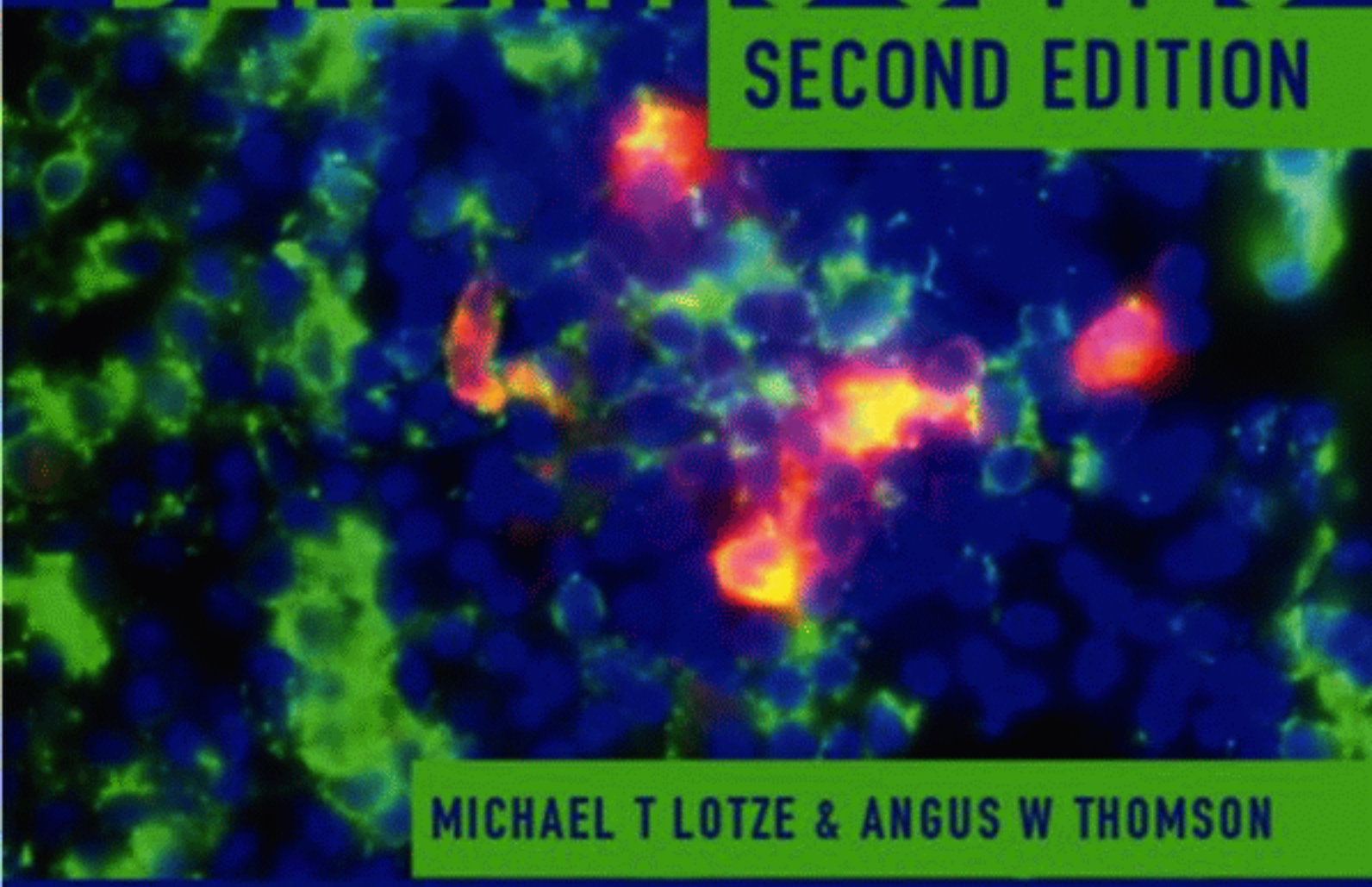
DENDRITIC CELLS

SECOND EDITION



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Foreword to the Second Edition

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> First, therefore, we must seek what it is that we are aiming at; then we must look about for the road by which we can reach it most quickly, and on the journey itself, if only we are on the right path, we shall discover how much of the distance we overcome each day, and how much nearer we are to the goal toward which we are urged by a natural desire. But so long as we wander aimlessly, having no guide, and following only the noise and discordant cries of those who call us in different directions, life will be consumed in making mistakes – life that is brief even if we should strive day and night for sound wisdom. Let us, therefore, decide both upon the goal and upon the way and not fail to find some experienced guide who has explored the regions towards which we are advancing; for the conditions of this journey are different from those of most travel. On most journeys some well-recognized road and inquiries made of the inhabitants of the region prevent you from going astray; but on this one all the best beaten and the most frequented paths are the most deceptive. Nothing, therefore, needs to be more emphasized than the warning that we should not like sheep follow the lead of the throng in front of us, travelling, thus, the way that all go and not the way that we ought to go.
>
> Seneca, *On the Happy Life (AD 58)*.

Drs Lotze and Thomson are to be congratulated on the tremendous success of the first edition of this book, which has necessitated a second edition after such a short time. It is indeed a pleasure to provide this brief foreword from the perspective of one who has followed the progress of cellular immunology at close quarters for 45 years, and thus is truly an eye-witness to history. It is amazing to me to think of how little the cellular basis of immune responses preoccupied the early giants of our discipline. For example, Paul Ehrlich (1900), though highlighting the possible importance of cell surface molecules in the antibody response, scarcely mentioned which cell he thought might be doing the job. To the extent that people worried about the question at all, the macrophage, well known for its role in phagocytosis of invading microorganisms, was presumed to be the antibody-former, for example by Jerne (1955). It was not until the beautiful studies by Fagraeus (1948) on the emergence of proliferating plasmablasts and plasma cells that the full spotlight was put on these specialized cells as fabricating antibody, as later proven by Leduc *et al.* (1955) and the author (Nossal, 1959). Similarly, the role of the lymphocyte in cell-mediated immunity became clear during the 1950s and early 1960s. As these cells were in no way implicated in antigen capture, there was a real gap in understanding needing to be filled. During the 1960s, a golden age in cellular immunology, the notion that three types of cells collaborated in immune responses gradually began to hold sway. These were the macrophage, the T cell and the B cell. The dendritic cell had not yet come into full focus.

Strangely enough, it was the author's collaboration with Gordon Ada (Nossal *et al.*, 1964, 1968) that first brought a peculiar kind of dendritic cell into prominence when we reported the extraordinary antigen-capturing potential of primary lymphoid follicles and the fact that germinal centres soon developed in close proximity to the antigen depot. Structural studies revealed that the antigen was actually captured on the surface of long intertwining dendritic processes, and furthermore that antigen was retained in this location for prolonged periods. Dr John Tew and his colleagues, particularly Andras Szakal and Marie Kosco subsequently deepened our understanding of follicular dendritic cells and their role in immunological memory (Tew *et al.*, 1990). Further clarity entered the field when Tew, Thorbecke and Steinman (1982) presented a sensible nomenclature for dendritic cells in immune responses. However, nothing can eclipse the tremendous role that Ralph Steinman (1991) has played in bringing dendritic cells into focus and prominence. It was his methods of identification and isolation of the 'regular' dendritic cells that allowed a subsequent veritable tidal wave of experimentation which has now led to the rich diversity of structure and function which makes the present volume such fascinating reading.

The reason why DCs are so prominent in current discussions in immunology relates to their extraordinary and perhaps unique ability to activate and maintain the survival of T lymphocytes. It first became clear that, on a quantitative basis, they were by far the most powerful stimulators of allogeneic T-cell responses. In the field of T-cell activation in response to recall antigens, they are also paramount. In regulation of antibody formation, dendritic cells pre-pulsed with antigen are by far the most immunogenic cells that can be found. *In vivo*, it is now clear that so-called interdigitating dendritic cells are also present at the initiation of the germinal center reaction. The sequence appears to be that DCs, perhaps after migrating from peripheral sites of antigen uptake, firstly activate T cells which in turn activate B cells, some of which migrate into the primary lymphoid follicle. The germinal center reaction is initiated when, in turn, these B cell blasts interact with follicular dendritic cell-bound antigen.

Twenty-five years after our initial interest in follicular dendritic cells, our attention was caught again by the germinal center reaction (summarized in Nossal, 1994). Technology had advanced to the stage where molecular biological and cell culture methods allowed the process of somatic mutation in germinal center B cells to be studied at the single cell level. This quickly confirmed the prior hypothesis of Kocks and Rajewsky (1989) that the somatic hypermutation which eventually leads to affinity maturation of the antibody response is not a feature of either primary or secondary B cells giving rise to a clone of antibody-forming cells. Rather, the process occurs during the separate clonal development of memory cells in germinal centers. The germinal center reaction permits an iterative process whereby only B cells with heightened affinity for the antigen in question gain access to antigen bound on follicular dendritic cells, permitting further stimulation and clonal proliferation, with multiple rounds of mutation and selection finally leading to greatly changed and higher affinity antibodies.

There are many respects in which the germinal center reaction resembles the original process of generation of primary B cells. Suppression and then re-emergence of Ig receptors are noted on the centrocyte surface followed by generation of new patterns of immunoglobulin expression conferred by high rates of mutation and not as originally believed by V gene translocations. A phase of substantial susceptibility to tolerance follows (Pulendran *et al.*, 1995). Indeed, if B cells are 'caught' in transit between the deeper layers of the germinal center, where cells are proliferating rapidly, and the areas of antigen deposition on the more superficial aspects of the germinal center encounter soluble antigen, they undergo apoptotic death. This resembles in principle what happens in the bone marrow if a B cell encounters 'its' antigen prior to being released from the marrow as a mature B cell. Presumably this mechanism prevents survival of B cells which could hypermutate, generating high-affinity anti-self reactivity.

If any further evidence of the great importance of DCs in the immune responses was required, it was provided by the work of Boyle *et al.* (1998) on DNA vaccines. One of the real problems hindering the

more rapid development of DNA vaccines has been the relatively weak nature of the immune responses they induce in many model systems. If, however, the gene construct encoding an antigen is modified so as to coexpress the molecule CTLA4, both B- and T-cell responses are tremendously enhanced. CTLA4 is a counterstructure to two receptors, CD80 and CD86, which are constitutively expressed on DCs. The strategy is therefore one which targets the product of DNA immunization directly to DCs. This strategy may well prove to have important practical applications.

No student of cellular immunology will be surprised to learn that there are different subsets of DCs beyond the variations we have briefly described. This aspect is at a fairly early stage of exploration and it is fitting that the present state of knowledge will be summarized by Dr Li Wu.

CONCLUSIONS

The editors are to be congratulated on having assembled a fascinating and authoritative series of chapters which summarize so many aspects of the anatomy, physiology, biochemistry and pathology of dendritic cells in considerable detail. The fact that this second edition follows so quickly after the first reflects the vigor and excitement of the field. Very few of us pondering the intricacies of the three cell interactions in immunology, which we thought to rotate around macrophages, T cells and B cells, would have thought as recently as 25 years ago that a book of these dimensions, with no fewer than 45 chapters, could be devoted to dendritic cells. A new, exciting, important and practical series of paradigms has been created in this short period. Let us hope that this book will stimulate still further effort, and that the third edition some years hence will also bring forth powerful new insights.

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Preface to the Second Edition

> The most beautiful experience we can have is the mysterious. It is the fundamental emotion which stands at the cradle of true art and true science.
>
> Albert Einstein, *The World As I See It.*

The substantial mystery surrounding antigen processing and presentation, initiation of the immune response and how the effector phase of the immune response vectored onto the dendritic cell (DC) over the last five years provided the impetus for our first edition. Much of the interest in DCs was made manifest in the increasing number of references and meetings devoted to this cell. We recall that there was a belief amongst outside advisors that a book dedicated to a single cell making for a paltry audience with limited scope would have equally limited value. This view, with the advantage of hindsight, was clearly wrong. We discovered, as our book was coming into print (quite rapidly from the time of initiation), that the field continued to grow with questions and answers leading inexorably to the next set of questions, apparently growing logarithmically. In addition, there was an appreciable interest in having much of the detailed information organized in one volume in a way that allowed credible exploration for the experienced dendritophile as well as the tyro. For those interested in these things, at the time of this edition, there have been 10,448 citations concerning DC in the literature since this cell was first described by Steinman and Cohn (1973) now almost 30 years ago; a prodigious literature to embrace and know – all on one cell! We have attempted to integrate many of these references by era (year) at the end of this volume, as an innovation for our readers and we hope that it will be of service. You can clearly see who are the authors of the DC citation classics by the number of chapters referencing them at the end of each citation! We have also introduced multiple new chapters and had several started anew to keep the volume contemporary and to reflect the new areas of biology and clinical application.

So what have we learned in the last three years? The answer is obviously quite a bit, both in the clinical arena into which DCs have been thrust, and in the area of basic science where gene discovery has uncovered a veritable treasure trove of new molecular targets limited to or preferentially in DCs (Hartgers *et al.*, 2000). The notion of lymphoid and myeloid DCs and the disparity between mouse and human biology *are finally being resolved*, although not yet to everyone's satisfaction. The critical role of the DC in self and transplantation tolerance (Kurts *et al.*, 2001; Thomson and Lu, 1999) and their association with a pathogen's ability to identify and target this cell, both as a means of immunosuppression as well as a means to shuttle virus or other microbes to distant sites (Servet-Delprat *et al.*,

2000) represent evolving areas. We have attempted to capture the current art, but additional information presents apace. The use of recently-identified tumor antigens to be presented by DC to cells of the immune system captured our interest and that of others. Moreover the number of clinical trials using these cells is now mature enough to support publication and presentation at national and international meetings. Now that we understand how tumor antigens interact with cells of the immune system, issues related to the source of antigen, adjuvanticity of DCs, and how tumors suppress DC function and maturation have become the central issues (Mayordomo *et al.*, 1995; Lotze and Jaffe, 1998). Finally, it probably is a given, but we should recognize that in the absence of other immune cells, the DC plays at best a bit part. Likewise, we cannot limn the shape of cellular or humoral immunity without reflecting on the critical role of DC in shaping and selecting our immunity. Perhaps we should argue that virtually all treatises on modern B or T cell biology, without reflection on the critical role of DCs, are at best incomplete.

And, at the end of this preface, we need to thank those individuals who have made our lives possible to lead meaningfully in the academic environment, providing support in every way they could to enable the preparation of this second edition. We would like to dedicate it to our administrative assistants – Maria Bond, Kathy Rakow, Margaret Corson, Joyce Caperilla, Shelly Conklin, as well as the staff of Academic Press, Lillian Leung, Jacqueline Read, and Tessa Picknett. In addition, Bridget Colvin's talent uncovered some remarkably 'DCesque' literary quotations and to Thomas Lotze for assistance with the compilation of the references at the end. If we have been at all successful, it has been because of their fine efforts. We thank them for their patience and prudence at all stages of this book's current evolution.

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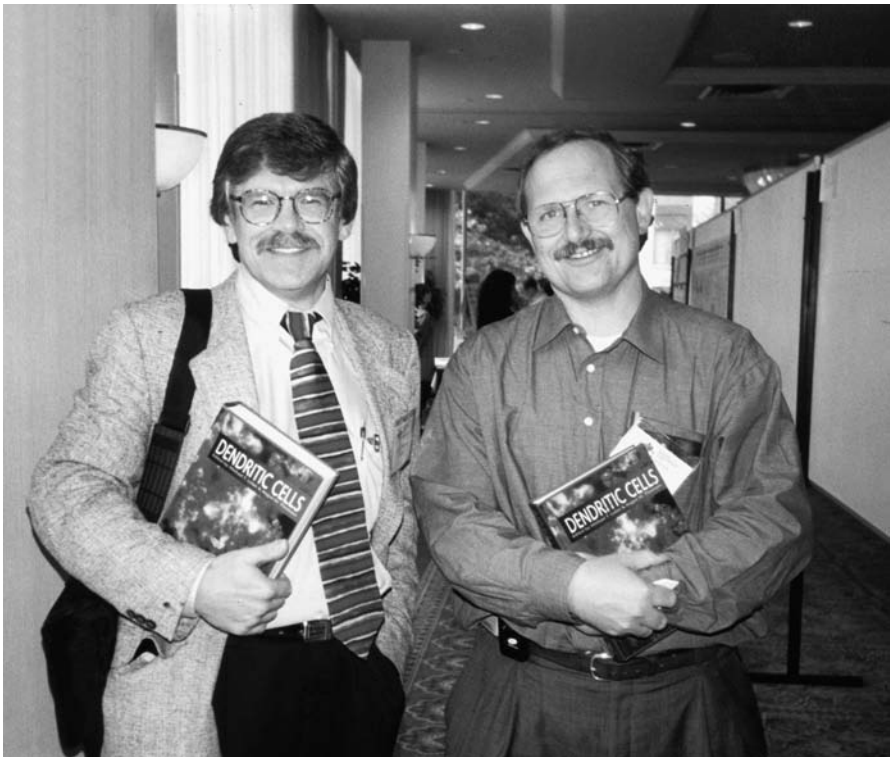

The editors (Dr Lotze at right, Dr Thomson at left) pictured with the first edition of the book.

Preface to the First Edition

Fighting in the forefront of the Greeks, the Athenians crushed at Marathon the might of the gold bearing Medes.

Simonides, c. 556–468 B.C.

This volume represents a special effort to bring togther in one place the information that would allow a scientifically oriented clinician, or even nonimmunologically oriented scientist, to appreciate the important role of this previously obscure cell. The notion of having an entire book dedicated to a single cell, albeit one as important as the dendritic cell (DC), is one which could be met with disapprobation in some quarters. It does, however, reflect the importance which the authors (who completed their tasks admirably) and the editors have placed on the extraordinary important role this cell has in dictating the initiation and persistence of the adaptive immune response. Indeed, all aspects of the 'fight' during the acute natural immune response but in particular the chronic infammatory immune response associated with cancer, chronic infections diseases, autoimmunity, and transplantation are importantly related to DC biology. While the first report of DC by Ralph Steinman and Zanvil Cohn, 25 years ago, posited an important role for this cell in immune regulation, this volume's role is not soley celebratory (but it does have those elements!).

It has thus been appreciated for the last 25 years that DC are specialized antigen-presenting cells (APC) with a unique ability to prime effective immune responses. This may give them a special importance in several human disease states known to have an immunological basis. While great strides were made in the understanding of the role of specific T cells and antibodies in mediating allergy, autoimmunity, graft rejection, infectious disease, and tumor immunity over this period, it was also clear that these effector cells and molecules represented the end stage of an immune response, the outcome of which was probably determined at its very initiation by the type of APC, the nature and state of the antigen, and the cytokine conditions under which the antigen was first presented. As is usually the case in science, testing of ideas must await development of new techniques and reagents. Several opportunities presented themselves in the 1970s with discovery and cloning of the first few cytokines, especially interferon-α and IL-2. This allowed for the first time use of immunologically important molecules themselves as means to manipulate immune responses or to support the growth and expansion of T cells and NK cells *in vitro*. This in turn led to the identification and characterization of the T cell receptor for antigen and examination of disease-specific T cell responses. T cells that mediate graft rejection, T cells specific for tumor antigens, and T cells specific for autoantigens were studied for their biology and function, as well as being used as reagents to fully define disease-specific antigens and the genes that encode them. The important role of cytokines, as well as the number of indentifiable cytokines, continued to rise

throughout that same period, now with possibly as many as 20–21 so-called 'interleukins' in addition to the colony-stimulating factors and interferons. Their pivotal role in directing the immune response was unveiled. An important outcome from this work has been the understanding of the polarity of the immune response at the T cell level, T_H1 vs T_H2 and Tc1 vs Tc2. The helper T cell response that develops as predominately T_H1 type (in the presence of and producing T_H1-type cytokines, IL-2, IFN-γ, GM-CSF) supports the development of cytotoxic T cells (CTL), often of the Tc1 type. Alternatively, the T_H2-type response (developed in the presence of and producing the T_H2-type cytokines, IL-4, IL-10) supports the development of humoral immunity and Tc2-type CTL. In systems where this can be tested *in vivo*, this dichotomy translates into a life or death, or disease or no disease situation. During this same period, understanding of immunoglobulin gene rearrangements and the advent of monoclonal antibody technology truly revolutionized all of the biological sciences, perhaps one of the greatest gifts that immunologists have yet bestowed on their nonimmunological colleagues.

So how is this complex adaptive system initiated and maintained? We now believe that the DC plays an important role in initiating and maintaining immune reactivity. The sequential steps in the choreography from its birth in the bone marrow, maturation there or in the thymus or secondary lymphatic tissues, traversion of the initial endothelial barriers into virtually all tissues, sensitivity to inflammatory initiators and tissue damage, injury or 'danger', and transformation into a 'mature cell' capable of migrating across the lymphatic or postcapillary venules to enter secondary lymphoid sites, interacting with the resident T cells and B cells to rapidly screen and select immunological suitors make it one of the most versatile of dance partners. Not only does it have the potential to come into contact with all cells in all tissues, its athletic potential as the 'track star of immunology' makes it a marathon contender of the first order. And, just like the marathon runner who crossed the Plains of Marathon, after delivering its message in the lynph node or spleen, this cell dies, to be born again, Athena-like, in the bone marrow every day.

The last five years have been particularly dizzying with application of cytokines now to the culture and maintenance of DC. Just as the advances in T cell and B Cell biology required means to grow and maintain these cells, the use of GM-CSF and IL-4 or TNF has made their study and application in preclinical and clinical disease models feasible. Prior studies, restricted by the relatively limiting numbers of these cells from any one site in tissue or blood, have now been extended by extraordinary new knowledge available from advances in these relatively simple and straightforward culture techniques. This new knowledge has emphasized the importance of the very early events in the priming of the immune response and turned full attention to the DC. As we prepare these comments, some of the important issues in DC migration and recruitment via specialized chemokines including MIP-3 and their receptors CCR6 and CCR7, as well as the preferred source of antigenic material for DC (apoplotic cells and bodies) are just coming to light. These and other recent insights will require some time to become integrated into the growing corpus of information and raise important additional questions about these centrally important cells. This volume celebrates them and, when it enters the second edition, hopefully many of the issues raised here will have been successfully addressed and new ones raised.

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C H A P T E R

1

The development of dendritic cells from hematopoietic precursors

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Experience is not what happens to a man. It is what a man does with what happens to him.
Aldous Huxley

INTRODUCTION

Blood cells are diverse types of cells that provide highly specialized functions such as tissue oxygenation, tissue repair, blood clotting or immune responses. The continuous demand for the supply of these various types of blood cells is provided by the proficient, yet tightly regulated development of hematopoietic progenitor cells. Cell fate specification of these uncommitted multipotential cells is therefore an important aspect of hematopoietic cell development, but one that remains incompletely understood. The study of the dendritic cell (DC) lineage specification has provided interesting insights into this area. DCs constitute a system of hematopoietic cells that are rare but ubiquitously distributed. Several DC types with different biological features have been identified in different tissues, including Langerhans cells (LCs) in the epidermis, interstitial DCs in various tissues, thymic DCs and DC populations found in other lymphoid organs. DCs have powerful functions in the immune system. They can capture and process antigens, then present the antigenic peptides and activate specific T cells (Steinman, 1991; Banchereau and Steinman, 1998).

Variations among the tissue distribution of DCs and differences in their phenotype and function indicate the existence of heterogeneous populations of DCs (Hart, 1997). DCs were originally considered to be of myeloid origin and closely related to monocytes, macrophages and granulocytes. However, recent studies suggested that DCs can be generated along distinct developmental pathways and can originate from precursors of different hematopoietic lineages, with at least two DC lineages being identified so far, namely the conventional myeloid-related DC and the newly defined lymphoid-related DC lineages. Because various populations of DCs in mice (Kronin *et al.*, 1996; Maldonado-Lopez

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et al., 1999; Pulendran *et al.*, 1999) or humans (Caux *et al.*, 1997) are able to induce distinct types of immune responses, it becomes important to determine the role of their origin in determining functional heterogeneity. An important question is how this heterogeneity arises at the developmental level.

EARLY DEVELOPMENTAL DECISION CHECKPOINTS IN THE HEMATOPOIETIC DEVELOPMENT OF DCs

The early developmental steps of DC formation from hematopoietic progenitor cells are not uniform and involve different types of progenitor cells, different developmental pathways and different signals. Understanding these early events is facilitated by the identification of early developmental checkpoints in the hematopoietic development of DCs.

Early hematopoietic progenitors

The existence and identification of lineage-restricted progenitor cells has been helpful in our understanding of hematopoietic cell fate specification. Multipotent yet lineage-restricted progenitor cells identified and characterized in mice and in humans can be distinguished from the most primitive hematopoietic stem cells (HSCs) based on differences in cell surface phenotype and the capacity and durability of multilineage engraftment. In the murine thymus an early lymphoid-restricted precursor population termed the 'low CD4 precursor' has been identified. This precursor population does not express hematopoietic lineage markers (Lin), but expresses low levels of CD4 and Thy-1 and high levels of the hematopoietic progenitor cell markers c-kit and Sca-1 (Wu *et al.*, 1991a). These precursors, although isolated from thymus, are not yet committed to the T-cell lineage and are able to produce T cells, B cells, natural killer (NK) cells and DCs (Wu *et al.*, 1991b; Ardavin *et al.*, 1993). However, they have no myeloid and erythroid differentiation potential (Wu *et al.*, 1991b).

In murine bone marrow (BM), clonogenic common lymphoid and common myeloid progenitors have also been identified recently (Kondo *et al.*, 1997; Akashi *et al.*, 2000). IL-7Rα expression is a main marker to distinguish these two progenitors. The common lymphoid progenitors (CLPs) are Lin^-, $IL^-7R\alpha^+$, c-kit^{lo} and Sca-1^{lo}. Such cells can generate all lymphoid cells at clonal level and some DCs (Wu *et al.*, unpublished), but not detectable myeloid or erythroid cells (Kondo *et al.*, 1997). The common myeloid progenitors (CMPs) are Lin^-, IL-$7R\alpha^-$, c-kit^+, Sca-1^-, $CD34^+$, $Fc\gamma R^{lo}$ (Akashi *et al.*, 2000). These cells can give rise to precursors for megakaryocytes/erythrocytes (MEPs) and precursors for granulocytes/macrophages (GMPs) (Akashi *et al.*, 2000). CMPs also produce DCs (Traver *et al.*, 2000)

The human equivalent of mouse CMPs has not yet been described. However, progenitor cells with features similar to those of the CLPs in mouse have been identified in human. The human BM progenitor cells expressing CD34, CD45RA, CD10 and IL-7Rα, but no lineage-associated markers, have differentiation potential restricted to the production of lymphocytes and DCs, but not of myeloid cells and erythrocytes (Galy *et al.*, 1995b; Ryan *et al.*, 1997). This CLP arises from a myeloid/lymphoid-restricted progenitor cell with limited erythroid differentiation potential that is contained in the $CD34^+CD45RA^+$ cell population (Galy *et al.*, 1995a). Thus, hematopoietic cell fate specification occurs incrementally. The existence of progenitor cells that can give rise to several lineages but not to all hematopoietic lineages represents possible developmental checkpoints of hematopoietic differentiation.

Developmental relationships of myeloid lineage and DCs

It is generally assumed that DCs have a 'myeloid' origin because they arise from hematopoietic progenitor cells with myeloid differentiation potential and they can be produced from monocytes, a typical myeloid cell. Monocytes can generate immunostimulatory DCs. This differentiation process occurs without proliferation

and is induced at a high frequency in culture by granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-4 (Romani *et al.*, 1994; Sallusto and Lanzavecchia, 1994; Zhou and Tedder, 1996). In spite of this relationship, DCs can also develop independently from monocytes in 'myeloid' cell growth conditions.

In mice, distinct pathways giving rise to granulocytes, monocytes/macrophages and DCs from a blood or BM precursor negative for MHC class II have been described (Inaba *et al.*, 1993). DCs can be generated along with phagocytic myeloid cells from cells within a single colony in semisolid medium cultures. GM-CSF, but not granulocyte (G)-CSF or macrophage (M)-CSF, is required for DC development in such systems (Inaba *et al.*, 1993). DCs generated under such conditions express MHC class II, display the characteristic morphology of DCs and are potent stimulators of resting T cells. They also have the capacity to home to T-cell regions in draining lymph nodes (Inaba *et al.*, 1993).

In the human system, it is also possible to grow pure colonies of DCs (DC-CFU) from BM in the presence of GM-CSF, TNFα and stem cell factor (SCF). These DC-CFU are distinct from mixed DC-myeloid CFU, therefore indicating that myeloid cells and DCs can have distinct clonogenic precursors at some point in their development (Young *et al.*, 1995).

The study of Langerhans cell (LC) production also indicates the existence of early developmental options within the DC lineage. By careful analysis of the *in vitro* differentiation of $CD34^+$ progenitor cells in culture, it is possible to recognize the existence of separate precursors of LCs that can be distinguished by their phenotype, by involvement of a recognizable monocyte stage and by their requirement for TGFβ (Caux *et al.*, 1996; Geissmann *et al.*, 1998; Jaksits *et al.*, 1999). Among $CD34^+$ cells, the expression of low levels of the IL-3Rα chain (Olweus *et al.*, 1997) or the expression of the cutaneous lymphocyte-associated antigen CLA (Strunk *et al.*, 1997) defines progenitor cells able to give rise to LCs in culture in the presence of GM-CSF and TNFα.

Two developmental pathways have been identified for the production of LCs and DCs from $CD34^+$ progenitor cells. One gives rise to HLA-DR^{bright} cells with LC morphology, phenotype and function, via a $CD14^-CD1a^+$ intermediate, after 4–5 days in culture with GM-CSF, TNFα and SCF (Caux *et al.*, 1996). Another differentiation pathway of $CD34^+$ cells was identified in these culture conditions that gives rise to a $CD14^+CD1a^-$ bipotential intermediate cell. This intermediate cell produces non-LC DCs but can be induced in the presence of TGFβ to differentiate into LCs (Jaksit *et al.*, 1999). Alternatively, this intermediate cell can differentiate along a macrophage pathway when recultured with M-CSF (Caux *et al.*, 1996; Szabolcs *et al.*, 1996). Thus, the divergence in developmental pathways leading to the production of LCs and non-LC DCs has one origin within the small population of $CD34^+$ progenitor cells. Both mouse and human studies indicate that there is a close lineage relationship between myeloid cells and DCs, and that some aspects of DC cell fate specification occur at the progenitor cell level.

Relationship between development of lymphocytes and some DCs

Further insight into DC lineage specification has been obtained in studies of lymphoid progenitor cell subsets. Mouse thymic DCs and a subpopulation of DCs in spleen and lymph nodes express several markers of lymphoid cells, such as CD8αα, CD2, BP-1 and CD25 (Vremec *et al.*, 1992; Wu *et al.*, 1995). This was the first suggestion of a relationship between these DCs and lymphoid cells. Indeed, when the intrathymic lymphoid restricted precursor, the 'low CD4 precursor' population (Wu *et al.*, 1991a, 1991b), was transferred intrathymically, thymic $CD8\alpha^+$ DCs were generated and when injected intravenously, both thymic DCs and the splenic $CD8\alpha^+$ DC population were generated (Ardavin *et al.*, 1993; Wu *et al.*, 1995, 1996). Unlike the bone marrow precursors, which produced both $CD8\alpha^+$ and $CD8\alpha^-$ DC populations in mouse spleen, the intrathymic precursor population can only generate the $CD8\alpha^+$ DCs (Wu *et al.*, 1996). This suggests that the $CD8\alpha^+$ DC population represents a lymphoid-related DC lineage.

In vitro studies showed that when CD8α$^+$ or CD8α$^-$ DC subsets were placed in short-term cultures to allow their further differentiation, they did not differentiate into one or the other (Vremec and Shortman, 1997 and unpublished). This again supports the theory that the CD8α$^+$ and CD8α$^-$ DC subsets represent separate DC lineages rather than DCs at different developmental stages of the same lineage.

Further studies on developmental potential of the precursor populations downstream from the earliest 'low CD4 precursors' in T-cell development, namely the CD3$^-$CD4$^-$CD8$^-$ triple negative (TN) thymocyte precursor populations, revealed that the early TN precursor population, the 'pro-T' cell population, also retained the potential to form DCs (Wu *et al.*, 1996). These pro-T cells resemble the 'low CD4 precursors' because their T-cell receptor (TCR) β gene is in germline state, they have the potential to produce T cells and DCs, but have lost the potential to form B cells and NK cells. This suggests a close relationship between T cells and DC development in the thymus. In contrast, the later TN precursor population, the pre-T cell, which has rearranged TCR β genes, is no longer able to produce any lineages other than T cells (Wu *et al.*, 1996; Lucas *et al.*, 1998). Therefore, it appears that full commitment to T cell lineage occurs at the stage of TCR β gene rearrangement.

Interestingly, DCs can also be generated in cultures from the intrathymic 'low CD4 precursors' or from pro-T cells in the presence of a set of cytokines which was different from the ones normally used for generating myeloid-derived DCs (Saunders *et al.*, 1996; Lucas *et al.*, 1998). The main difference from the myeloid-derived DC cultures was the lack of requirement for myeloid cell growth factor GM-CSF to stimulate proliferation or DC differentiation. The cytokines required for DC generation from the thymic precursors include TNFα, IL-1, IL-3, IL-7, SCF, Flt-3L and CD40L. DCs could be generated from single low CD4 precursor cells in these cultures with a cloning efficiency of about 70%. Thus, the majority of the thymic lymphoid precursors are able to produce DCs (Saunders *et al.*, 1996).

Interestingly, a recent report by Bjorck and Kincade (1998) showed that mouse bone marrow CD19$^+$ pro-B cells could also develop into DCs with T-cell stimulatory properties when cultured under conditions similar to those used for DC production from the thymic lymphoid precursors. This further illustrates the close relationship between some DCs and committed lymphoid progenitor cells and shows a potential link between DCs and the B lineage. However, it is not known whether DC differentiation from B-cell precursors occurs *in vivo*.

Similarly, in the human system, relationships between DCs and lymphoid progenitor cells have been found. Hematopoietic progenitor cells expressing CD45RA, the high-molecular-weight isoform of CD45, display a greater level of commitment for differentiation into lymphocytes (T, B and NK cells) than in the HSCs population (Galy *et al.*, 1995a) and also seem to be more committed toward LCs than most progenitor cells as they contain CLA$^+$ cells, the precursors for LCs (Strunk *et al.*, 1997). In bone marrow, the CD34$^+$CD45RA$^+$ progenitor cells are distinct from primitive HSCs phenotypically and functionally as they produce lymphocytes and myeloid cells (granulocytes and monocytes) but they are markedly depleted of erythroid progenitor cells, indicating the loss of some developmental options compared with HSCs (Craig *et al.*, 1994; Galy *et al.*, 1995a). This population of CD34$^+$CD45RA$^+$ cells contains a common lymphoid progenitor (CLP) expressing CD10 but no lineage-associated markers (Lin), such as CD19, CD2, CD14, CD15, CD56 and glycophorin A. Such CLPs represent approximately 5% of progenitor cells in adult bone marrow. Their differentiation potential is restricted to the production of lymphocytes and DCs as they cannot produce myeloid cells (granulocytes or monocytes), erythrocytes, mast cells or platelets in spite of stimulation with multiple growth factors (Galy *et al.*, 1995b).

The ability of these CLPs to produce T cells rapidly (although not durably) and their overall differentiation potential into lymphocytes and DCs suggest that such cells could leave the bone marrow to colonize the thymus (Ardavin *et al.*,

1993; Márquez *et al.*, 1995; Res *et al.*, 1996). Indeed, $CD34^+Lin^-CD10^+$ cells are found in the human thymus and can be isolated from steady-state circulating blood to produce DCs *in vitro* (A. Galy, unpublished observations). It is not yet clear whether such CLPs are able to give rise to all of the types of thymic DCs which include interdigitating cells as well as plasmacytoid T-cell DCs (Res *et al.*, 1999). Yet, the existence of this small progenitor cell population which contains clones of bipotential progenitors of lymphocytes and DCs strongly and directly argues for a tight developmental link between lymphocytes and DCs. Interestingly, the origin of DCs may be less of a determinant of their phenotype or function than previously thought. This is evidenced by recent studies showing that $CD8\alpha^+$ DCs as well as $CD8\alpha^-$ DCs can be produced from CMPs (Traver *et al.*, 2000 and Wu *et al.* manuscript submitted) suggesting that the expression of CD8α does not delineate the ultimate lineage origin of DCs. An important area is to define what signals control the development of DCs.

SIGNALS REGULATING THE HEMATOPOIETIC DEVELOPMENT OF DCs

Functions of the transcription factor Ikaros family in DC hematopoiesis

Several signaling pathways, in which Ikaros is differentially involved, regulate DC development *in vivo* and *in vitro*. The *Ikaros* gene family was first implicated in DC hematopoiesis by studies in mutant mice. *Ikaros* is abundantly expressed in lymphoid tissues and encodes for a family of Kruppel-like zinc finger DNA-binding proteins. Potential Ikaros-binding sequences have been identified in many T cell- and B cell-associated genes such as the promoter and enhancer regions of CD3γ, δ and ε, the TCR α and β genes, the CD4 silencer, in the NFκB sites of the IL-2Rα, interferon β and MHC class II genes, in the HIV-LTR, in the LYF element of the TdT promoter, the EBF sites of the Igα promoter, and in the promoters of granzyme B, B29, TNFR p75 and BP-1 (Wargnier *et al.*, 1995; Babichuk *et al.*, 1996; Molnar *et al.*, 1996; Santee and Owen-Schaub, 1996; Thompson *et al.*, 1996).

Mice homozygous for an *Ikaros* null allele lack B and NK lymphoid cell development and display specific alterations in T-cell development and a strong reduction in numbers of DCs in lymphoid organs (Georgopoulos *et al.*, 1994; Wu *et al.*, 1997).

Deletions of the DNA encoding the Ikaros DNA-binding domain from the mouse germline that generate an Ikaros mutation with dominant negative properties ($DN^{-/-}$) cause more serious lymphoid and DC defects (Wu *et al.*, 1997). Proteins produced by the dominant negative locus interact and interfere with proteins produced by the wild-type *Ikaros* locus or with other family members, and compromise their activity (Sun *et al.*, 1996; Morgan *et al.*, 1997; Kelley *et al.*, 1998). Hematopoietic defects in $DN^{-/-}$ animals include a severe block in lymphopoiesis and a general depletion of DCs in lymphoid organs although monocytes and skin LCs are abundantly present, suggesting that several signaling pathways, in which Ikaros is differentially involved, regulate DC development *in vivo*.

The human equivalent of Ikaros is highly homologous to its murine counterpart with almost complete identity in the DNA-binding region and protein interaction domains (Molnar *et al.*, 1996). Ikaros mRNA is detectable in human $CD34^+$ cells (Galy *et al.*, 1998) and murine dominant negative proteins interfere with the normal function of Ikaros proteins in human cells. One dominant negative Ikaros protein, Ik7, is the product of a gene targeting deletion of exons 3 and 4 which causes a strong reduction in the DNA-binding ability of heterocomplexes formed between Ik7 and other members of the Ikaros family of proteins through their C-terminal zinc finger modules (Sun *et al.*, 1996). When the dominant negative Ik7 protein was overexpressed in human hematopoietic cells that were cultured in conditions promoting the development of