The Biology of Dendritic Cells and HIV Infection

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Edited by

Sandra Gessani
Istituto Superiore di Sanità
Rome, Italy

Filippo Belardelli
Istituto Superiore di Sanità
Rome, Italy

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Sandra Gessani
Istituto Superiore di Sanità
Department of Cell Biology
and Neurosciences
Viale Regina Elena, 299
00161 Rome
Italy
gessani@iss.it

Filippo Belardelli
Istituto Superiore di Sanità
Department of Cell Biology
and Neurosciences
Viale Regina Elena, 299
00161 Rome
Italy
belard@iss.it

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.

Rome, Italy

Sandra Gessani
Filippo Belardelli

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Although many individuals have offered help or suggestions in the preparation of this book, several deserve special mention. We are grateful to Cinzia Gasparrini, Alessandro Spurio, Barbara Varano, and Paola De Castro for their invaluable editorial assistance. We would also like to give great thanks to all the people who read parts or all of the chapters and advised us on how to make them better.

Rome, Italy

Sandra Gessani
Filippo Belardelli

Contents

Contributors

Gosse J. Adema
Tumor Immunology, Medical Centre Nijmegen,
Nijmegen, The Netherlands

Massimo Alfano
AIDS Immunopathogenesis Unit, San Raffaele Scientific Institute,
Center of Excellence on Physiopathology of Cell Differentiation,
Milan, Italy

Jacques Banchereau
Baylor Institute for Immunology Research,
Dallas, Texas, USA

Filippo Belardelli
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,
Rome, Italy

Mara Biasin
Chair of Immunology, DISP LITA Vialba, Milan University Medical School,
Milan, Italy

Imerio Capone
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,
Rome, Italy

Damien Chaussabel
Baylor Institute for Immunology Research,
Dallas, Texas, USA

Mario Clerici
Chair of Immunology, DISP LITA Vialba, Milan University Medical School,
Milan, Italy

John E. Connolly
Baylor Institute for Immunology Research,
Dallas, Texas, USA

Lucia Conti
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,
Rome, Italy

Manuela Del Cornò
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,
Rome, Italy

Anthony L. Cunningham
Centre for Virus Research, Westmead Millennium Institute,
Westmead; and University of Sydney,
Sydney, Australia

I. Jolanda M. de Vries
Departments of Pediatric Hemato-Oncology, Medical Centre Nijmegen;
and Tumor Immunology, Medical Centre Nijmegen,
Nijmegen, The Netherlands

Heather Donaghy
Centre for Virus Research, Westmead Millennium Institute,
Westmead, New South Wales, Australia

Alexander H. Enk
Department of Dermatology, University of Heidelberg,
Heidelberg, Germany

Laura Fantuzzi
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,
Rome, Italy

Carl G. Figdor
Tumor Immunology, Medical Centre Nijmegen,
Nijmegen, The Netherlands

Maria Cristina Gauzzi
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,
Rome, Italy

Sandra Gessani
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,
Rome, Italy

Silvia Ghezzi
AIDS Immunopathogenesis Unit, San Raffaele Scientific Institute,
Center of Excellence on Physiopathology of Cell Differentiation,
Milan, Italy

Michel Gilliet
Department of Immunology, MD Anderson Cancer Center,
Houston, Texas, USA

Sabrina Haupt
Institute of Clinical and Molecular Virology,
German National Reference Centre for Retroviruses,
Erlangen, Germany

Guillaume Hoeffel
Institut Cochin, Département d'Immunologie, INSERM U567, CNRS,
UMR 8104, IFR 116, Université Paris V René Descartes,
Paris, France

Anne Hosmalin
Institut Cochin, Département d'Immunologie, INSERM U567, CNRS,
UMR 8104, IFR 116, Université Paris V René Descartes,
Paris, France

Joannes F.M. Jacobs
Departments of Pediatric Hemato-Oncology, Medical Centre Nijmegen,
Nijmegen, The Netherlands

Peter Kelleher
Department of Immunology, Imperial College School of Medicine,
Chelsea and Westminster Hospital,
London, United Kingdom

Nicolai A. Kittan
Institute of Clinical and Molecular Virology,
German National Reference Centre for Retroviruses,
Erlangen, Germany

Adriano Lazzarin
Infectious Disease Clinic, San Raffaele Scientific Institute,
Milan, Italy

Jay A. Levy
Department of Medicine, Division Hematology/Oncology,
University of California,
San Francisco, California, USA

Mary F. Lipscomb
Department of Pathology, University of New Mexico School of Medicine,
Albuquerque, New Mexico, USA

Yong-Jun Liu
Department of Immunology, MD Anderson Cancer Center,
Houston, Texas, USA

Karsten Mahnke
Department of Dermatology, University of Heidelberg,
Heidelberg, Germany

Concepción Marañón
Institut Cochin, Département d'Immunologie, INSERM U567, CNRS,
UMR 8104, IFR 116, Université Paris V René Descartes,
Paris, France

Barbara J. Masten
Department of Pathology, University of New Mexico School of Medicine,
Albuquerque, New Mexico, USA

Steven Patterson
Department of Immunology, Imperial College School of Medicine,
Chelsea and Westminster Hospital,
London, United Kingdom

Cândida F. Pereira
Tumor Immunology, Medical Centre Nijmegen,
Nijmegen, The Netherlands

Guido Poli
Vita-Salute San Raffaele,
Milan, Italy

Melissa Pope
Center for Biomedical Research, Population Council, New York,
New York, USA

Cornelus J.A. Punt
Medical Oncology, Radboud University, Medical Centre Nijmegen,
Nijmegen, The Netherlands

S. Ring
Department of Dermatology, University of Heidelberg,
Heidelberg, Germany

Anne-Claire Ripoche
Institut Cochin, Département d'Immunologie, INSERM U567, CNRS,
UMR 8104, IFR 116, Université Paris V René Descartes,
Paris, France

Paola Rizza
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,
Rome, Italy

Barbara Schmidt
Institute of Clinical and Molecular Virology, German National Reference
Centre for Retroviruses, Erlangen, Germany; and Department of Medicine,
Division Hematology/Oncology, University of California,
San Francisco, California, USA

Vassili Soumelis
Inserm U520, Institut Curie,
Paris

Paul J. Tacken
Tumor Immunology, Medical Centre Nijmegen,
Nijmegen, The Netherlands

Giuseppe Tambussi
Infectious Disease Clinic, San Raffaele Scientific Institute,
Milan, Italy

Stuart Turville
Center for Biomedical Research, Population Council, New York,
New York, USA

Elisa Vicenzi
AIDS Immunopathogenesis Unit, San Raffaele Scientific Institute,
Center of Excellence on Physiopathology of Cell Differentiation,
Milan, Italy

Julie A. Wilder
The Lovelace Respiratory Research Institute,
Albuquerque, New Mexico, USA

John Wilkinson
Centre for Virus Research, Westmead Millennium Institute,
Westmead; and University of Sydney,
Sydney, Australia

Part I

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

Chapter 1

Dendritic Cell Biology: Subset Heterogeneity and Functional Plasticity

Vassili Soumelis, Yong-Jun Liu, and Michel Gilliet

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 interferor (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).

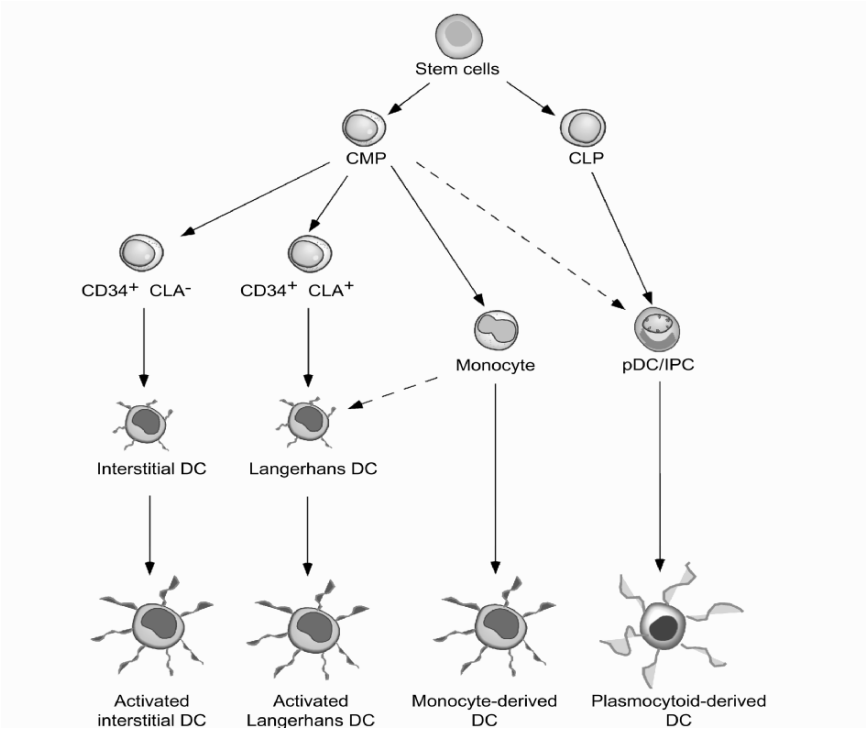

FIGURE 1.1. Pathways of human DC development.

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; Rissoan *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; Vandenabeele *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,