
11.8.3 Langerhans Cells	431
11.8.4 Plasmacytoid DCs	432
11.9 Concluding Remarks.....	432
References	433

**Chapter 12 HIV Exploitation of DC Biology to Subvert
the Host Immune Response 447**
*Manuela Del Cornò, Lucia Conti, Maria Cristina Gauzzi,
Laura Fantuzzi, and Sandra Gessani*

12.1 Introduction	447
12.2 The Host Response to Viral Infection	448
12.2.1 Innate Immune Response	448
12.2.2 Adaptive Immune Response	451
12.3 Virus-induced Phenotypic and Functional Alterations of Human DCs	454
12.3.1 Interference with DC Generation and Survival	456
12.3.2 Loss of DC Morphology	456
12.3.3 Interference with DC Maturation	457
12.3.4 Modulation of DC Migration	458
12.4 DCs in HIV Pathogenesis: Protective or Defective?	459
12.4.1 HIV-1 Effects on DC Differentiation/Maturation	464
12.4.2 Modulation of Cytokine/Chemokine Secretion	469
12.4.3 Regulation of DC Chemotactic Functions.....	472
12.4.4 Effect on DC Survival.....	473
12.4.5 Effect on DC Cytotoxic Activity.....	473
12.5 Concluding Remarks	474
References	474

**Chapter 13 Cross-Presentation by Dendritic Cells: Role
in HIV Immunity and Pathogenesis 485**
*Concepción Marañón, Guillaume Hoeffel, Anne-Claire
Ripoche, and Anne Hosmalin*

13.1 Introduction	485
13.2 Antigen Presentation Pathways	485
13.2.1 MHC Class II–restricted Antigen Presentation	486
13.2.2 MHC Class I–restricted Antigen Presentation	486
13.3 Direct Presentation of HIV from Infected DCs	489
13.4 Cross-Presentation of HIV	490
13.4.1 Cross-Presentation of Defective HIV	490
13.4.2 Cross-Presentation from Apoptotic, HIV-infected Cells.....	491
13.4.3 Cross-Presentation from Live, HIV-infected Cells	492
13.4.3.1 Evidence of Cross-Presentation from Live, HIV-infected Cells.....	492
13.4.3.2 Mechanisms of Antigen Internalization from Live Cells into DCs	494
13.5 Comparison of Cross-Presentation from Live or from Dead Cells.....	496
13.5.1 Uptake and Antigen-processing Pathways	496
13.5.2 Role of DC Maturation	497

13.5.3 Is Death Necessary to Cross-Presentation?.....	498
13.6 Role of Cross-Presentation from Infected Cells in HIV Immunity and Pathogenesis	499
13.7 Concluding Remarks	501
References	502

**Chapter 14 Immunotherapy of HIV Infection: Dendritic
Cells as Targets and Tools..... 515**
*Imerio Capone, Giuseppe Tambussi, Paola Rizza, and Adriano
Lazzarin*

14.1 Introduction	515
14.2 The Rationale for Combining HAART with Immunotherapy in the Treatment of HIV-1 Infection	516
14.2.1 Drug Therapy Limitations	516
14.2.2 Immune Control of HIV-1	517
14.3 Overview of the Clinical Trials of Immunotherapy for the Treatment of HIV-1 Infection	519
14.3.1 Introduction.....	519
14.3.2 IL-2 Treatment in HIV-infected Patients.....	520
14.3.3 The Use of Immunomodulatory Drugs in Primary HIV Infection	521
14.3.3.1 Use of Cyclosporin A Alongside HAART.....	522
14.3.3.2 Mycophenolic Acid in Patients Undergoing Supervised Interruption of Therapy	523
14.4 The Dendritic Cell as Target for the Development of HIV-1 Vaccines	524
14.5 Dendritic Cells as Tools for the Development of Therapeutic Vaccines Against HIV	526
14.5.1 Introduction	526
14.5.2 Studies in Hu-PBL-SCID Mouse Model.....	529
14.5.3 Studies in Non-Human Primate Model	530
14.5.4 Clinical Studies with DC-based Vaccines in HIV-infected Individuals	531
14.6 Concluding Remarks	533
References	534

Index.....541

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Part I

General Aspects of Dendritic Cell Biology, Functions, and Clinical Application

Chapter 1

Dendritic Cell Biology: Subset Heterogeneity and Functional Plasticity

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1.1 Introduction

The immune system is central to the homeostasis of living organisms and has evolved to protect us against a broad variety of microbial pathogens. Cells of the innate immune system represent the frontline of the defense against invading pathogens and play a key role in controlling infections during the time needed for the induction of an adaptive immune response. In addition, innate immune responses are essential to the efficient priming of naïve T and B cells and the generation of an appropriate adaptive immune response. In this respect, dendritic cells (DCs) have the unique capacity to link innate and adaptive immunity, since they sense invading pathogens at the site of infection, capture and process foreign antigens (Ag), and then migrate to the secondary lymphoid organs where they activate naïve T cells in an Ag-specific manner. The three major defining criteria of mature DCs were proposed by Steinman in 1991 (Steinman, 1991): (1) dendritic morphology; (2) constitutive expression of high levels of major histocompatibility complex (MHC) class II molecules; and (3) capacity to induce strong proliferation of naïve CD4⁺ T cells in a mixed lymphocyte reaction (MLR). Other important features of DCs include the lack of lineage-specific markers, the expression of a variety of costimulatory molecules that will provide signals for the activation and the polarization of T cells (Lanzavecchia and Sallusto, 2001), and the ability to interact, either directly or indirectly, with various immune cell types of the innate or adaptive immune systems (Banchereau and Steinman, 1998; Banchereau *et al.*, 2000; Pulendran *et al.*, 2001a).

DCs form a dynamic “system” with several levels of complexity and diversity according to their lineage origin, stage of differentiation, as well

as anatomic location. In each of these different states, DCs exhibit great plasticity, which allows them to adjust their functional properties depending on the nature of the pathogens (Liu *et al.*, 2001).

In this chapter, we summarize current knowledge of the basic developmental, phenotypic, and functional characteristics of DC subsets, trying to underline their differences and complementarities. We chose to focus on human DCs, given the scope of this book. Studies on mouse DCs are only described briefly when bringing important knowledge on the general physiology of DCs. DCs from non-human primates are not specifically discussed but share most major characteristics of human DCs, including subset diversity, phenotype, and function. This general overview should serve as a basis to understand more specific chapters on the role of DCs in HIV infection.

1.2 Origin and Development of Human DC Subsets

DCs are continuously produced from hematopoietic stem cells within the bone marrow, and FLT-3 ligand represents the key DC growth and differentiation factor *in vivo* (Pulendran *et al.*, 2001a). The principal developmental pathways of human DCs from hematopoietic stem cells of the bone marrow are illustrated in Figure 1.1. CD34⁺ stem cells differentiate into common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). CMPs appear to differentiate into CLA⁺ and CLA⁻ populations, which subsequently differentiate into CD11c⁺CD1a⁺ and CD11c⁺CD1a⁻ DC, respectively (Strunk *et al.*, 1997). Whereas CD11c⁺CD1a⁺ DCs migrate into the skin epidermis and become Langerhans cells, CD11c⁺CD1a⁻ DCs migrate into the skin dermis and other tissues and become interstitial DCs (Ito *et al.*, 1999). TGF- β plays a critical role in Langerhans cell (LC) development as demonstrated by the fact that the *in vitro* generation of Langerhans cells from CD34⁺ progenitors can be greatly enhanced by TGF- β (Caux *et al.*, 1999) and Transforming growth factor (TGF)- β knockout mice lack Langerhans cells (Borkowski *et al.*, 1996). In their peripheral locations, both Langerhans cells and interstitial DCs are immature DCs that are readily activated by products of microbial invasion. Langerhans cells and interstitial DCs display different phenotypes and functions (Caux *et al.*, 1997). While Langerhans cells express CD1a, Lag-antigen, E-cadherin, and Birbeck granule-associated Ag langerin (Romani *et al.*, 2003), interstitial DCs express CD2, CD9, CD68, and factor XIIIa. Interstitial DCs, but not Langerhans cells, have the ability to take up large amounts of antigens by the mannose receptors and to produce IL-10, which may contribute to naïve B-cell activation and IgM production in the presence of CD40-ligand (CD40L) and IL-2.

In addition to these two subsets of immature myeloid DCs (MDCs), stem cells also give rise to two types of DC precursors: monocytes and plasmacytoid DC precursors (pDCs) (Liu *et al.*, 2001). DC precursors are defined by their low expression levels of costimulatory molecules, their failure to induce significant naïve T-cell activation, the lack of DC morphology and mobility in culture, and the ability to colonize non lymphoid tissues in the absence of stimulation. Monocytes are of myeloid origin, and express the myeloid antigens CD11b, CD11c, CD13, CD14, and CD33 (Liu *et al.*, 2001), mannose receptors, and CD1a, b, c, and d.

Stem cells differentiate into CLPs and CMPs. CMPs appear to differentiate into CLA⁺ and CLA⁻ populations, which subsequently differentiate and migrate into the skin epidermis to become Langerhans cells and the skin dermis to become interstitial DCs, respectively. In their peripheral locations, both Langerhans cells and the interstitial DCs are immature DCs that can be readily activated by products of microbial invasion. Stem cells also give rise to two types of DC precursors: monocytes and pDCs. Monocytes differentiate into immature DCs in culture with granulocyte-macrophage colony-stimulating factor (GM-CSF) and Interleukin (IL)-4 and can be further activated into mature DCs by stimulating with proinflammatory cytokines such as Tumor Necrosis Factor (TNF)- α , microbial products such as Lipopolysaccharide (LPS) or T cell-derived CD40L. Monocytes may also differentiate into Langerhans cells *in vivo* (Fig. 1.1, dashed line). pDCs represent the natural type 1 interferon (IFN)-producing cell (IPC) and have the ability to differentiate into mature DCs in culture with IL-3 or upon viral activation through Toll-like receptor (TLR)7 or 9.

Whereas the presence of macrophage colony-stimulating factor (M-CSF) leads to the differentiation of monocytes into macrophages, culture with GM-CSF and IL-4 leads to their differentiation into immature DCs with the ability to produce large amounts of IL-12 upon subsequent trigger with CD40L. These immature monocyte-derived DCs (MoDCs) resemble interstitial DCs and can be further activated into mature DCs by stimulating with proinflammatory cytokines such as TNF- α , microbial products such as LPS or T cell-derived CD40L. Addition of TGF- β to GM-CSF and IL-4 can drive the *in vitro* differentiation of monocytes into langerin⁺, Birbeck granules⁺ Langerhans cells (Geissmann *et al.*, 1998). This suggests that monocytes form a circulating pool of precursors capable of differentiating into various phagocytic cells in the tissue depending on the local factors they will encounter. *In vivo* monocyte-to-DC transformation may occur after transmigration from the blood to tissues across the endothelial barrier (Randolph *et al.*, 1998; Randolph *et al.*, 1999).

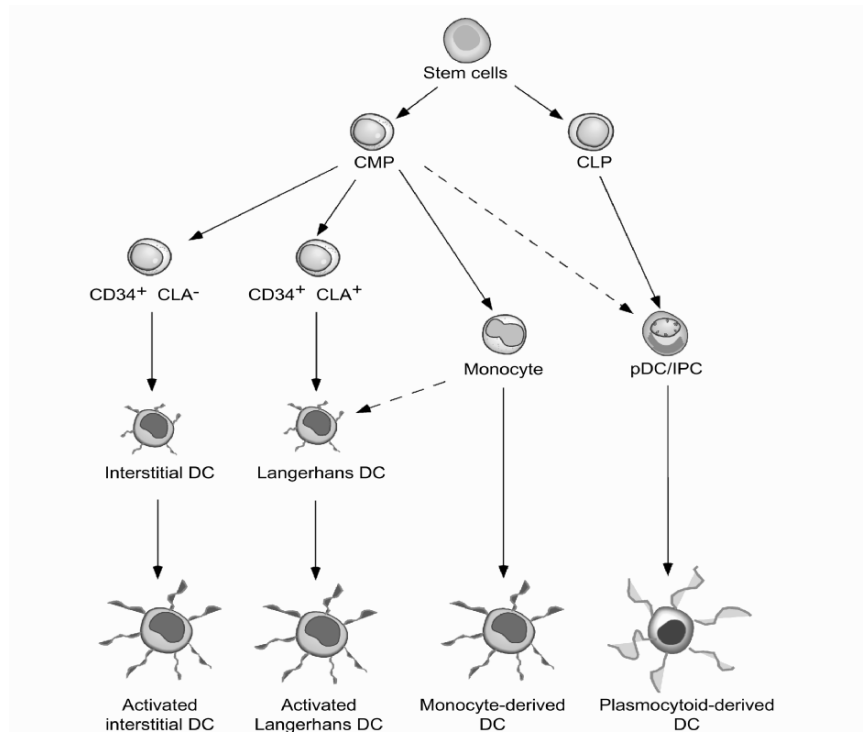


FIGURE 1.1. Pathways of human DC development.

The second DC-precursor subset generated from stem cells is the pDC subset. pDCs represent the natural type 1 IFN-producing cell (IPC) (see Section 1.4) and have the ability to differentiate into mature DCs in culture with IL-3 or upon viral activation through TLR7 or 9 (see Section 1.5). pDCs have plasmacytoid morphology and are lineage- $CD4^+CD11c^-$ cells that express BDCA2 (a novel C-type lectin receptor) and BDCA4 (which is identical to neuropilin-1), two specific markers of blood pDCs (Dzionek *et al.*, 2000; Dzionek *et al.*, 2001). pDC also express CD45RA, lack myeloid markers, and express high levels of the IL-3 receptor (CD123). The developmental path and molecular regulation of pDCs are not fully understood. To date, FLT3L ligand is the only known cytokine that is critical for pDC development from hematopoietic stem cells (HSCs) in humans and mice (Blom *et al.*, 2000; Chen *et al.*, 2004; Gilliet *et al.*, 2002). The ability of FLT3L to promote pDC development *in vivo* was confirmed by experiments showing that administration of FLT3L into human volunteers led to an increase in the number of peripheral blood pDCs (Pulendran *et al.*, 2000) and that FLT3L-transgenic mice have

increased numbers of pDC, whereas FLT3L-deficient mice have less pDCs (Manfra *et al.*, 2003). Whereas the other human DC subsets described above are of myeloid origin (called myeloid DCs; MDCs), it has been suggested that pDC/IPC are of lymphoid origin. This notion has been supported by findings such as their expression of many lymphoid markers, the lack of surface myeloid markers, and the production of mRNA for germ-line IgK and for pre-T cell receptor (Grouard *et al.*, 1997; Rissouan *et al.*, 1999c). Moreover, two separate studies further support the lymphoid origin of pDCs in humans and mice: (a) overexpression of the dominant-negative transcription factors Id2 or Id3 in human CD34⁺ hematopoietic progenitor cells blocks development of pDCs, T cells, and B cells, but not of myeloid DCs (Spits *et al.*, 2000); (b) knock down of Spi-B mRNA in human CD34⁺ hematopoietic progenitor cells strongly inhibits their potential to differentiate into pDCs (Schotte *et al.*, 2004). However, more recent studies revealed that FLT3⁺ cells within either CLPs or CMPs could differentiate into both MDCs and pDCs in cultures and *in vivo* (Chicha *et al.*, 2004). As a result of these seemingly divergent findings, several different hypotheses have been proposed regarding the developmental origin of pDCs, including the existence of a common DC precursor in blood that can give rise to all DC subsets, pDCs arising as a branch of the committed lymphoid lineage (Corcoran *et al.*, 2003), and lineage conversion (Zuniga *et al.*, 2004).

1.3 Anatomic, Phenotypic, and Functional Features of DC Subsets

Although the *in vitro* differentiation pathways of human DC subsets from different precursors are being well characterized, the correspondence with DCs observed *in vivo* or *in situ* in the tissue (resident or “naturally occurring” DCs) is not always clear (Shortman and Liu, 2002). However, both human and mouse studies have greatly advanced our understanding of the heterogeneity of DCs in peripheral tissue, which is based on the subset, activation state, and distinct microenvironments depending on the anatomic localization.

1.3.1 Thymic DCs

The thymus is a primary lymphoid tissue where T-cell differentiation and selection occurs and leads to the generation of naïve CD4⁺ and CD8⁺

T cells with a diverse TCR repertoire, and also naturally occurring CD4⁺CD25⁺ regulatory T cells (Apostolou *et al.*, 2002; Jordan *et al.*, 2001; Watanabe *et al.*, 2005) as well as some of the double-negative invariant T-cell subsets, such as NKT cells (Benlagha *et al.*, 2005; Tilloy *et al.*, 1999) or mucosa-associated invariant T (MAIT) cells (Treiner *et al.*, 2003). Human thymus contains pDCs and two subsets of mature CD11c⁺ MDCs: CD11b⁻CD45RO^{low} DC that lack myeloid markers, and a minority of CD11b⁺CD45RO^{high} DCs expressing many myeloid markers (Bendriss-Vermare *et al.*, 2001; Vandenberghe *et al.*, 2001). Thymic pDCs were shown to produce type I IFN in HIV-1-infected thymus, which exerts antiviral effects (Gurney *et al.*, 2004) and upregulates MHC class I expression on thymocytes (Keir *et al.*, 2002). Whether thymic pDCs play a role in the differentiation and/or selection of T-cell subsets is not known.

Thymic MDCs differ from other peripheral MDC subsets in two major ways: (1) they derive from an intrathymic precursor and die within the thymus, suggesting a nonmigratory behaviour (Ardavin *et al.*, 1993); (2) they mostly present self-Ag rather than foreign Ag (Steinman *et al.*, 2003). Thymic MDCs may be involved in the induction of central tolerance through the process of negative selection as well as the generation of the naturally occurring CD4⁺CD25⁺ regulatory cells (Watanabe *et al.*, 2005) (see Section 1.6).

1.3.2 Blood DCs

Human DC subsets in the blood are well characterized because of tissue accessibility. Human blood contains two types of DC precursors, monocytes and pDCs, which can be induced to differentiate into DCs after *ex vivo* culture (Grouard *et al.*, 1997; Rissoan *et al.*, 1999a; Sallusto and Lanzavecchia, 1994) and have been described in Section 1.2. In addition, human blood contains a subset of immature CD11c⁺ MDCs (O'Doherty *et al.*, 1994). Blood CD11c⁺ MDC subsets are considered naïve cells that are migrating from the bone marrow to the peripheral tissue. This assumption is based on their immature phenotype and on the fact that DCs do not recirculate from peripheral tissue to blood, as suggested by mouse studies (Austyn *et al.*, 1988; Kupiec-Weglinski *et al.*, 1988). It is currently believed that blood CD11c⁺ MDCs locate to the secondary lymphoid organs and peripheral tissues as resting interstitial DCs and that they are related to the *in vitro* generated monocyte-derived or CD34-derived interstitial DCs (Shortman and Liu, 2002). MDCs are lineage-CD4⁺CD11c⁺ cells expressing CD45RO, myeloid markers, such as CD13 and CD33, and the MHC-like molecule CD1c. These phenotypic markers allow clear distinction of blood MDCs from blood pDCs (which lack CD11c but express CD123 and BDCA2) and can be used to purify the two subsets for *in vitro* studies (Duramad *et al.*, 2003; Rissoan *et al.*, 1999a; Soumelis *et al.*, 2002).

Blood pDCs express L-selectin and migrate to the secondary lymphoid organs through the high endothelial venules (Yoneyama *et al.*, 2004).

1.3.3 Skin and Mucosal DCs

In the skin and mucosa, DCs form a dense network of resident cells, both within pluri-stratified epithelia (epidermis, anogenital and oropharyngeal epithelium) as well as in subepithelial areas. The best-studied epithelial DC is the Langerhans cell (LC) of the epidermis. As described in Section 1.2, LCs are immature MDCs expressing CD1a, Lag-antigen, E-cadherin, and Birbeck granule-associated Ag langerin (Romani *et al.*, 2003). LC activation induces their migration out of the epidermis, as it is observed *in vivo* in mouse studies (Baldwin *et al.*, 2004; Ratzinger *et al.*, 2002; Romani *et al.*, 2003), *ex vivo* in cultures of human skin explants (Larsen *et al.*, 1990; Schuler *et al.*, 1993), and *in situ* in skin inflammatory diseases, where LC density is markedly decreased in areas of inflammation (Soumelis *et al.*, 2002).

DCs in subepithelial areas, such as the dermis, have a phenotype of interstitial DCs (Shortman and Liu, 2002). In the mucosa of the intestinal tract, DCs are absent in the epithelium but are abundant in the subepithelial region. Here DCs are lined-up under the basal membrane and protrude thin dendrites through the epithelium into the intestinal lumen to sample for foreign Ag (Kelsall and Rescigno, 2004; Rescigno *et al.*, 2001).

In contrast with these MDC subsets, pDCs are not resident cells of normal skin and mucosa (Gilliet *et al.*, 2004; Wollenberg *et al.*, 2002) but are present in HPV-related cervical cancer (Bontkes *et al.*, 2005), skin melanoma lesions (Salio *et al.*, 2003), lupus erythematosus (Farkas *et al.*, 2001), psoriasis (Nestle *et al.*, 2005), allergic contact dermatitis (Bangert *et al.*, 2003) and in the nasal mucosa as early as 6 h after allergen challenge (Jahnsen *et al.*, 2000), suggesting an active recruitment of blood pDCs to the site of peripheral inflammation. Furthermore, pDC recruitment to the skin has been observed in a therapeutic setting in which skin tumors were treated topically with TLR7 agonist imiquimod (Urosevic *et al.*, 2005). As will be discussed further, pDC trafficking has many similarities with T cells, both being attracted to the site of inflammation by chemokines (SDF-1/CXCR3-ligands).

1.3.4 DCs in Solid Organs

DCs are found in small numbers in all organs except brain (Hart and Fabre, 1981). They have characteristics of interstitial DCs. In each organ,