Human Dendritic Cells and the Regulation of Allergic Immune Responses

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AKADEMISK AVHANDLING
som för avläggande av teknologie doktorsexamen vid Tekniska fakulteten vid Lunds Universitet kommer att offentlig försvaras i Segerfalkssalen, Wallenberg Neurocentrum, Sölvegatan 17, Lund,
Fredagen den 8 april 2005, kl. 10.00

Fakultetsopponent är Dr Françoise Rousset,
L’Oréal Recherche, Aulnay-sous-bois, Frankrike
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**Abstract**

Dendritic cells (DC) are a heterogeneous lineage of bone-marrow-derived leukocytes that serve as the link between innate and adaptive immunity. They are professional antigen-presenting cells that play an important protective and regulatory role in both health and disease. This thesis is based upon six original papers that deal with the function and transcription of human DCs, with focus on their role in the inflammatory immune responses, such as allergic rhinitis. Transcriptional profiling of DCs with microarrays has been an extensively utilized technique in the projects presented in this thesis. We have evaluated the gene expression profiles and functions of two in vitro models of human DCs, namely the monocyte-derived DCs and the differentiated cell line MUTZ-3, and the transcriptional regulation induced in these models by pro-inflammatory signals. We have also studied the effect of allergenic stimulation on the transcription and function of DCs derived from healthy and allergic individuals. Two allergens were used, the detergent enzyme lipase and grass pollen, to evaluate the direct effect on phenotype and gene expression of DCs in addition to the subsequent ability of allergen-challenged DCs to amplify and modify the autologous effector T cell response. We demonstrate that the transcriptional responses of DCs and effector T cells to allergenic stimulation are different between allergic and healthy individuals. These transcriptional profiles involved in the immune recognition of allergens will be further evaluated in order to understand the interplay between DC's and T cells in allergic rhinitis. Furthermore, in addition to the in vitro models studied, we have performed phenotypical and transcriptional characterizations of in vivo DCs isolated from peripheral blood and tonsilar tissue. We suggest that follicular DCs in tonsils may have previously unacknowledged costimulatory functions in the germinal center reaction, as they express CD137. An extensive transcriptional profiling of freshly sorted DC subsets from blood and tonsils identified DC-subset selective gene expression and pinpoint their relationships. We demonstrate innate specialization of these subsets and show that the environment in tonsils determines the transcriptional activity of myeloid DCs. In conclusion, these studies have provided insight in the transcription and phenotype of in vivo immature/mature DC populations as well as in the immune response induced by allergens or inflammatory signals.

**Key words:** Dendritic cells, microarray, T cells, transcriptional profiling, inflammation, allergic rhinitis

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CONTENTS

ORIGINAL PAPERS ............................................................................................................. 5
ABBREVIATIONS .................................................................................................................. 6
INTRODUCTION ...................................................................................................................... 7
HUMAN DENDRITIC CELLS .................................................................................................. 9
   Definition and origin ........................................................................................................... 9
   Subtypes, phenotypes and distribution ............................................................................... 10
   Lifecycle and migration ................................................................................................... 14
   DC maturation ................................................................................................................ 16
   Functional plasticity of DC subtypes and the concept of DC1 and DC2 ......................... 17
DC/T CELL INTERPLAY ....................................................................................................... 20
   T cell development and migration ................................................................................... 20
   T cell memory ................................................................................................................ 21
   DC-T cell interaction ....................................................................................................... 22
ALLERGIC DISEASE .......................................................................................................... 24
   Characteristics of allergic rhinitis .................................................................................... 24
   Epidemiology .................................................................................................................. 24
   Symptoms and pathology of allergic rhinitis ................................................................... 24
   Lymphoid structures and architecture of the upper respiratory tract ............................. 25
   Cellular mechanisms of allergic immune response ......................................................... 26
   DCs and CD4+ T cells in allergic responses .................................................................... 28
   Evidence for DCs as important regulators of allergic disease in vivo .............................. 28
   Uptake of allergens and modification of DC function ........................................................ 29
   Th2 cells .......................................................................................................................... 33

TRANSCRIPTIONAL ANALYSIS IN DC AND ALLERGY RESEARCH ............................ 35
   Introduction to high-density oligonucleotide microarray technology ............................ 35
   Transcriptional profiling of human DCs ........................................................................... 35
   Characterization of in vivo DCs from tonsils and peripheral blood ............................... 35
   Transcriptional analysis of the maturational program in DCs in vitro ............................. 37
   Understanding molecular mechanisms in allergic disease using microarrays ............. 37
   DCs as immune sentinels for allergens; a transcriptional approach ............................... 39
   Transcriptional activity in T cells induced by allergen-stimulated DCs ......................... 41

MYELOID CELL LINES ...................................................................................................... 43
   MUTZ-3 as a model system for DC biology .................................................................... 43
   Prospects and pitfalls with MUTZ-3 as a DC model in allergy research ...................... 45

CONCLUDING REMARKS AND FUTURE PERSPECTIVE ............................................. 47

ACKNOWLEDGEMENTS .................................................................................................... 51
REFERENCES ...................................................................................................................... 55
ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-VI)


*Scand J Immunol* 2003 Apr;57(4):305-10

*Clin Exp Allergy* 2005 Feb;35(2):199-206

*Int Immunol* 2005 Mar 3; [Epub ahead of print]

V Larsson K, Lindstedt M and Borrebaek C. Functional and transcriptional profiling of a differentiated MUTZ3 myeloid cell line: A model system for DC biology in allergic inflammation. 
*Submitted* 2005

VI Lindstedt M, Lundberg K and Borrebaek C. Gene family clustering identifies functionally associated subsets of human *in vivo* blood and tonsillar dendritic cells. 
*Submitted* 2005

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# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Ab</td>
<td>Antibody</td>
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<tr>
<td>Ag</td>
<td>Antigen</td>
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<td>AHR</td>
<td>Airway hyperresponsiveness</td>
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<tr>
<td>APC</td>
<td>Antigen presenting cell</td>
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<tr>
<td>BCR</td>
<td>B cell receptor</td>
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<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
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<tr>
<td>CI</td>
<td>Calcium ionophore</td>
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<tr>
<td>CLP</td>
<td>Common lymphoid progenitor</td>
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<tr>
<td>CMP</td>
<td>Common myeloid progenitor</td>
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<tr>
<td>DC</td>
<td>Dendritic cell</td>
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<tr>
<td>ECP</td>
<td>Eosinophil cationic protein</td>
</tr>
<tr>
<td>EPO</td>
<td>Eosinophil peroxidase</td>
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<tr>
<td>FACS</td>
<td>Fluorescence activated cell sorter</td>
</tr>
<tr>
<td>FDC</td>
<td>Follicular dendritic cell</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>GC</td>
<td>Germinal center</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage-colony stimulating factor</td>
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<tr>
<td>HEV</td>
<td>High endothelial venule</td>
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<tr>
<td>HPC</td>
<td>Hematopoietic progenitor cell</td>
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<tr>
<td>IDC</td>
<td>Interdigitating dendritic cell</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LC</td>
<td>Langerhans cell</td>
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<tr>
<td>LN</td>
<td>Lymph node</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>MACS</td>
<td>Magnetic-activated cell sorting</td>
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<tr>
<td>MALT</td>
<td>Mucosa-associated lymphoid tissue</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MoDC</td>
<td>Monocyte-derived DC</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>OVA</td>
<td>Ovalbumin</td>
</tr>
<tr>
<td>PAMP</td>
<td>Pathogen-associated molecular pattern</td>
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<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
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<tr>
<td>PDC</td>
<td>Plasmacytoid dendritic cells</td>
</tr>
<tr>
<td>PGE2</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>PHA</td>
<td>Phytohemagglutinin</td>
</tr>
<tr>
<td>PMA</td>
<td>Phorbol myristate acetate</td>
</tr>
<tr>
<td>TCR</td>
<td>T cell receptor</td>
</tr>
<tr>
<td>Th cell</td>
<td>T helper cell</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<tr>
<td>Treg</td>
<td>Regulatory T cell</td>
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<tr>
<td>TSLP</td>
<td>Thymic stromal lymphopoietin</td>
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<tr>
<td>Wt</td>
<td>Wild-type</td>
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INTRODUCTION

The hematopoietic cells of the immune system serve as our defense against harmful foreign macromolecules and invading pathogens, such as viruses, fungi and bacteria. They operate in a delicate way with highly individual tasks, making them specialists in their field. The immune system is divided into two arms, the innate and the adaptive immune response, and both are essential for survival in a world overwhelmed with microbial pathogens. Innate immunity comprises the first line of defense and covers several components; physical barriers (skin, mucosa), physiological factors (pH, temperature), protein secretion (lysozyme, complement) and phagocytic cells (macrophages, neutrophils). The cells of the innate immune system are triggered immediately upon recognition of foreign harmful antigens and have the possibility to either engulf microorganisms or release bactericidal molecules that kill the organism directly. The second defense system, the adaptive immunity, consists of cells with a refined capacity to recognize and battle reoccurring antigens. The activation of these specialized T and B cells is slower, but on the other hand results in the development of long-term specific immunological memory.

Dendritic cells (DC), the central player of this thesis, act as the gatekeeper between the innate and adaptive immune system. The primary function of these cells is to maintain tolerance to harmless antigens and self molecules under immunological steady-state conditions, and trigger immunity in the presence of danger in terms of invading pathogens or other harmful antigens. DCs are an important part of the innate immune system, since they upon trigger rapidly produce large amounts of cytokines and other mediators, which either directly activate cells in the vicinity or attract other cells to the site of inflammation. Their outstanding ability to present antigen and selectively respond to various pathogens and environmental factors, with a subsequent quality control and fine-tuning of the cellular communications, clearly demonstrate their deterministic role in the adaptive immunity.

The maintenance of balance between tolerance and immunity is a complex process that can easily be disturbed. In some cases, priming of the immune system by DCs is an adverse effect and consequently, they play an essential role in many human disorders such as allergy, autoimmune- and inflammatory diseases. The reason for breakdown of immunological tolerance in these diseases is unknown, however, recent findings clearly point to the involvement of DCs. DCs display a capacity to orchestrate the decision-making process in Th1 or Th2 development. In allergy, it is the mounting of a Th2 response that cause the detrimental effects of e.g. induced IgE production and mast cell degranulation. Even if Th2 cells and its products induce many of the pathogenic features of allergic disease, they still depend on the instruction from antigen-presenting DCs to develop and act. DCs provide the first step of immune recognition of allergens and thus represent the decision maker of the immunological outcome. Hence, in the absence of activation signals, allergen-challenged DCs induce the proper T cell unresponsiveness and
tolerance. In contrast, in the presence of these signals, the outcome of TCR-ligation may instead result in induction of T cell activation, division and Th2 differentiation. Understanding the basis of DCs in these immunological processes may be key for novel therapeutic approaches of allergic disease.

Knowledge in DC biology has been impeded by the scarcity of DCs in vivo and difficulties in DC isolation, especially in the human system. Nevertheless, much has been gained with the development of in vitro DC models, even though the gathered information needs to be validated in vivo.

The work presented in this thesis deals with aspects of how the function and transcription of human DCs are regulated in the inflammatory immune response, with emphasis on their role in allergic settings (Paper I, III and IV). This thesis also cover transcriptional evaluations of two in vitro models for DC studies, which will be used further to assess the role of DCs in the immune recognition and response to allergens (Paper I and V). Last but not least, we present transcriptional and phenotypical characterization of the in vivo DC subsets in peripheral blood and tonsillar tissue (Paper II and VI).
HUMAN DENDRITIC CELLS

Definition and origin

Dendritic cells originate from CD34+ hematopoietic progenitor cells (HPC) in the bone marrow. The CD34+ cells differentiate into common lymphoid progenitors (CLP) and common myeloid progenitors (CMP), which may divide the DC family of subset into either lymphoid or myeloid DCs (Liu, 2001). Differentiation of CMPs results in either the immature interstitial CD11c+/CD1α DC subset localized in the skin dermis or the CD11c+/CD1α+ Langerhans cells which reside in the skin epidermis (Fig. 1). CMPs also give rise to monocytes, which serve as an additional DC precursor, whereas CLPs seem to differentiate into only one DC subset, the plasmacytoid DCs (Liu, 2001). However, the origin of plasmacytoid DCs (PDC) is still controversial and supportive observations have been provided for both a lymphoid and myeloid origin (Galibert et al., 2001). On one hand, CD123+ (IL-3Rα+) PDCs can be generated from M-CSF-R+/CD34+ myeloid precursors stimulated with IL-3 and GM-CSF in vitro (Olweus et al., 1997), which support a myeloid origin. Also, early plasmacytoid dendritic cell leukemia/lymphoma has been shown to express myeloid markers such as CD33 (Giacoumidis et al., 2004). At the other end, PDCs express lymphoid-related markers like CD7, pre-TCRα and CD3e. Also, ectopic expression of the transcriptional inhibitor Id3 blocks PDC, as well as T and B cell, development but not NK and CD14+ cell development, supporting a common lymphoid origin of PDCs, T and B cells (Spits et al., 2000). Also, the MHC class II machinery in PDCs is more comparable to the one in lymphoid cells, as PDCs expression of CIITA rely on the B cell promoter pIII, whereas myeloid DCs depend on pI (LeibundGut-Landmann et al., 2004). In addition, there seems to be a high degree of plasticity in DC development. Recently, it was shown that CD123+ PDCs can be subdivided into subsets exhibiting either lymphoid or myeloid characteristics, and it was suggested that PDCs can undergo cell fate conversion from lymphoid to myeloid cells both phenotypically and functionally (Comeau et al., 2002).

A major breakthrough in the field of DC biology occurred in 1992, when in vitro culture systems were identified which could be used to generate large numbers of dendritic cells (Caux et al., 1992; Inaba et al., 1992). To date, two culture systems have mainly been used for the generation of human myeloid DCs in vitro. Firstly, human in vitro DCs can be generated from peripheral monocytes in the presence of GM-CSF and IL-4 into monocyte-derived DCs (MoDC) (Sallusto and Lanzavecchia, 1994) or, secondly, by culturing CD34+ haematopoietic progenitors from cord blood (Caux et al., 1992) or bone marrow (Reid et al., 1992) with GM-CSF and TNF-α. Many versions of these protocols have appeared since, further emphasizing the potential of monocytes and CD34+ cells to differentiate into several DC subtypes. For instance, adding TGF-β to the cytokine cocktail results in DC differentiation towards cells with features of Langerhans cells (LC), such as expression of Langerin (Geissmann et al., 1998; Ratzinger et al., 2004).
Subtypes, phenotypes and distribution

Dendritic cells are distributed throughout the human body, patrolling virtually every tissue and organ for the presence of antigen. The human DC network is heterogeneous and only limited data are available about DCs isolated from different human tissues (Bendriess-Vermare et al., 2001; McIlroy et al., 2001; Summers et al., 2001; Vandenabeele et al., 2001) and lymph (Hunger et al., 2001). The majority of studies on DCs in vivo are performed in mice, for understandable reasons such as scarcity of human biomaterial and ethical considerations concerning in vivo human experiments. However, several DCs populations have been characterized and described in human tonsils and peripheral blood (MacDonald et al., 2002b; Summers et al., 2001). Even though it is uncertain whether the pool of DCs in various tissues represent distinct subpopulations or if a limited number of bone marrow-derived subpopulations can become specialized depending on the local tissue environment, there are still some general distinctions regarding phenotypical properties and distribution of human DC subpopulations.

Circulating DCs constitute heterogeneous populations of developing DCs on the move from the bone-marrow to peripheral tissues (Fig. 1). Various types of DCs have been characterized in human blood and, hitherto, at least five distinct subpopulations, consisting of the myeloid CD34+, CD16+, BDCA1+, BDCA3+ and the plasmacytoid CD123+ DCs, are believed to populate the circulatory system (MacDonald et al., 2002b). The transcriptional and phenotypical differences of these subsets are addressed in Paper VI.

Langerhans cells (LC) are located above the basal layer of epithelial cells in the oral, nasal and pulmonary mucosa, as well as the epidermis of skin, and are characterized by the presence of a unique cytoplasmic organelle, the Birbeck granule, and the associated C-type lectin Langerin (Valladeau et al., 2000). LCs acquire their characteristics upon localization in tissue and are not detectable in blood (MacDonald et al., 2002b). They are specialized at collecting antigen in the periphery (Fig. 1), including self-antigen, and carry processed peptides to the draining lymph nodes for T cell presentation (Romani et al., 2003). LCs express E-cadherin, CD1a, CD68 and common myeloid antigens, such as CD11b, CD11c and CD33. LCs use a diverse repertoire of receptors for endocytosis of foreign antigens, in particular Fcγ/Fce receptors and DEC205 (Girolomoni et al., 2002). In a model of Candida albicans-infected skin, increased numbers of LCs were detected in dermis, in comparison to normal skin (Katou et al., 2000), which suggests a role for LCs in the specific recognition of antigen. The dermal LCs, probably migrated from C. albicans-challenged epidermis, expressed CD83 and CD86 and were in close contact with memory CD4+ T cells. In contrast to the activation in the periphery, it was recently shown in chronically inflamed human skin that CD83+ immature LCs also are recruited in afferent sinuses and T cell areas in draining lymph nodes, indicating that migration and maturation can be independently regulated events (Geissmann et al., 2002).

Interstitial DCs is a collective term for all immature DCs located in the interstitial spaces of virtually all human organs and tissues drained by the afferent
lymphatics, such as heart, liver, lung, intestine and other mucosal surfaces. Many different interstitial cells have been characterized and whether they represent one unique cell lineage or many different is still unknown. A mature population of DCs (Langerin-/DC-LAMP+/CD83+), probably representing the interstitial DCs from dermis, also accumulate in lymph nodes draining inflamed skin along with immature DCs (Geissmann et al., 2002). Dermal/interstitial DCs and LCs share many myeloid phenotypic markers, such as CD11c and CD33, but many functional and phenotypical differences have recently been described. For instance, dermal DCs, in contrast to LCs, express DC-SIGN (Ebner et al., 2004). Also, HPC-derived dermal DCs were shown to directly stimulate NK cell proliferation and cytotoxicity, whereas LCs required exogenous IL-2 or IL-12 (Munz et al., 2004). In contrast, LCs proved superior in stimulating cytolytic T lymphocyte activity against a recall viral antigen as compared to dermal DCs (Ratzinger et al., 2004).

**Figure 1.** DC subsets and lifecycle. FDC, follicular dendritic cells; GCDC, germinal center DCs; mDCs, myeloid DCs

*Veiled DCs* are located in the afferent lymphatics, which transport antigen from the periphery to the draining lymph nodes (Moser, 2003). They represent a heterogeneous population of cells, e.g. Langerhans cells and interstitial DCs, with a sheet-like morphology. In addition to the maturing antigen-experienced DCs, the
veiled DCs observed in human cannulated afferent lymph (Hunger et al., 2001) most likely also consist of steady-state migrating DC loaded with tissue antigens with a mission to induce tolerance in the regional LNs (Gad et al., 2003). For instance, a distinct DC population has been described in vivo in rat that constitutively endocytoses and transports apoptotic intestinal epithelial cells to the T cell areas of mesenteric lymph nodes in the absence of infection, suggesting a role for these DCs in maintaining self-tolerance (Huang et al., 2000).

Figure 2. Cytospins of dendritic cells isolated from human tonsils and stained with antibodies specific for HLA-DR (red) and IFN-α (green).

Plasmacytoid DCs (PDC) represent a DC subset of various names, such as CD11c+ DCs, naturally IFN-producing cells, DC2 and plasmacytoid monocytes/T cells, based on their phenotype and specialized function (Briere et al., 2002). PDCs are rare and only found immature in normal skin, in small clusters of up to 5 cells beneath the basement membrane of the epidermis (Ebner et al., 2004). They constitute approximately 20% of the total numbers of DCs in blood and 50% of the DCs in tonsils (unpublished observations). Unstimulated and fresh isolated from blood or tonsils, their morphology is more comparable to lymphocytes than the myeloid DCs (Fig. 2). Two new PDC-specific markers have been identified which simplifies their isolation: the c-type lectin and antigen receptor BDCA2, and the neuronal receptor BDCA4 (Dzinek et al., 2000; Dzinek et al., 2002). Unlike blood myeloid DCs, they produce high levels of type I IFNs (-α and -β) in response to viral stimulation (Cella et al., 1999; Siegel et al., 1999). PDCs express a different spectrum of toll-like receptors (TLRs) compared to myeloid DCs, which makes them specialized toward a different repertoire of microbial compounds than myeloid DCs (Briere et al., 2002). PDC express TLR1, 6, 7, 9 and 10, whereas myeloid DCs express TLR1, 2, 3, 4, 6, 7 and 8. Other features of PDCs include spontaneous death in culture, if not rescued by IL-3, and poor ability to phagocytose latex-beads and endocytose FITC-dextran (Grouard et al., 1997). PDCs are able to prime naive T cell responses toward either Th1 or Th2 effector responses, depending on the stimulation (Cella et al., 2000; Rissoan et al., 1999). Recently, it was demonstrated that human blood PDCs and CD11c+ DCs, activated
with influenza virus, exhibited equivalent ability to expand specific CTLs and CD4+ Th1 cells (Fonteneau et al., 2003), pinpointing a prominent role for PDCs in the adaptive immunity against viruses. Adding to the complexity, it was recently shown that PDCs from peripheral blood can be subfractionated into lymphoid-like and myeloid-like plasmacytoid cells, exhibiting diverse phenotypes and different allostimulatory capacity (Comeau et al., 2002). The authors speculate that PDCs, rather than forming a separate DC lineage, represent a population of lymphoid cells undergoing conversion from lymphoid to myeloid cell types.

Interdigitating DCs (IDC) constitute a heterogeneous mix of various DC subtypes, located in the T cell areas of secondary lymphoid organs (Steinman et al., 1997). CCR7 expression enables selective recruitment of some of these DCs into the T cell zone in response to the chemokines CCL19 (ELC), produced by other DCs, and CCL21 (SLC), expressed by the cells of the high endothelial venules (HEVs) (Cyster, 1999). Immunohistological analysis shows, for instance, that DC-LAMP is an IDC-specific marker, which can be used to separate IDCs from FDCs and GCDCs (Fig. 3A). Summers et al. phenotypically defined the different IDCs in tonsillar tissue based on their expression of HLA-DR, CD11c, CD123 and CD13, and reported that four distinct populations could be identified in the T cell zone (Summers et al., 2001).

Germinal Center DCs (GCDC) is the only DC subtype, apart from follicular dendritic cells (FDCs), which is present in the B cell follicle. They were identified in 1996 as a subset of CD11c+ cells located in close contact with T and B cells in germinal centers (GC) (Grouard et al., 1996) (Fig. 3B). GCDCs had a high stimulatory capacity for T cells and could be separated from CD71+ tingible body macrophages and CD40high IDCs. The subset has been described to drive plasma B cell differentiation and isotype switching towards IgG1 (Dubois et al., 1999). However, these cells were FACS sorted based on the phenotypic characteristics of CD4+/CD11c+/CD3-/CD20-/CD1a-, with the depletion of CD40high cells. This isolation procedure may have enriched for a mixture of myeloid DCs in tonsils, since GCDCs later were shown to express similar levels of CD40 as IDCs (Summers et al., 2001). Other GCDC products have been identified based of immunohistochemical stainings, e.g. DC-CK1 (Lindhout et al., 2001; Summers et al., 2001), however there is still no marker identified that is expressed exclusively by GCDCs. In conclusion, there seems to be little evidence that GCDCs represent a separate DC subtype in GCs.

Follicular dendritic cells (FDC) are restricted to the primary and secondary lymphoid follicles (Fig. 1 and 3C) and are believed to play an essential role in GC formation in addition to selection, differentiation and maintenance of memory B cells (van Nierop and de Groot, 2002). They are stromal cells of the connective tissues and originate from mesenchymal precursors. FDCs differ from the bone-marrow-derived DCs as they do not internalize and process antigens, neither do they present antigenic peptides on self-made MHC class II molecules to T cells. They do, however, carry MHC class II-expressing microvesicles, so-called exosomes, on their surface, possibly produced by B cells, which may play a role in the fine tuning of the immune responses (Denzer et al., 2000). FDCs form a large
network throughout the follicle with their long cytoplasmic extensions that trap antigen in immune complexes, so-called iccosomes, with their abundant complement receptors and FcRs, e.g. FDC-specific CD21 long isoform (DRC-1) and FcγRIIB (van Nierop and de Groot, 2002). Potential memory B cells with high affinity B cell receptors are rescued from apoptosis in the GC, provided they bind naive antigen presented by FDCs. Recently, FDCs were shown to produce CXCL13 (BCA-1), which specifically attracts the CXCR5+ follicular T cells (Estes et al., 2004). In Paper II, we describe that FDCs also express CD137, a co-stimulatory molecule of the TNF-family which, upon receptor binding, can transmit signals leading to differentiation and activation of T cells (Vinay and Kwon, 1998). The CD137 expression on FDCs may serve as an additional way that FDCs use to interact with CD137L+ GC B cells.

**Figure 3.** Immunohistochemical staining of *in vivo* DC subtypes and FDCs. A. Myeloid DC-LAMP+ DCs (green) are located in the T cell zone, separated from the CD137+ FDCs (red) in the germinal center. B. In contrast, CD11c+ (green) DCs are present in both T cell zone and germinal center. C. DRC-1+ (green) FDCs reside exclusively in the follicular area.

Lifecycle and migration

Production of DCs during hematopoiesis occurs independently of antigen and in a steady-state manner (Liu, 2001). The various DC precursors continuously migrate from the bone-marrow into the blood circulation where they constitute a sparse population, steering for the peripheral tissues. Transendothelial migration and recruitment of immature DC to peripheral tissues is mediated by the expression of several inflammatory mediators. Among the chemotactic factors that have been suggested to participate in selective migration of DCs in non-lymphoid tissues are platelet-activating factor (PAF), fMLP, and C5a (Sozzani et al., 1997), MIP-3α (Dieu-Nosjean et al., 2000), β-defensins, possibly through CCR6 (Yang et al., 1999), and CCL22 (Katou et al., 2001). In addition, there is a constant turn-over of DCs in the periphery in the absence of inflammatory signals. Under steady-state conditions, LCs can linger in the skin for months before they are replaced (Merad et
al., 2002). Inflammatory signals, however, induce migration of LCs to LNs and the recruitment of new circulating LC precursors, a process dependent on secretion of CCR2-binding chemokines in the skin.

At an immature stage, DCs act as the sentinels of the periphery, sampling the environment for self and nonself antigens, such as dead cells, cellular debris and microorganisms. Uptake can occur via pinocytosis, phagocytosis or receptor-mediated endocytosis, through numerous receptors for specialized antigen uptake, such as members of the C-type lectin-family (DEC205, DC-SIGN, Dectin-2, mannose receptor) (Figdor et al., 2002) and FcRs (Moser et al., 2002). Immature DCs express a number of chemokine receptors, such as CCR1, CCR2, CCR5, CCR6 and CXCR1, which they use to remain in the peripheral tissues or home to sites of inflammation (Sallusto and Lanzavecchia, 2000; Steinman, 2003). Depending on the antigenic properties, DCs may start a maturation process with concomitant migration to LNs. A complete switch in the functional properties of DCs occurs during maturation and they change from being perceptive, endocytosing cells to being proficient in presenting antigen in the context of MHC class I and II. The functional changes are preceded by a comprehensive transcriptional reprogramming, as demonstrated in Paper I.

However, the migration of DCs to lymphoid tissues under homeostatic conditions is a process that appears not to require maturation. A functional aspect of this immature DC migration, separated from homing of DCs loaded with antigens from the periphery, is the fact that DCs also need a route to locally acquire soluble antigen which reach LNs though leakage out of conduit networks from the afferent lymphatics (Itano and Jenkins, 2003). An essential mediator for entry of skin DC into the lymphatic vessels within the dermis during homeostasis is the CCR7 chemokine receptor (Ohl et al., 2004). Accordingly, DCs in CCR7-deficient mice fail to migrate from the skin into the regional LNs (Forster et al., 1999). Activation and maturation of DCs in the periphery by danger signals such as bacterial or viral products induce immunity in the regional LNs, whereas DCs loaded with self and other nonself antigens migrate in an immature state and induce tolerance by triggering anergy or apoptosis in T cells (Hawiger et al., 2001). It is also suggested that tolerogenic DCs, in a “semi-mature” state loaded with with self-peptide/MHC and expressing costimulatory molecules but lacking signals for induction of immunity, induce IL-10+ CD4+ T regulatory cells (Tregs) (Lutz and Schuler, 2002). Pulmonary DCs exposed to respiratory antigens have been shown to transiently produce IL-10 and stimulate the development of IL-10-producing regulatory T cells (Treg), which may be an additional route for the maintenance of tolerance (Akbari et al., 2001). This process was further evaluated in a model of allergen-induced airway hypersensitivity and was demonstrated to involve ICOS ICOS-ligand costimulation between T cells and DCs (Akbari et al., 2002).

The mechanisms of migration used by DCs to enter LNs from peripheral tissues depend not only on the maturation status of DCs but also on specific DC subtype. PDCs are recruited to draining LNs either from the afferent lymphatics or directly from the circulation through high endothelial venules (HEV), in a CXCL9 and E-selectin dependent manner (Yoneyama et al., 2004). In contrast, neither
immature nor mature myeloid DCs have been shown to enter LNs through HEVs, suggesting distinct functional properties of PDC in LNs. In addition to CCR7, several chemotactic mediators have been shown to participate in the migration of various DCs to LNs, such as osteopontin for LCs (Weiss et al., 2001). DCs derived from monocytes use the CCR7 and CCR8 pathways during mobilization from skin to LNs (Qu et al., 2004). Once inside the LN, chemokines dictate the appropriate localization of DCs and lymphocytes. For instance, a subpopulation of activated skin DCs expressing CXCR5 migrates predominantly to the B cell area where FDCs express the ligand CXCL13 (Saeki et al., 2000; Wu and Hwang, 2002). The chemokine SLC (CCL21) mediates the entry of naïve T cells and antigen-stimulated DCs, both expressing the receptor CCR7, into the T cell zones of secondary lymphoid organs (Gunn et al., 1999). Within LNs, DCs in the T cell zone constitutively produce CCL19 (ELC), which is strongly chemotactic for naïve CCR7+/CD4+ T cells (Ngo et al., 1998).

The new technology of two-photon laser microscopy (Denk et al., 1990) enables time-resolved, 3-dimensional imaging of single cells within intact tissue. This exciting technique has been used to image migrating DCs, actively participating in the immune responses, in intact lymph nodes (Lindquist et al., 2004; Miller et al., 2004a). Mature DCs that arrive in lymph nodes from tissues were found to be more motile than steady-state DCs, and were rapidly dispersed and arranged in a network throughout the T cell area, possibly to enable maximum antigen-specific T cell contact (Lindquist et al., 2004). The incoming DCs were mainly found at the boundary between the B and T cell zones. DCs were estimated to make approximately 5000 T cell contacts per hour, each lasting ~3 min, and the encounters occurred preferentially on DC dendrites many microns from the DC body, minimizing steric hindrance among T cells (Miller et al., 2004a).

Once DCs have fulfilled their mission to contact antigen-specific T cells, they are believed to die by apoptosis in the lymphoid tissues (Matsue and Takashima, 1999). This controls the induction of immune responses, as it limits the availability of antigen for T cells. Recently, it was demonstrated that DC turnover and function in the LNs are controlled by two pathways; a survival pathway involving Bcl-XL and a molecular timer dependent on the antiapoptotic factor Bcl-2 (Hou and Van Parijs, 2004). The Bcl-XL-dependent survival pathway was induced after 24 h of stimulation by components from the innate (TLRs) and acquired (CD40L and TRANCE) immune systems, whereas the Bcl-2-dependent pathway was induced at later time points (3–4 days) by components of the innate but not adaptive system and limited the lifespan of cells activated by TLR ligands.

DC maturation

In order to break down tolerance, DCs need signals of danger to fully mature. DCs become activated by inflammatory mediators produced by tissue damage, by microbial ligands through their ingenious repertoire of pattern-recognition receptors (PRRs), such as the TLRs, or by direct interaction with other cells through cell surface receptors, e.g. CD40 or CD137 (Gad et al., 2003). During maturation, DCs
functional and phenotypical features changes dramatically, e.g. their ability to capture antigen diminish and their T cell stimulatory function increase. The extensive transformations comprise e.g. upregulation of immunogenic peptide/MHC complexes, costimulatory molecules (CD80, CD86), TNF family members (CD137L, OX40L, CD70), chemokine receptors (CCR7), release of cytokines and chemokines, and morphological changes that enables effective interaction with T cells (Steinman, 2003). Huang et al. performed an extensive study of the gene regulation in DCs after stimulation with various pathogens, such as *E. coli*, *C. albicans* and influenza virus (Huang et al., 2001). Specific transcription of 286-685 genes in response to each pathogen was reported, in addition to a core response of 166 genes induced by all three pathogens. In Paper I, we describe the transcriptional response of DCs to pro-inflammatory cytokines and present data of the global and time-ordered reprogramming of DCs during their course of maturation. The transcriptional reorganisation may reflect the effect of pathogen-induced inflammation on DC maturation in tissues, since up to 70% of the pathogen-induced transcripts found by Huang et al. also were found in the DCs stimulated by inflammatory mediators. A majority of these core response genes can be regarded as general activation genes, such as IL-1β, TNF-α and IL-6, which we suggest act in an autocrine fashion to enhance the activation. The endogenous mediators released in pathogen-infected, inflamed or damaged tissue, in addition to the direct information received from pathogens through e.g. TLRs thus play an instructive role for DCs. As shown in Paper IV, DC maturation can also be induced by effector T cells, including those DCs that do not carry specific antigen. However, T cell induced DC maturation in the absence of specific antigen does not initiate IL-12 cytokine production (Sporri and Reis e Sousa, 2003), ensuring that T cells themselves do not prime for T helper differentiation.

**Functional plasticity of DC subtypes and the concept of DC1 and DC2**

In 1999, DC subsets were suggested to be predetermined to polarize T cells to Th1 and Th2 cells, based on their cytokine production and stimulatory capacity (Rissoan et al., 1999). These Th1- and Th2-priming DCs were called DC1 and DC2, respectively. DC1s (MoDCs) were shown to produce large amounts of IL-12 after CD40L activation, whereas DC2s (PDCs) did not. MoDCs induced IFN-γ production in allogeneic naive T cells in contrast to PDCs which induced IL-4, IL-5 and IL-10 production. Also, mediators that selectively expand DC1 or DC2 *in vivo*, such as GM-CSF or Flt3L, were shown to influence Th1 or Th2 responses generated *in vivo* (Pulendran et al., 1999). The view that certain DC populations are programmed for unique functions was later modified, as the flexibility of DC subsets became clear (see below), and the concept of DC1/DC2 was extended to generally refer to Th1- and Th2-priming DCs, irrespectively of subset. However, the restricted IL-12 production observed in humans DC1s raised the question of which DC2-produced factors influence the character of Th2 differentiation, and whether the inability of DC2s to produce IL-12 is sufficient to drive Th2 (Moser and Murphy, 2000). The concept of a third signal necessary to drive Th1 or Th2 was
suggested by Kapsenberg’s group, as different DC subsets were shown to exert specialized functions depending on the local environment and antigen encountered (de Jong et al., 1999). DCs respond to triggering signals in a highly specific fashion and in a sense determine the quality of the immune response. Recognition of certain antigens, such as intracellular pathogens (bacteria, viruses and protozoa) (Cella et al., 2000; Palucka and Banchereau, 2002), enables DCs to polarize T cells towards the Th1 pathway, whereas other stimuli, such as schistosomes (MacDonald et al., 2002a), filarial nematodes (Whelan et al., 2000), histamine (Gagliardi et al., 2000) or thymic stromal lymphopoietin (TSLP) (Soumelis et al., 2002), mediates DC-induced Th2 responses. DCs cultured in the presence of prostaglandin E2 (PGE2) or IL-10 promote Th2 polarization, whereas CD40L, lipopolysaccharide (LPS), poly I:C and IFN-γ induce Th1-polarising DCs. The overlapping functions of different subset are demonstrated by the fact that both PDCs and myeloid DCs produce IL-12 and type I IFNs, depending on the stimulatory mediators. The PDCs are by far the principal producer of type I IFNs after microbial challenge (Siegal et al., 1999). However, also myeloid DCs stimulated with either herpes simplex virus type-I or schistosoma mansoni eggs produce type I IFNs (Pollara et al., 2004; Trottein et al., 2004). In addition, cultured peripheral blood PDCs and myeloid DCs were demonstrated to produce comparable amounts of IL-12 in response to LPS and CD40L stimulation (Cella et al., 1999), in contrast to the report by Rissoan et al. mentioned above. Nonetheless, despite overlapping functions of DCs, each subtype may still exert different functional biases (Shortman and Liu, 2002). For instance, ligation of TLR7 on myeloid DCs and PDCs lead to IL-12 and IFN-α production, respectively, demonstrating that TLR7 exerts its biological effects in a DC subset-specific manner (Ito et al., 2002).

In contrast to a subset selective commitment, individual DC subsets also display a high degree of flexibility and are accessible to environmental instructions (Edwards et al., 2002). For example, LPS from various bacteria activate DCs to produce different cytokines and induce distinct types of T cell responses, where differences in TLR4 signaling for these pathogens may be accountable for these discrepancies (Pulendran et al., 2001). Ligation of various TLRs mainly promote Th1 development (Kapsenberg, 2003). However, other so far unknown PPRs may promote pathogen-induced Th2 responses (Kaisho et al., 2002). The flexibility of DCs is further demonstrated by the fact that DCs can discriminate between different forms of a pathogen and initiate both Th1 and Th2 responses by differential expression of IL-12 and IL-4 (d’Ostiani et al., 2000). For example, yeast from the fungus Candida albicans stimulate murine DCs to produce IL-12 and drive Th1 polarization, whereas ingestion of hyphae inhibits IL-12 and prime for IL-4 production by DCs. Whether the latter is a murine or pathogen-specific feature is unclear, since no other report, to my knowledge, has been published which describes IL-4-producing human DCs. The antigen dose and strength of signal can also determine the outcome of the T helper response. High levels of antigen have been shown to predominantly stimulate a Th1 response, whereas low doses of the
same antigen favour Th2, regardless of DC subset (Boonstra et al., 2003). Whether this is true in physiological situations remains to be determined.

In the transcriptional study mentioned above by Huang et al., stimulation of MoDCs with either bacteria, yeast or fungi yielded both a common core response as well as pathogen-specific responses, clearly demonstrating plasticity, at least on a transcriptional level, within one DC subtype (Huang et al., 2001). In a recent report, the ability of DCs to discriminate between microbial and helminth antigens was examined (Cervi et al., 2004). Stimulation with *Propionibacterium acnes* (Pa) induced IL-12 and Th1 differentiation when injected into naive mice, whereas *Schistosoma mansoni* eggs (SEA) induced Th2 differentiation. When copulsed with both Pa and SEA, DCs lost the ability to produce IL-12 in response to Pa, but were still capable of inducing non-overlapping Th1 and Th2 responses. The antigens were internalized by different pathways and ended up in discrete intracellular compartments; Pa in LAMP2+ and SEA in LAMP2low/v- vesicles. In a recent report from the same group, the effect of SEA on TLR ligand-induced DC activation was examined in IL10−/− mice with transcriptional profiling (Kane et al., 2004). DCs were stimulated with SEA, LPS or SEA+LPS and the transcriptional activity was evaluated. SEA induced a moderate response of only 29 affected genes, whereas LPS induced 551 genes. Used in combination, SEA suppressed the activity of LPS, and had a negative effect on the transcription of e.g. *IL-12h, OX40L, CD83, Bcl10* and *Ccl3*. SEA did not directly induce DC maturation, based on analysis of maturation markers, but induced changes in expression in a small number of genes. It is currently unclear how any of the genes might confer the properties accompanied with SEA-exposure, such as the Th2 polarizing ability. Interestingly, these results are in line with our observations from the studies on allergen-stimulated DCs (Paper III and IV), where transcriptional activity were noted despite lack of DC maturation.
DC/T CELL INTERPLAY

T cell development and migration

T cells originate from a lymphoid CD34+ progenitor in the bone-marrow and migrate early in development in small numbers as T cell precursors to the thymus where all the important events during their development occur, such as proliferation, differentiation and rearrangement of the T cell receptor (TCR) gene loci (Rothenberg et al., 2003). Naive CD4+ T cells that survive the selection in thymus enter the blood stream and circulate continuously through the blood, lymph, and secondary lymphoid tissues where they scan the environment for dendritic cells expressing peptide-loaded MHC molecules (Reinhardt et al., 2001). Naive, as well as central memory T cell traffic into lymphoid organs is controlled in part by CCR7 (Forster et al., 1999) and occurs via HEVs that serve as doorways from the blood stream. It is estimated that a naive T cell on average recirculate for months before a specific interaction occurs which allows embarkment to effector cell differentiation (Jenkins, 2003). T cell clonal expansion is induced by the recognition of peptide-MHC and appropriate co-stimulatory molecules, after which the differentiated T cells either migrate out of the lymphoid organs to sites of inflammation or to B cell areas where they provide help for B cells. After contact with antigen-bearing DC in the lymph node, naive T cells become activated and starts migrating rapidly (Stoll et al., 2002). After 2-4 days they become visible in the efferent lymphatics. Most naive T cells that enter LNs do not find their specific antigen, and therefore continue to recirculate after a period of 12-18 h (Cyster, 2004). The exit of both naive and memory T lymphocytes from peripheral lymphoid organs and the return to circulation are dependent on expression of sphingosine-1-phosphate receptor 1 (S1P1) (Matloubian et al., 2004).

Differentiation of CD4+ T cells following specific TCR-MHC/peptide contact occurs in several transient steps. The real-time behavior of naive CD4+ T cells and in vivo-labeled antigen-carrying DCs was recently imaged using two-photon microscopy (Miller et al., 2004b). T cells and DCs made short-lived contacts in the first 2h of entry into LNs, with each interaction lasting an average of 11-12 min and mainly occurring on dendrites. The next stage was formation of dense T cell clusters around DCs, a dynamic process characterized by increased duration of interaction. Thereafter, the clusters dissociated and T cell motility increased, a stage which was followed by proliferation and only infrequent, brief contacts with DCs were made. By 40h, most T cells had divided at least once and were spread out in the T cell zone. A similar pattern of interaction was also demonstrated for CD8+ T cells responding to migratory DCs with transient serial engagements during the first activation phase, stable contacts during phase two and high motility and rapid proliferation during phase three (Memper et al., 2004).

After DC-T cell interactions and depending on the repertoire of costimulatory molecules and other mediators present in the environment, CD4+ cells differentiate into either effector or central memory cells (Sallusto et al., 2004).
Some CD4+ T cells develop into follicular helper cells and migrate into the B cell area, possibly through upregulation of CXCR5 (Cyster, 2004). Depending on the stimulus, the level of antigen-specific T cells in the LNs during primary immune responses reach its peak 3-7 days after antigen exposure (Jenkins, 2003). Activated effector memory T cells selectively migrate from the local secondary lymphoid organs to tissues where the antigen was first encountered (Dudda and Martin, 2004). Thus, CD4+ T cells activated in cutaneous lymph nodes upregulate P-selectin ligand for migration to the skin, while those responding to antigen in the intestinal lymph nodes selectively express α4β7 for homing to the intestinal lamina propria (Campbell and Butcher, 2002). Upregulation of α4β7 on CD8+ T cells were shown to be mediated by DCs from Peyer’s patch, but not by DCs from spleen or peripheral LNs, suggesting that DCs authorize the T cells to access anatomical sites (Mora et al., 2003). Shortly thereafter, it was demonstrated that the presence of matured DCs was required for establishing T cell gut tropism, and that CCR9 too played a critical role for homing to the gut (Johansson-Lindbom et al., 2003b). Recently, it was shown in vitro that memory CD8+ T cells with an already committed skin-homing phenotype can be stimulated, by gut-derived DCs, to replace their skin-homing molecules by gut-homing molecules, and vice versa, demonstrating that the most recently encountered DCs dictate the T cell homing (Mora et al., 2005). Whereas activated T cells in the LNs undergo clonal expansion and migrate to peripheral tissues in response to ag challenge, the ag-experienced T cells in the nonlymphoid tissues cease to divide and become unable to migrate back to the LNs (Harris et al., 2002).

**T cell memory**

Based on the expression of CCR7, the memory pool of human CD4+ T cells can be divided into two functionally distinct subsets: the CCR7+ effector memory (T_{EM}) and CCR7- central memory (T_{CM}) subset (Sallusto et al., 1999). The T_{EM} express receptors needed for homing to peripheral tissues, and exert immediate effector functions, whereas T_{CM} express CD62L, needed for homing to LNs, and lack immediate effector functions. The T_{CM} population is predominant in human blood (Sallusto et al., 2004). They display phenotypic similarities with naive T cells but are more responsive to TCR stimulation than naive cells and can undergo terminal differentiation upon restimulation with antigen (Lanzavecchia and Sallusto, 2000). Local cytokine milieu, TCR affinity, duration of TCR stimulation, antigen load and costimulatory molecules are factors that determine the outcome of T cell differentiation (Sallusto et al., 2004). The T_{CM} population is suggested to arise from an intermediate stage, preceding the T_{EM} population, which are generated by subthreshold stimulation. The T_{EM} subset might arise early in the immune response, when antigen load is high and DCs secrete large amounts of polarizing cytokines, whereas the T_{CM} subset may arise later in the response when DC numbers and antigen load is low and DCs have exhausted their cytokine-producing capacity.

The T_{EM} pool contains both Th1 and Th2 cells, distinguished by the chemokine receptors CCR5 and CXCR6 (Th1) and CRTH2 and CCR3 (Th2). It
was recently demonstrated that CXCR3, CXCR5 and CCR4 could identify functional subsets within the human CD4+ T<sub>CM</sub> pool (Rivino et al., 2004). The CXCR3<sup>+</sup> and CCR4<sup>+</sup> cells represented pre-Th1 and pre-Th2 cells, respectively, which differentiated into effector Th1 and Th2 cells spontaneously in response to the homeostatic cytokines IL-7 and IL-15. In contrast, CXCR5<sup>+</sup> cells were nonpolarized cells that required TCR triggering and signalling by polarizing cytokines to differentiate. In humans, T<sub>CM</sub> and T<sub>EM</sub> subsets differ in their turnover rates, with disappearance rate of 4.3% and 11.3% per day, respectively, demonstrating that the T<sub>EM</sub> population represents a more short-lived population (Macallan et al., 2004). In comparison, the disappearance rate for naive T cells was too small to estimate.

Even though CCR7 is a valid marker for separating the CD4<sup>+</sup> memory cells, both CD62L (Ahmadzadeh et al., 2001; Hengel et al., 2003) and CD27 (De Jong et al., 1992; Hintzen et al., 1993) are markers that have been used by other groups, including ours, to separate effector from non-polarized memory T cells. In our laboratory, we have assessed the transcriptional activity in CD27<sup>+</sup> and CD27<sup>+</sup> memory CD4<sup>+</sup> T cell populations from peripheral blood (Schiot et al., 2004). We demonstrate that the CD27<sup>+</sup> cells represent a transcriptionally more active and differentiated effector population, based on the expression of several transcription factors (T-bet), chemokines and their receptors (CX3CR1, CCL5, CCL4), adhesion molecules (CD11b, catenin alpha 1) and activation markers (HLA-DR, HLA-DP). The memory CD4<sup>+</sup> T cells are a heterogeneous population and neither CCR7, CD62L nor CD27 define a distinct CD4<sup>+</sup> population. In peripheral blood CD4<sup>+</sup> T cells, approximately 30% of the CCR7<sup>+</sup> cells are also CD27<sup>+</sup>, and over 50% of the CD27<sup>+</sup> cells also lack CCR7 (Campbell et al., 2001). Thus, there is extreme diversity among the cells lacking these molecules, whereas a relative uniformity can be seen among the cells expressing all three of these markers together.

DC-T cell interaction

DCs are involved in every aspect of T cell responses, in initial priming of naive T cells, generation of effector and memory cells and mounting of central and peripheral tolerance. The interaction between these cells is a complex story, much dependent of the antigenic properties and induction of DC maturation, as discussed previously. The interaction is an active process which involves a spectrum of molecules and cytoskeletal rearrangements, besides the specific binding of TCR to peptide/MHC. The large-scale molecular complex built around the DC/T cell interface, the immunological synapse, involves e.g. CD28, CTLA-4, CD80/CD86, CD3, CD4, CD45 (Huppa and Davis, 2003). The initial contact requires binding of adhesion molecules, such as LFA-1, ICAM-1 DC-SIGN and ICAM-3. Another element in the initial interaction between these cells during primary responses is neuropilin-1, expressed by both DCs and resting T cells, as blocking neuropilin-1 antibodies inhibit DC clustering with resting T cells and DC-induced proliferation of resting, but not of activated, T cells (Tordjman et al., 2002). The magnitude and quality of the CD4<sup>+</sup> T cell response in vivo depend on the number of antigen-
carrying DCs and tissue inflammation (MartIn-Fontecha et al., 2003). Inflammatory triggers not only promote recruitment of immature DCs into tissues, but also boost migration of mature CCR7+ DC into LNs, by inducing upregulation of CCL21 on lymphatic endothelial cells. As discussed in Paper I, the kinetics of DC activation is also vital, since DCs early upon activation produce cytokines and mediators which may differ from those produced later during an immune response (Langenkamp et al., 2000). Thus, the time-ordered activation of DCs changes the outcome of the effector T helper responses.

Another interesting feature of DCs, which adds a level of complexity in the DC/T cell interaction, is the secretion of exosomes, small nanovesicles loaded with peptide/MHC, costimulatory molecules and various molecules which probably addresses the exosomes to target cells (Thery et al., 2002). The exosomes may enable membrane exchange between different DCs, thereby increasing the number of peptide-bearing DCs and amplification of T cell responses. Membrane structures may also be transferred from DCs to T cells, which could licence the T cells for immunoregulatory tasks.

Interestingly, a few recent reports also address the instructive role of T cells on DC function, which reveal that a reciprocal crosstalk occurs between these cells. For instance, MHC class II crosslinking on immature MoDCs promotes their maturation, with upregulation of CD80, CD86, CD83, CD40 etc, and thereby enhances their stimulatory function (Lokshin et al., 2002). On the other hand, crosslinking of MHC class II on mature DCs induced apoptosis. In Paper IV, we demonstrated that DCs, both unstimulated and P. aeruginosa-stimulated from healthy and allergic individuals, upregulated the activation markers CD80 and CD86 after T cell contact only. Thus, the gradual upregulation of costimulatory molecules during the 7-day culture period was not antigen-dependent, but was induced merely by the presence of the nearby T cells. Likewise, newly activated murine CD4+ T cells can induce an increase in DC costimulatory activity in the absence of any innate priming (Sporri and Reis e Sousa, 2003). Furthermore, they can amplify secretion of bioactive IL-12 p70 by DCs exposed to an appropriate innate stimulus but cannot initiate IL-12 synthesis by bystander DCs that do not present Ag. Therefore, DC maturation triggered by CD4+ T cells and production of polarizing cytokines, induced by other signals, seem to be two independently regulated processes. Alpan et al. suggested that DCs may function as temporal bridges of information between memory and naive T cells (Alpan et al., 2004). Memory T cells induced after oral immunization were shown to “educate” DCs, with IL-4 and IL-10 but not CD40L, which in turn induced naive T cells to produce the same set of cytokines. Moreover, ligation of soluble CD28 to CD80/CD86 on mouse DCs induced IL-6 and IFN-γ production (Orabona et al., 2004). Soluble CD28 was also demonstrated to have adjuvant activity in vivo as CTL activity increased during tumor challenge. Antifungal resistance was also enhanced by DC vaccination in combination with soluble CD28. The “education” or “licensing” of dendritic cells by CD4+ T cells was also required for CTL immunity in some situations. For instance, antigen-specific CD4+ T cell-licensing of DCs was shown to be essential for the generation of effector and memory CTLs to herpes simplex virus-1 in vivo (Smith et al., 2004).
ALLERGIC DISEASE

Characteristics of allergic rhinitis

Epidemiology
Allergic diseases are caused by a dysregulated immune response against common, ubiquitous allergens such as house dust mite, pollen or animal dander. The disorders are categorized by the organ of disease manifestation and include asthma, dermatitis, rhinitis and anaphylaxis. Allergic disease represents a major health problem in the industrial world today and the prevalence is steadily increasing. In 2000, it was estimated that up to 150 million people globally are affected by asthma alone and that the deaths from this condition has reached more than 180,000 annually (WHO, 2000). In addition, allergic disease has a substantial socio-economic impact. In 2000, the world-wide economic costs associated with asthma were estimated to exceed those of tuberculosis and HIV/AIDS combined. Allergic rhinitis is a common illness in affluent, western countries with a high degree of industrialization, affecting up to 40% of children and 30% of adults (Holgate and Broide, 2003).

The cause of allergy is still only in its infancy of being elucidated, and existing data regarding the underlying causes of rhinitis are difficult to deduce. There are supportive data for many factors which may affect the onset of allergic disease, including genetic susceptibility, environmental factors, such as microbial exposure and allergen dose, age of allergen exposure and subset/function of dendritic cells initiating the Th2 polarization. Although the genetic component is important, it seems that interactions with the environment early in life, a concept called the hygiene hypothesis, are crucial determinants for developing rhinitis (Kabesch and Lauener, 2004). Early-life exposures of microbial endotoxins, with immunological Th1 programming, may have a protective effect. For example, early childhood contacts with animals, such as growing up on a farm or keeping indoor pets, confer protection against atopic sensitization (Liu, 2004). Thus, allergy may stem from a lack of shift from allergen-specific Th2 to protective Th1 responses. Altered activity of regulatory T cells in atopic individuals has also been suggested as relevant for disease development (Romagnani, 2004).

Symptoms and pathology of allergic rhinitis
Allergic rhinitis is an inflammatory disorder of the upper airways, clinically characterized by inflammation of the nasal mucosa and symptoms such as sneezing, rhinorrhea, itching and nasal blockage (Borish, 2003). Depending on the duration and season of allergen exposure, allergic rhinitis is classified as seasonal (intermittent) or perennial (persistent). Pollens are common seasonal allergens, whereas dust mite, molds and animal dander belong to the group of perennial allergens (Skoner, 2001). 20% of cases with allergic rhinitis are strictly seasonal, 40% perennial and 40% mixed. Allergic rhinitis is diagnosed if a patient displays symptoms of disease and produce allergen-specific IgE antibodies, which is
determined with skin prick test or serum IgE immunoassays. Remodeling of the nasal mucosa is a histopathological feature of allergic rhinitis. Examples of structural alterations are thickening and degeneration of the epithelium, change in the proportion of different collagen types, increase in number of mucosal glands and infiltration of eosinophils, mast cells, mononuclear cells and lymphocytes (Salib et al., 2003; Salib and Howarth, 2003). An increase of LCs and other DCs has also been observed in the nasal mucosa during allergen provocation in patients with allergic rhinitis to grass pollen (Godthelp et al., 1996). Other characteristics are higher levels of nasal fluid eosinophils and eosinophil granulae proteins in symptomatic patients than in controls (Benson et al., 2002a). In addition to inflammation in the nasal airways, an increased systemic inflammation is observed. Systemic circulation of activated T cells and mononuclear cells, producing cytokines such as IL-6 and TNF-α, may be responsible for symptoms such as fatigue (Borish, 2003). Some TH2 cells also migrate to the bone marrow and stimulate production of inflammatory effector cells, such as basophils, eosinophils and mast cells. Increased bronchial hyperreactivity and increased expression of adhesion molecules as well as eosinophil infiltration in the lower airways are other examples of distant inflammatory changes (Braunstahl and Fokkens, 2003; Braunstahl et al., 2000; Braunstahl et al., 2001).

**Lymphoid structures and architecture of the upper respiratory tract**

The mucosa-associated lymphoid tissues (MALT) in the nasal airway consist of both organized discrete lymphoid structures and less organized clusters of lymphoid tissue. The majority of antigens enter through the mucosal route, making the tissues incredibly important for the immune system. The airway mucosa in healthy individuals has a similar structure in nose and bronchi. The respiratory epithelium, consisting of ciliated columnar cells and interspersed mucus-producing goblet cells, rest on a basement membrane (Salib et al., 2003). The submucosa, underneath the epithelium, consists of mucous glands, vessels, nerves, structural cells, such as fibroblasts, and immune cells (e.g. dendritic cells).

In addition, there are many organized lymphoid structures in the upper respiratory/nasal tract which serve as the guardians of the oropharynx. Waldeyer’s ring is the composition of lymphoid tissue in the throat consisting of the palatine and lingual tonsils and the adenoids (Nave et al., 2001). The palatine tonsils are the only easily accessible human lymphoid organ as routine tonsillectomy is performed on children with recurrent infections or hypertrophy. Tonsils differ from conventional lymph nodes, which are present virtually all over the body, in that they do not contain afferent, only efferent, lymphatic vessels (Fig. 1 and 4). Instead of being lymph-borne, ingested or inhaled antigen reach these tonsilar structures by a posterior flow of mucus and become trapped in blind-ending tubular crypts in the surrounding epithelium. The antigens are endocytosed by specialized epithelial M-cells, which transport the antigen in vesicles to the basolateral membrane and exocytose the antigen in subepithelial spaces (Fujimura, 2000; Fujimura et al., 2004). The tonsil can be divided into 4 compartments histologically: the crypt
epithelium, extrafollicular area, follicular mantle zone and intrafollicular area, which all participate in the immune response (Fig. 4). B cell maturation and differentiation take place in the follicular germinal centers (GC) (Nave et al., 2001), as well as some activation of the DC-primed, but not yet polarized, CXCR5+ follicular Th cells (Johansson-Lindbom et al., 2003a; Mackay, 2000; Moser et al., 2002). The GCs consist of a dark zone with proliferating centroblasts, a light zone with centrocytes, FDCs, follicular helper T cells and GCDCs, and a surrounding mantle zone, populated predominantly by naive B cells (van Kempen et al., 2000). The extra/interfollicular area is mainly populated by T cells and various subtypes of DCs. Many HEVs are present in the T-cell area, which constitute a way of specific entry of blood-borne naive and memory T and B cells (Miyasaka and Tanaka, 2004), as well as PDCs (Yoneyama et al., 2004).

**Figure 4.** Immunology of the human tonsil. M-cells, membranous cells; T\(_N\), naive T cell; T\(_M\), memory T cell; B\(_N\), naive B cell; B\(_P\), plasma B cell

**Cellular mechanisms of allergic immune response**

The allergic response is a consequence of complex signaling cascades and interactions between many cells of the immune system. Repeated exposure of allergenic compounds is required to trigger a hypersensitivity response such as
allergic rhinitis in atopic individuals. The allergic response can be divided into two phases; the sensitization phase and the effector phase (Wills-Karp and Hershey, 2003). The first encounter with otherwise harmless antigens such as inhalation of pollen, animal dander and detergent enzymes, results in sensitization and mounting of an inappropriate immune response towards the antigen (Fig. 5). Allergens that cross the first line of defense, the airway epithelial cells, are taken up by nasal and bronchial mucosal antigen-presenting cells, mainly DCs, situated above and beneath the basement membrane of the respiratory epithelium (Lambrecht and Hammad, 2003). DCs process the allergens and present them to allergen-specific naive CD4⁺ T cells in the draining lymph nodes which subsequently become polarized proliferating effector Th2 cells that produce cytokines such as IL-4, IL-5, IL-9 and IL-13. Within the Th2-cytokine environment, allergen-specific B cells switch their antibody production towards IgE. Circulating allergen-specific IgE binds to various FcεRI⁺ effector cells of the immune system, such as tissue mast cells and blood basophils. It is not until repeated exposure of allergens, during the effector phase, that the clinical symptoms emerge. In this phase, DCs process and present internalized allergens also to specific memory CD4⁺ T cells generated during the sensitization phase. The activated effector memory T cells further amplify the IgE production by producing Th2 cytokines. Simultaneously, intact allergen directly activates mast cells and basophils by binding to surface IgE bound to FcεRI. Allergen-induced crosslinking of FcεRI initiates a signaling cascade that cause exocytosis of preformed mediators such as histamine, leukotrienes and prostaglandins, as well as production of various cytokines, e.g. IL-4 (Bradding et al., 1993; Salib et al., 2003). T-cell derived cytokines, such as IL-5, also promote eosinophil growth, differentiation and activation. Large number of activated eosinophils migrate into areas of allergen-challenge and release for instance the toxic mediators eosinophilic cationic protein (ECP) and peroxidase (EPO), which may be responsible for tissue damage in later stages of the effector phase (Venge, 2004). Eosinophils also produce IL-4 and IL-13 which may further enhance the allergic response (Nonaka et al., 1995; Woerly et al., 2002). Other relevant cell types and their products participating in the allergic inflammation are neutrophils (lactoferrin and myeloperoxidase), monocytes (lysozyme and IL-6) and endothelial cells (E-selectin) (Venge, 2004). Production of Th2 cytokines, degranulation of mediators from mast cells and basophils, as well as activation of eosinophils etc. are all events that trigger the allergic inflammation. Other characteristics of allergic rhinitis include exudation of plasma proteins in the nasal and bronchial airways as a result of increased vascular permeability (Svensson et al., 1995) and the involvement of neuropeptides and nerve fibres in the nasal mucosa (Hepp et al., 2004; Korsgren et al., 2003).
Figure 5. Cellular processes involved in the immune response in allergic rhinitis

DCs and CD4+ T cells in allergic responses

Evidence for DCs as important regulators of allergic disease in vivo

DCs play a unique role in the initiation of specific immune responses, in addition to their role in determining the differentiation and polarization of the antigen-specific T cells responses. Even though other cells have been suggested to participate in antigen-presentation, such as B cells, macrophages, epithelial cells and even eosinophils, it is becoming increasingly clear that DCs are the most important professional APCs at mucosal surfaces (Lambrecht and Hammad, 2003). For instance, B-cell deficient mice sensitized with ovalbumin still develop allergic airway inflammation (Korsgren et al., 1997). Similarly, eosinophils seem to amplify Th2 responses but lack antigen-presenting capacity to naive T cells (van Rijt et al., 2003). In an experimental mouse model of human severe combined immunodeficiency, reconstituted with peripheral blood mononuclear cells from patients sensitive to Dermatophagoides pteronyssinus (Dpt), human DCs were shown to be mainly located in the alveolar spaces in lungs of mice developing an inflammatory pulmonary infiltrate (Hammad et al., 2000). After exposure of Dpt, the number of airway DCs decreased and subsequently, increased production of IgE was detected, as compared to control mice. Recently, adoptive transfer of DCs
from mice with food allergy to cow’s milk was also shown to induce allergen-specific IgE in naïve syngenic mice in absence of antigen challenge (Chambers et al., 2004). Interestingly, allergen-specific IgE response was induced without altering of the Th1/Th2 balance, indicating that Th2-skewed responses were not involved in the early phases of allergic responses. Furthermore, OVA-sensitized transgenic mice with selectively depleted airway DCs, but not macrophages and B cells, exhibited suppressed eosinophilic airway inflammation after exposure of OVA, compared to control mice, demonstrating the contribution of DCs to disease pathogenesis (Lambrecht et al., 1998). Moreover, no allergen-induced boosting of OVA-specific IgE levels was detected in the absence of DCs.

In humans, the number of myeloid DCs rapidly increase in bronchial mucosa of atopic individuals following local allergen challenge (Jahnsen et al., 2001). In parallel, blood myeloid DC numbers decrease following allergen exposure (Upham et al., 2002), indicating a recruitment of DCs from the circulation into the airway mucosa. Once DCs have been challenged with allergen, they either induce a local response by remaining in the lung (Constant et al., 2002) or rapidly travel to the regional lymph nodes for initiation or amplification of the allergic immune response (Upham, 2003). Recently, the direct effect of DCs in Th2 sensitization was shown in transfer experiments during which allergen-loaded DCs, administered systemically with subsequent aeroallergen challenge, induced Th2 immunity (Graffi et al., 2002; Hammad et al., 2002). In a model of experimental asthma, DCs were shown to populate the respiratory tract submucosa within 2 h after ovalbumin (OVA) aerosol challenge (Huh et al., 2003). Within 24 h, antigen-carrying DCs disappeared from the airway wall and instead appeared in the regional lymph nodes.

Investigations of the direct effect of allergen-challenged DCs on sensitization in humans are for ethical reasons not feasible. However, one study demonstrated the role of DC-induced Th2-dependent sensitization to bovine serum albumin (BSA), with BSA-specific IgE production and anaphylactic reactions, adverse effects observed during cancer therapy with peptide-loaded autologous DCs (Mackensen et al., 2000).

Uptake of allergens and modification of DC function
DCs utilize several receptors for antigen internalization, e.g. DEC205, DC-SIGN, langerin and the mannose receptor (Lambrecht, 2001). On the other hand, only limited information is available covering the mechanisms behind allergen uptake. DC can phagocytose antigens such as whole bacteria and apoptotic cells, which may be the dominant mechanisms for uptake of particulate allergens. Moreover, recombinant Bet v1 and Phl p1 were shown to be internalized by LCs through macropinocytosis (Noirey et al., 2000), while the mannose receptor was involved in uptake of Der p1 (Deslee et al., 2002). In sensitized individuals, allergens bound to IgE may also be internalized through FceRI by receptor-mediated endocytosis (Lambrecht, 2001). Many of CD1a+ DCs that infiltrate the mucosa of allergic individuals have been shown to carry IgE molecules on their surface (Godthelp et
Peripheral blood from allergic subjects also contains more FcRε+ DCs than controls (Maurer et al., 1996). In addition, the proportion of FcRε-expressing airway DCs has also been demonstrated to be higher in atopic asthmatics as compared to healthy individuals (Maurer et al., 1996; Tunon-De-Lara et al., 1996), which may contribute to a modified function of DCs after allergen exposure.

Besides different routes of antigen/allergen internalization, many additional factors may influence DC phenotype and function in allergic settings (Fig. 6). The intrinsic properties of DCs may differ in sensitized and healthy individuals, such as their ability to produce cytokines and other mediators in response to allergenic stimulation. For instance, Th2 polarization by MoDCs stimulated with Der p1 was shown to depend on the allergic status of the donors (Hammad et al., 2001). In sensitized individuals, CD86 expression and IL-1β, IL-6, TNF-α and IL-10 production were induced in vitro, whereas CD80, rather than CD86, and IL-12 were induced in MoDCs from healthy individuals. CD86 seems to be more important for the induction of Th2 responses, as compared to CD80 (Ranger et al., 1996), and treatment of mice with anti-CD86, but not anti-CD80, inhibited allergen-specific IgE and IgG levels, Th2 cytokine levels, airway eosinophilia and airway hyper responsiveness (AHR) (Haczku et al., 1999). Others have reported a comparable activity of allergen-pulsed DCs from allergic and healthy donors (Bellinghausen et al., 2000), while after coculture with T cells, both Th1 and Th2 recall responses are produced, depending on the allergic status of the donors. An altered ability of DCs in atopic individuals to produce various mediators has also been suggested, such as decreased IL-12 synthesis (Reider et al., 2002) and increased PGE2 production (Long et al., 2004). Furthermore, monocyte-derived DCs from sensitized individuals show increased production of the Th2-attracting chemokines TARC and MDC in response to Der p1-challenge, as compared to healthy individuals (Hammad et al., 2003). In comparison, we have confirmed upregulation of TARC and MDC transcription in DC-T cell cocultures from allergic patients after Phil p-stimulation (Paper IV), in addition to increased chemokine protein secretion in supernatant (unpublished observation). There are a few reports on IL-4 and IL-13 production by DCs (d'Ostiani et al., 2000; Johansson et al., 2000; Kelleher et al., 1999), which would provide an obvious link to Th2 development, however, we were unable to detect any IL-4 or IL-13 induced in DCs by allergen or inflammatory trigger, on a transcriptional level.

Even though allergen stimulation in many cases do not induce DC maturation (Paper III and IV), some immunogenic allergens are known to directly trigger maturation in DCs from healthy individuals. For instance, the yeast Malassezia furfur induce upregulation of CD83, CD80 and CD86 on MoDCs, as well as TNF-α, IL-1β and IL-18 production (Buentke et al., 2001). Some contact sensitizers, such as NiSO4, also induce DC maturation (Arrighi et al., 2001). In a transcriptional analysis of DCs stimulated with the contact allergen dinitrobenzensulfonic acid (DNBS), it was demonstrated that hundreds of genes were differentially regulated in DNBS-stimulated DCs as compared to unstimulated
DCs (Ryan et al., 2004). 118 genes were commonly up/downregulated by different concentrations of the contact allergen. Whether these genes represent a general response to that specific chemical or if they are involved in the allergic response are unclear since only healthy patients were studied.

The homing of allergen-pulsed DCs to lymph nodes has been shown to involve CCR7 expression (Hammad et al., 2002), which require some activational trigger for upregulation. Activation and migration of DCs are crucial for priming of T cell responses, however, the mechanism responsible for maturation of allergen-challenged DC is not clear. Recognition of pathogen-associated molecular patterns (PAMPs) through TLRs have been suggested as essential for DC activation, even in allergen-specific responses (Eisenbarth et al., 2003). However, it seems that Th2-type immunity is the functional outcome when DC maturation is induced without strong polarizing signals (Dodge et al., 2003; Stumbles et al., 1998). In OVA-sensitized mice, local antigen-challenged DCs transiently upregulate CD86 and MHC class II during continuous communication with memory T cells, indicating that T cells are responsible for the observed DC activation (Huh et al., 2003). Whether the local airway memory T cells have an instructive effect on DCs, in terms of antigen-presentation to other T cells, e.g. resting central memory T cells, in the regional lymph nodes, remains to be shown. In paper IV, we also demonstrate a T cell-dependent maturation of DCs. In contrast, we show that the maturation occurs independently of antigen and allergic status of the donors. The induced migratory properties of allergen-stimulated DCs and thus the enhanced contact with memory T cells in vivo may account for the observed difference from our in vitro system (Paper IV).

The initial response to allergens with subsequent modulation of DC function may also be influenced by the local tissue environment, e.g. from epithelial cells, after allergen challenge. Tissue factors, in addition to allergens, have the capacity to directly induce the formation of a DC2, i.e. a type 2-inducing DC. Recently, the direct role of epithelial cells in modulating DC function in allergic inflammation was demonstrated. Human thymic stromal lymphopoietin (TSLP) is expressed by epithelial cells of inflamed tonsils and by skin keratinocytes in atopic dermatitis (Soumelis et al., 2002). TSLP-activated DCs prime naive T cells to produce proallergic cytokines (IL-4, IL-5, IL-13 and TNF-α), while downregulating IFN-γ and IL-10. TARC and MDC were also induced in DCs stimulated with TSLP. PGE₂ is another factor that promote Th2 cytokine production, by impairing the ability of maturing DCs to produce bioactive IL-12 p70 (Kalinski et al., 1997). PGE₂ synergizes with IL-1β and TNF-α in the induction of functional and phenotypical maturation of DCs (Kalinski et al., 1998) and induce IL12 p40 production, which as a monomer or homodimer function as an IL-12 antagonist (Kalinski et al., 2001). PGE₂ is produced by airway epithelial cells (Vancheri et al., 2004), which may influence the local balance of Th1/Th2 polarizing agents. Airway epithelial cells have many features of accessory cells, such as expression of HLA-DR, costimulatory molecules, functional FcγR and molecules of the ag-processing machinery, and display an ability to internalize ag and to stimulate
memory T cells (Oei et al., 2004). These features may authorize them for important immunomodulatory functions, such as amplification of memory T cells during secondary immune responses. The route of antigen entry also seems to be of importance for the generation of T helper cells. Sensitization to inhaled antigen via the respiratory route appears to be Th2 skewed (Constant et al., 2000), possibly because the antigen recognition by DCs occurs in an environment rich of airway epithelial cell-product such as PGE₂.

The concept of a third signal, separated from MHC/TCR and CD28 ligation, provided by DCs that influence Th2 differentiation has been discussed. However, there is still no DC-induced strictly Th2-inducing factor identified. Nonetheless, several costimulatory molecules have been implicated in Th2 responses, such as ICOSL (Eisenbarth et al., 2003), OX40L (de Jong et al., 2002; Salek-Ardakani et al., 2003) and sCD30 (Bengtsson et al., 1997). The fact that ICOS-ICOS-ligand costimulation between T cells and DCs is critical for Th2-driven inflammation (Gonzalo et al., 2001) and Treg cell-mediated tolerance (Akbari et al., 2002), as pointed out above, is intriguing and suggest that these processes are related. Moreover, the cell surface Notch ligand family members delta and jagged on DCs were recently shown to influence Th1 and Th2 differentiation, respectively (Amsen et al., 2004). These ligands were differentially expressed by DCs stimulated with either cholera toxin, PGE₂ or LPS. Also, ectopic expression of jagged and delta polarized naive T cells into Th2 or Th1 cells, respectively.

Many additional factors have been suggested to influence Th2 polarization by DCs (Fig. 6), such as allergen characteristics and cytokine production (Lambrecht, 2001). The various DC subsets may also play different deterministic roles. The contribution of myeloid DCs in the allergic immune response is now recognized (Lambrecht and Hammad, 2002), and evidence is emerging on the role of PDCs in allergy. PDCs and BDCA1⁺ myeloid DCs both express FcεRIα and the level of expression is increased in allergic patients on both subsets (Foster et al., 2003). Interestingly, the BDCA3⁺ DCs do not express FcεRIα. CD11c⁺ DCs and PDCs are equally potent at stimulating Phil p-dependent T cell proliferation and Th2 cytokine production (Farkas et al., 2004). The T cell stimulatory ability of allergen-pulsed PDCs can be inhibited by addition of the adjuvant CpG, targeting TLR9, which induce IFNα/β production. Recently, a protective regulatory role of PDCs in controlling asthmatic reactions to harmless antigens was demonstrated in murine models (De Heer et al., 2004). Continuously, both myeloid and plasmacytoid DCs internalized inhaled antigen in the lung and presented it to draining node T cells. Depleting PDCs during inhalation of normally inert antigen resulted in IgE sensitization, airway eosinophilia and Th2 cell cytokine production. Also, adoptive transfer of PDCs before sensitization prevented asthmatic reactions, suggesting a suppressive effect on the generation of effector T cells. However, a similar function of human PDCs remains to be determined. Nonetheless, PDCs numbers in nasal mucosa increase in response to repeated allergen provocation (Jahnsen et al., 2000), and atopic patients have more circulating PDCs than healthy controls (Matsuda et al., 2002; Uchida et al., 2001), indicating that PDCs also play an essential role in human allergic reactions. In contrast, circulating PDC numbers of allergic
children, especially asthmatics, have been reported to be significantly lower than in healthy children (Hagendorens et al., 2003). Furthermore, PDCs has been found to express the tryptophan-catabolizing enzyme idoleamine 2,3 dioxygenase (IDO) (Munn et al., 2002), which may enable PDCs to inhibit T cell proliferation and reduce T cell responses in vivo. Therefore, the recruitment of PDCs after allergen challenge may be due to an attempt by the immune system to downregulate antigen-specific T cell-responses (Novak et al., 2004).

While DCs appear to be essential in the decision process to mount an inappropriate allergen-specific Th2 response, accompanied by allergic reactions, the DC-derived signals giving rise to tolerance or allergy are not completely understood. Seemingly, both PDCs and myeloid DCs play a crucial role in this process.

Figure 6. Factors that influence Th2 skewing by DCs

Th2 cells

Animal models have been extensively used to confirm the contribution of Th2 cells, in the pathogenesis of allergic diseases. For instance, adoptive transfer of OVA-specific Th2 cells results in transfer of disease characteristics such as airway eosinophilia and mucus hypersecretion (Cohn et al., 1998; Haczku et al., 1997). Conversely, removal of CD4+ T cells prevents existing OVA-induced pathology (Gavett et al., 1994). The role of IL-4, IL-5 and IL-13 in disease pathogenesis have been demonstrated in various experiments involving knockouts, transgenic or antibody-treated mice (Wills-Karp, 1999). In humans, the number of CD4+ memory cells, exhibiting Th2 deviation, increases in the nasal mucosa during allergic rhinitis.
(Del Prete et al., 1993; Karlsson et al., 1994). In addition, allergic asthmatics display a Th2 cytokine profile in bronchoalveolar lavage and peripheral blood, as compared to nonallergic asthmatics (Robinson et al., 1992; Walker et al., 1992). The activated Th2 cells are in part responsible for the observed systemic inflammation triggered in allergic rhinitis (Togias, 2004). Th2 cells develop from naive CD4+ T cells under the influence of IL-4 acting at the level of antigen presentation. The transcription factor c-maf and signal transducer and activator of transcription (STAT) 6 are involved in the IL-4 production and signaling pathways, respectively (Romagnani, 2004). GATA-3 is a transcriptional factor for Th2 differentiation, which inhibits IFN-γ production and upregulates Th2 cytokines. The Th2 cytokines orchestrate many of the reactions typical for the allergic inflammation. For instance, IL-5 regulates the eosinophil infiltration whereas IL-9 promotes mast cell proliferation and differentiation as well as mucus production (Renauld, 2001). Further, IL-4 and IL-13 induce eosinophilia in addition to promoting IgE isotype switching.

Several additional markers have been discovered which further discriminate between different effector CD4+ T cell responses. Th2 express several chemokine receptors necessary for allergen-induced recruitment to the target tissues. For instance, CCR4 and CCR8 are preferentially expressed on human Th2 cells (Romagnani, 2002). Furthermore, the prostaglandin D2 receptor DP2 (CRTH2) is also a chemokine receptor, absent on Th1 and Th0 cells, that might be an important contributor to the recruitment of Th2 cells (Luster and Tager, 2004). The percentage of CRTH2+ T cells in PBMC from patients with atopic dermatitis has been shown to correlate with severity of the disease (Iwasaki et al., 2002).

In a recent report by Akdis et al., it was demonstrated that Th1, Th2 and Treg cells, specific to common environmental allergens such as Bet v1 and Der p 1, were present in both healthy and allergic individuals, however in different proportions (Akdis et al., 2004). In healthy individuals, the Tregs (Tr1) were the dominant allergen-specific subset, whereas in allergic patients, high frequencies of Th2 cells were detected, indicating that the immune response to the allergens was characterized by a delicate balance in frequency of these specific T cells. Furthermore, blocking the suppressor activity of Tregs ex vivo enhanced the Th2 cell activation. CD25+/CD4+ Tregs in non-atopic donors may prevent pathological Th2 activation by aeroallergens, whereas the allergy may arise from a failure to suppress Th2 cells (Ling et al., 2004).
TRANSCRIPTIONAL ANALYSIS IN DC AND ALLERGY RESEARCH

Introduction to high-density oligonucleotide microarray technology

The microarray technology has revolutionized and reshaped the area of biomedical research during the past decade. The technique has become a major tool for high-throughput analysis of gene expression and is valuable for a multitude of applications, such as discovery of novel drug targets and understanding of molecular mechanisms and regulatory pathways involved in various biological processes. High density oligonucleotide arrays, developed by Affymetrix Inc., and sometimes referred to as GeneChips, are now applicable for transcriptional analysis in more than 20 different species (for detailed information visit www.affymetrix.com). During 2004, the first array was launched which covers the entire human genome, with more than 55,000 transcripts represented on a single chip. The manufacturing of the high-density oligonucleotide arrays involves solid-phase oligonucleotide synthesis with photolithographic techniques similar to those used in the microelectronics industry. Unique 25-mer oligonucleotide chains are synthesized on a feature size of 11 microns in the more comprehensive arrays. The 25-mer probes are designed to cover approximately 600 bp of the 3’ of each gene and each probe set consists of 11 probe pairs. Each probe pair includes a Perfect Match probe (PM) and a Mismatch probe (MM), where the MM is identical to the PM with the exception of a single base mismatch in its center and functions as a control for non-specific hybridization. The intensity signal of each probe set is calculated from the PM/MM ratios and mirrors the amount of a specific mRNA in the sample. Even though one array can provide information whether or not a gene is transcribed, it is not until a comparative analysis is performed between different samples that the significance of transcription can be determined. Careful design of experiments and replicate samples are essential for informative data and if used correctly, the high-density oligonucleotide arrays constitute a powerful fishing tool. The technology has been central in many projects described in this thesis. For instance, we used microarrays to assess the influence of inflammatory mediators on the transcriptional activity of in vitro DC models (Paper I and V). The technology was also useful for analysis of the effect of allergens on DCs and T cells (Paper III and IV) and in the characterization of various DC populations in tonsillar tissue and peripheral blood (Paper VI). Recent scientific progress in these areas, with regards to transcriptional profiling, will be discussed in greater detail below.

Transcriptional profiling of human DCs

Characterization of in vivo DCs from tonsils and peripheral blood

The evident heterogeneity of the human DC subpopulations, as discussed in the chapter entitled Human Dendritic Cells, have generated many conflicting and thus confusing reports regarding phenotype and function of these subtypes. In paper VI,
we therefore aimed at defining the DC subpopulations at a transcriptional level, in order to further characterize and understand the relationships between these populations. In addition, we aimed to identify alternative molecular markers which can be used for DC isolation and subset characterization. Furthermore, the objective was to pinpoint the genes differentially expressed within each DC subset from peripheral blood and tonsillar tissue, to study gene regulation during their lifecycle, such as differences in molecules needed for differentiation and homing of circulatory DCs as compared to lymphoid tissue DCs. Three CD11c<sup>high</sup> DC populations from murine spleen have recently been isolated and characterized with transcriptional analysis (Edwards et al., 2003). Even though the data offer new insights into the biology of DCs, the murine and human DC systems are diverse and difficult to compare. The majority of transcriptional studies performed with human DCs have elucidated the response induced by various pathogenic or inflammatory signals <i>in vitro</i>. We consider it important to also characterize the DC populations in the absence of strong maturational signals, in order to define the innate differences between the unique populations. The two major obstacles of transcriptional studies of ex vivo DCs have been the lack of DC-specific mAbs for positive cell sorting and the high amount of mRNA needed for chip hybridization. However, the human DC-specific BDCA-markers (Dziewiecki et al., 2000) and the novel repetitive <i>in vitro</i> transcription protocol (www.affymetrix.com) enabled us to perform global transcriptional analysis of rare DC populations (< 40,000 cells). Rissoan et al. used a cDNA subtraction technique with 650 sequences to identify human PDC-restricted transcripts, with MoDCs as control cells (Rissoan et al., 2002). Interestingly, they found that 25% of the 650 sequences were transcripts previously associated with B-cell transcription. In paper VI, we took this study one step further and identified genes that were preferentially expressed by PDC, BDCA1<sup>+</sup>, BDCA3<sup>+</sup> and CD16<sup>+</sup> myeloid DCs from peripheral blood. The individual DC populations were also compared to their counterparts isolated from tonsils, with the exception of CD16<sup>+</sup> DCs which could not be found in tonsils. With hierarchical clustering on differentially expressed genes in these population, we were able to identify their transcriptional relationship. PDCs from blood and tonsils were clearly separated from the myeloid cells and the BDCA1<sup>+</sup> and BDCA3<sup>+</sup> DC populations clustered together, independently of tissue type. The CD16<sup>+</sup> DCs clustered with the other myeloid DC subsets. Interestingly, large clusters of DC subtype-specific gene expression was identified in the blood DCs, suggesting that these populations are unique and functionally specialized. In an assessment of their expression of chemokine receptors, TLRs and c-type lectins, we further demonstrated subset-specific transcription. Interestingly, the differential expression patterns observed in the myeloid DC subsets in blood were overrun in the tonsillar myeloid subsets, possibly due to the environmental triggers such as pathogenic- or inflammatory signals in the lymphoid tissue.
Transcriptional analysis of the maturational program in DCs in vitro

The maturation of DCs is characterized by an extensive reorganization of the proteome and a switch in their functional properties. The dramatic and global changes from immature, antigen-capturing DCs to mature, antigen-presenting DCs are advantageously analyzed with microarrays since it allows examination of thousands of transcripts simultaneously. Large amounts of data have during recent years been generated which provide insights in the transcriptional response of in vitro-derived human DCs stimulated with various pathogenic and inflammatory products (Huang et al., 2001; Ju et al., 2003; Messmer et al., 2003). In Paper I, we studied the kinetics of transcription during the maturation process in MoDCs in response to pro-inflammatory stimulation. Genes with different kinetics of transcription were identified and grouped into transient, sustained or progressively upregulated, and downregulated transcripts. Among the transient, but strongly upregulated genes after 8 h of stimulation, we found a broad panel of chemotactic mediators and interleukins, emphasizing their potential to attract other immune cells to site of inflammation early during their maturation. The group of progressively induced transcripts included DEC205 and the costimulatory molecules CD86, OX40L and RANK. Moreover, CD137 and CD80 transcripts remained elevated at both the early and late time points. A switch in expression of adhesion molecules became evident during maturation; E-cadherin, N-cadherin, CD18, CD49e and CD31 were downregulated whereas CD44, CD48, CD54 and CD58 were induced. Furthermore, downregulation of CCR1 in parallel to CCR7 upregulation illustrate that DCs modify their migratory properties during maturation. This study showed that DCs respond with waves of coordinated gene expression over time during maturation. The reprogramming is extensive and alters the patterns of gene expression globally. Furthermore, the novel finding that both in vitro and tonsilar DCs express CD137 transcripts (Paper I), prompted us to analyse the expression of CD137 with immunohistochemistry on tonsillar tissue sections. This resulted in the discovery of high CD137 expression by FDCs, which we describe in Paper II.

Understanding molecular mechanisms in allergic disease using microarrays

The clinical diagnosis of allergic rhinitis depends on display and history of symptoms, which may be complemented with skin prick test and detection of allergen-specific IgE in blood. There is still no single marker identified which distinguish an allergic inflammation from other inflammatory airway disorders. Other parameters, such as level of ECP in sputum or blood, plasma proteins in nasal fluid, number of eosinophils in sputum etc, can be used to characterize the disease (Venge, 2004). It would be valuable to identify the differential mechanisms involved in the immune response in allergic versus healthy individuals, since it would enable new therapeutic approaches. Improved understanding of the upstream cellular and molecular processes underlying the pathogenesis of rhinitis may enable inhibition of the allergic cascade in an early stage, instead of treating patients with inhibitors of downstream mediators, such as histamine. Consequently,
DCs and T cells would be the optional cells to target, since these cells clearly orchestrate the allergic immune responses. Some interesting approaches of targeting immune cells and their products involved in the allergic inflammation are currently under development for therapeutic purposes, and a few have reached clinical trials (Holgate and Broide, 2003). For instance, the therapeutic potential of inhibitors such as IL-4 and IL-13 antagonists/mAbs, CCR3-antagonist and eotaxin-mAbs are being evaluated in human studies.

There is a limited amount of reports concerning transcriptional differences between healthy and allergic individuals and most of these have been performed with tissue or blood samples. In these complex mixtures of cells, only a few cells may be responsible for the bias towards an allergic immune response. In addition to the risk of diluting important transcripts beyond the limit of detection in these studies, one may also be detecting differences in these samples due to structural or other disease-unrelated factors. However, the quantity of RNA necessary for chip hybridization makes it difficult to purify isolated cell populations from certain tissues. In vitro cell culture techniques solve the issue of RNA quality and cell quantity, however, in these studies one may instead introduce results that are not representative of the in vivo situation. Therefore, it is important to view data generated from global transcriptional analysis in vitro as a first step to identify novel molecules and regulatory processes. The transcriptional analysis should be followed by assessment of the corresponding gene products and their functional contributions.

In contrast to cancer research, few microarray studies have been published in the field of allergy, partly because of the complexity of the disease manifestation and the numerous cellular players involved. However, several different approaches have been reported and within a few years, important pieces of the puzzle will be added. Transcriptional analysis have, for instance, been used to decipher the expression profile in nasal biopsies from patients with seasonal allergic rhinitis in comparison to healthy controls (Benson et al., 2002b). VEGF-A was identified as differentially expressed and an increase of VEGF-A protein in nasal fluids was confirmed after allergen provocation in allergic patients. Interestingly, in our study (Paper IV), increased expression VEGF-A was also identified in the CD4+ memory T cells stimulated with allergen-challenged DCs in allergic individuals, as compared to healthy controls.

In addition to nasal biopsies, various other tissues and cell types involved in allergic processes have been analyzed by transcriptional profiling. For instance, Dolganov et al. identified 75 genes differentially expressed in bronchial biopsies from asthmatic patients, as compared to controls (Dolganov et al., 2001). As expected, many of the identified transcripts coded for Th2 cytokines, e.g. IL5, IL9 and IL13. Increased expression of the Na+-K+-Cl- cotransporter (NKCC1) was also identified and increased protein expression could be verified by immunohistochemistry in asthmatic subjects, with restricted localization to goblet cells. Furthermore, the differential gene expression profile of skin lesions from patients with either atopic dermatitis (AD) or psoriasis have been assessed with microarrays (Nomura et al., 2003). 18 genes were found to be preferentially
expressed in AD patients and 62 genes in psoriasis patients. Among the AD-transcripts, various CC chemokines were identified, e.g. MCP-4 with chemotaxtractant activity for CCR3+ eosinophils, basophils and mast cells. Heishi et al. used a different approach and assessed the difference in gene expression of PBMCs, stimulated in vitro with PHA/PMA, from patients with AD compared to controls (Heishi et al., 2002). Also, anti-CD3/CD28-activated peripheral blood CD4+ T cells from individuals with atopic asthma (AA) and AD were compared to healthy individuals with DNA arrays (Wohlfaht et al., 2003). Moreover, the transcriptional profiles of developing Th1 and Th2 cell, polarized in vitro with the addition of exogenous cytokines, have been analyzed by a number of groups (Hamalainen et al., 2001; Lund et al., 2003; Rogge et al., 2000).

**DCs as immune sentinels for allergens; a transcriptional approach.**

There is no doubt that DCs play a major role in the immune system as sentinels for pathogenic antigens and inflammatory triggers. DCs are also essential in the allergic response, in the presentation of allergens to T cells and the amplification of Th2 cells in the effector phase, as discussed in the chapter entitled Allergic Disease. We therefore aimed to identify the mediators involved in the transcriptional response by DCs to allergenic compounds, an area in which published data is very limited. The long-term goal is to identify markers involved in the DCs response to environmental irritants and allergens, and to incorporate these in a DC test system for prediction of compound allergenicity.

Recently, the changes in gene expression in peripheral blood-derived dendritic cells following exposure to a contact allergen were described (Ryan et al., 2004). However, the study covered direct allergen effects on DCs from presumably healthy donors without comparing the response in DCs from allergic individuals. Another recently published study reported that stimulation with the allergenic yeast *Malassezia sympodialis* affected the transcription in DCs derived from healthy individuals and patients with atopic dermatitis (Gabrielsson et al., 2004). Using cDNA arrays containing 406 genes, several genes were recognized as preferentially expressed in DCs from atopic patients, e.g. IL-8 and MDC.

We have performed two studies focusing on allergen induced transcriptional regulation in DCs (Paper III and IV). In the first study, we used allergenic recombinant detergent enzymes and studied the gene transcription in DCs from atopic factory workers. In spite of significant safety measures, allergy to industrial enzymes remains a major concern and the prevalence increases. The study aimed at elucidating the molecular mechanisms underlying allergic immune responses to the industrial enzyme lipase, and a mutated hypoallergenic version of lipase, developed by Novozyme A/S (Denmark). DCs, from individuals with documented lipase allergy, were stimulated with the lipases and the transcriptional activity of DCs was assessed. We also studied their ability to induce proliferation and polarization of memory T-cells. Out of 5 atopic skin prick test positive individuals, only one displayed T cell proliferation and Th2 cytokine production in response to lipase-stimulated DCs. The responding donor, referred to as nr 4, also
exhibited the highest levels of serum lipase-specific IgE. Flow cytometry showed that only donor 4 had cell surface bound IgE, possibly facilitating uptake of lipase for enhanced antigen-presentation (Fig. 7). The transcriptional analysis of DCs revealed differences in gene expression between donor 4 and the other donors in response to lipase stimulation. Furthermore, hypoallergenic lipase and wild-type lipase induced different genetic signatures in these DCs, with 42 or 57 regulated transcripts, respectively. For instance, FcεRII was downregulated in DCs from donor 4 in response to 8 h lipase stimulation, possibly due to a switch from being Ag-capturing to Ag-presenting DCs, as a consequence of Ag-Ab internalization. IgE enhance antibody responses via FcεRII by an unknown mechanism (Heyman, 2000). However, increased internalization of allergen through FcεRII may enhance presentation, Th2 responses and thus IgE production. Since only one of the 5 atopic individuals responded in our assays, any general conclusions regarding the transcriptional activity in lipase-challenged DCs are difficult to draw. Lack of donor material is one of the major obstacles in these kinds of studies. Factory workers are for safety reasons withdrawn from the production of detergent enzymes as soon as they display symptoms of rhinitis and positivity in a skin prick test, which complicates studies in the field of occupational allergy.

![Figure 7](image_url)

**Figure 7.** Cell surface bound IgE on DCs from allergic patients, as determined by flow cytometry. DCs from individuals with high (A) or low (B) serum levels of lipase-specific IgE were stained with primary rabbit anti-human IgE Abs, followed by secondary anti-rabbit-FITC Abs (filled grey histograms). DCs incubated with secondary Abs were used as a control of unspecific staining (empty black histogram).

The conceptual important finding that the allergens induced transcriptional activity in DCs (Paper III) led us to the second study (Paper IV), where we further characterized the gene regulation in DCs. To increase the statistical significance in our cohort, we now used both healthy and allergic donors stimulated with grass pollen allergen *Phl p*. Healthy and atopic individuals were carefully selected from a large panel of donors and only those displaying clearly defined parameters were enrolled in this study. The allergic patient inclusion criteria were (i) clinical history
of strictly seasonal allergic rhinitis, (ii) symptoms associated with seasonal allergen exposure, (iii) positive skin prick test and (iv) elevated levels of grass pollen-specific IgE in serum. The global transcriptional analysis of Phl p-challenged DCs clearly revealed that pollen induced differential transcript profiles in DCs from allergic and healthy donors. Of interest, the CCR4+ Th2 cell attracting chemokine MDC (CCL22) was upregulated in allergen-challenged DCs derived from allergic individuals, a finding also reported by Gabrielson et al., as mentioned above. Various interleukins/interleukin receptors (IL15, IL6, LTB, IL7R, IL2RG) and chemokines/chemokine receptors (IL8RB, CCL4, CCL8, CCL23, CCR6) were downregulated in DCs from the allergic individuals. In addition to signalling molecules, several transcripts coding for receptors (PTGER3, FCGR2A, C3AR1, CD209L, HRH4, OLR1, LILRB2) were downregulated. Oxidized low density lipoprotein receptor 1 (OLR1), also called scavenger receptor E1, and CD209L (DC-SIGNR) are members of the C-type lectin superfamily which may function as receptors for antigen internalization. TNFRSF11A (RANK) and SPN (leukosialin, CD43) were found among the upregulated transcripts in DCs from allergic individuals, as well as transcripts coding for the two lysosomal proteins LAPT4MB and LAMP1 (CD107a). The individual contribution of these transcripts in the response of DCs to allergen in allergic disease is currently unknown. Further investigations are warranted to elucidate details of the molecular interplays that may explain the present observations. Nevertheless, our results show that DCs change their transcriptional activity after allergen-challenge and that this property is different between allergic and healthy individuals.

Transcriptional activity in T cells induced by allergen-stimulated DCs

In Paper IV, we demonstrated that peripheral memory CD4+ T cells from healthy and allergic donors respond differently after stimulation with allergen-loaded DCs, with respect to cytokine production, proliferation and surface marker expression. To further dissect the molecular mechanisms involved in the DC/T cell response to allergen, we used high-density oligonucleotide arrays to produce a comprehensive picture of gene regulation following coculture of memory CD4+ T cells with allergen-stimulated DCs. The rational was to assess transcription induced solely by the specific interaction between T cells and DCs, without the addition of polarizing cytokines. DCs and T cells were cocultured at a ratio of 1/20 and allowed to interact for 7 days prior to collecting the cell samples for microarray analysis. Since the T cells proliferated during the 7-day culture period, only an insignificant amount of collected cells was estimated to be DCs. Therefore, we expected the T cell transcripts to outnumber the DC transcripts. However, a contribution of DC mRNA in the final results can not completely be excluded. Many genes were found to be differentially expressed in the comparative analysis between healthy and allergic individuals. In DC/T cells from the allergic patients, we found well-established markers for Th2 responses, in addition to genes previously not described in the context of allergy. Due to space limitations in Paper IV, many interesting transcripts were not thoroughly discussed, which deserves attention. For instance,
IL-17A, IL-17R and IL-17RB transcripts were upregulated in the allergic individuals. IL-17A levels in bronchial tissue are elevated in asthmatic patients (Chakir et al., 2003). Furthermore, IL-17A−/− mice display decreased AHR as well as decreased IL-4, IL5 and IgE levels in response to OVA (Nakae et al., 2002). IL-17 seems to be involved in many processes in allergic diseases (Kawaguchi et al., 2004). For instance, IL-17-stimulated bronchial epithelial cells produce the chemokine CCL2 (MCP-1). Interestingly, MCP-1 was also found to be upregulated in T cells from allergic individuals. The receptor of MCP-1, CCR2, is expressed by DCs (Vecchi et al., 1999) and effector Th cells (Sallusto et al., 1998) on a transcriptional level. MCP-1 downregulates IL-12 production by DCs stimulated with CD40L and inhibits IFN-γ but not IL-13 induction in naïve T cells, thereby inhibiting Th1 cell development (Omata et al., 2002). Thus, IL-17-producing memory T cells may in an autocrine fashion, by synthesising MCP-1, promote recruitment of local DCs and Th cells and hamper further Th1 cell development.

The TNF receptors RANK (TNFRSF11a) and osteoprotegerin (OPG, TNFRSF11b) were upregulated in the DCs and T cells from allergic individuals, respectively. These receptors bind their mutual ligand OPG/RANKL/TRANCE (TNFSF11), which was expressed at similar transcript levels in both healthy and allergic individuals (unpublished observations). Cellular responses to RANKL depend on the expression level of RANK and its decoy receptor OPG. TRANCE is constitutively expressed in memory, but not naïve, T cells and acts as a survival factor for DCs by binding to RANK (Heishi et al., 2002). TRANCE stimulation of DCs induces the expression of the proinflammatory cytokines IL-1β, IL-6, IL-15 and IL-12p40. The decoy receptor OPG blocks the actions of TRANCE and may thereby modulate the interaction between DCs and T cells. Immature DCs coproduce TRANCE and RANK, whereas TRANCE is lost once DC mature, indicative of a dependence on exogenous TRANCE for the survival of mature DC (Cremer et al., 2002). In addition, TRANCE-RANK interaction was recently shown to be required for IL-12 production and initiation of Th1 responses in CD40L−/− mice (Padigel et al., 2003). The finding that T cells from atopic individuals, stimulated with allergen-challenged DCs, express higher levels of OPG that healthy controls, indicate that OPG may be counterbalancing the effects of TRANCE production.

Furthermore, histamine receptor H4 (HRH4) was upregulated in the allergic group. Chemotaxis of eosinophils and mast cells via histamine in chronic allergic inflammation is triggered through HRH4 (Daugherty, 2004). Various selective HRH4 antagonists are presently evaluated in animal models for antiallergy therapy. Our finding that T cells stimulated with allergen-loaded DCs also express HRH4 mRNA make them a potential target for additional antagonistic effects.
MYELOID CELL LINES

The immunoregulatory properties that DCs possess make these cells highly interesting candidates for many applications in both health and disease. The development of human DC-vaccines clearly demonstrate their potential, as injected tumor antigen-loaded DCs can enhance tumor immunity (Paczesny et al., 2003). Autologous monocyte-derived DCs have been used as antitumor adjuvants in the majority of clinical trials with DC vaccinations, and many of these trials have demonstrated induced anti-tumor immune responses with tumor regression (Davis et al., 2003). These in vitro generated DCs have proven to be a very useful cellular source, which has greatly contributed to the increased knowledge in DC biology during the last decade. The low frequency of DCs in vivo and the difficulties involved in their isolation are major obstacles for experimental work on human in vivo DCs for functional studies. To be able to both minimize the use of human material and generate large numbers of DCs, we have assessed the potential of monocytic cell lines to develop into DC-like cells. Apart from being an unlimited source of cells, a fully functional human DC line would be of great benefit in the standardization of experiments. Since DCs undergo an extensive reorganization of their proteome and functional characteristics during maturation, it is essential that also the DC line have these properties. Therefore, the cell line should acquire an immature phenotype after differentiation and display a mature DC phenotype after activational trigger.

Several groups have evaluated the possibility of human myeloid cell lines to differentiate into DCs. Calcium ionophore (CI)-treatment of the promyelocytic HL-60 cell line induces morphological changes, upregulation of CD80, CD83 and CD86 and enhanced T cell stimulatory capacity (Koski et al., 1999). However, HL-60 do not express HLA-DR even after mobilization of intracellular calcium, a common feature of acute promyelocytic leukemia (APL) which accounts for approximately 10% of cases of acute myeloid leukemia (AML). Also, the CD34+ myelomonocytic cell line KG-1 acquire DC characteristics after stimulation with GM-CSF and TNF-α or phorbol myristate ester (PMA), such as a DC-like morphology, expression of costimulatory molecules, MHC class I and II, ability to pinocytose macromolecules and to activate resting T-cells (St Louis et al., 1999). TNF-α was also shown to induce CD1a expression in a small subpopulation (4-9%) of KG-1 cells (Hulette et al., 2001). Moreover, KG-1 cells are able to cross-present exogenous Ag in an MHC class I-restricted fashion to CD8+ T cell hybridomas (Ackerman and Cresswell, 2003). Likewise, the human monocytic cell line THP-1 displays some DC characteristics, e.g. HLA-DR (low levels), CD80 and CD86 expression, after culture with GM-CSF and IL-4 (Yoshida et al., 2003).

MUTZ-3 as a model system for DC biology

Even though THP-1 and KG-1 display responsiveness to cytokine treatment and acquire some characteristics of DCs after stimulation, they do not have the ability
to differentiate into immature DCs. To address this problem, we have turned to MUTZ-3, which originate from peripheral blood cells from a 29-year old man diagnosed with acute myelomonocytic leukemia in 1993 (Hu et al., 1996). The cell line expresses CD13, CD14, HLA-DR and CD68, markers of the monocyte/macrophage lineage, and displays a morphology similar to monocytes. It requires the addition of either SCF, GM-CSF, M-CSF or IL-3 for sustained cell growth and survival.

MUTZ-3 is able to differentiate into cells similar in phenotype to interstitial- or LC-like DCs upon stimulation with GM-CSF and TNF-α (Masterson et al., 2002). Interestingly, differentiated MUTZ-3 DCs possess functional antigen processing and presentation pathways, demonstrated by antigen-specific presentation of viral peptides and tetanus toxoid in a MHC class I and II restricted manner, respectively, to HLA-matched T cells. MUTZ-3 DCs were also shown to present antigen via CD1d to NKT cells, and to produce IL-12 or IL-10 in response to IFN-γ or dexamethasone, respectively. During differentiation, MUTZ-3 looses the expression of CD14 and acquires CD1a, a hallmark of DC differentiation.

In our laboratory, we have assessed various human myeloid cell lines for their ability to differentiate into DCs and fully mature after stimulation. Human cell lines, such as Monomac-6, K-562, HL-60, THP-1, KG-1 and MUTZ-3 (all from either ATCC or DSMZ, for detailed information visit www.atcc.org or www.dsmz.de), were stimulated with growth factors, interleukins and maturational stimuli. Similar to monocytes, MUTZ-3 is also responsive to GM-CSF and IL-4. In Paper V, we compared the ability of monocytes, KG-1, THP-1 and MUTZ-3 to differentiate into immature DCs and mature after stimulation with pro-inflammatory cytokines and LPS. MUTZ-3 DCs mirrored MoDCs by upregulating MHC class II and co-stimulatory molecules, and displayed a similar ability to stimulate allogeneic T cells. In addition, the transcriptional activity of MUTZ-3 DCs and MoDCs after stimulation with pro-inflammatory cytokines was compared. Transcriptional profiling of MUTZ-3 DCs demonstrated that these cells expressed many DC markers in their immature form, such as CD13, CD14a, CD40, DC-SIGN and DEC205. Stimulated MUTZ-3 DCs upregulated many activation-dependent transcripts, such as CCR7, CD137L, DC-LAMP, IL-12p40, OX40L and RANK. As for MoDCs, several transcripts were also suppressed during maturation of MUTZ-3 DCs, exemplified by CD31 (PECAM1) and CCR1. Even if the majority of transcripts were similarly expressed in MUTZ-3 and MoDCs, a few discrepancies were detected. Slower temporal gene expression was observed in some cases in MUTZ-3, as for instance, the expression levels of some transcripts that were downregulated in MoDCs upon maturation, such as DC-SIGN, CD14a and CD32, remained unaltered in MUTZ-3. Also, a lower frequency of MUTZ-3 DCs upregulated CD1a and CD86 upon differentiation and maturation, respectively, as compared to MoDCs. All together and despite minor discrepancies, MUTZ-3 DCs closely mirror MoDCs in many respects, which qualifies it as the most attractive cell line for in vitro studies of DC biology.
Prospects and pitfalls with MUTZ-3 as a DC model in allergy research

We have aimed at developing a human *in vitro* test system which can be used instead of monocyte-derived DCs or *in vivo* DCs sorted from blood or tonsillar tissue. The test system will be used to further understand the role of DCs in allergy and to test the function of novel markers involved in the allergic reaction. In the future, one could utilize the system as a pre-screening method to test the allergenicity of new recombinant proteins and low-molecular substances, which are constantly produced by the pharmaceutical, food and detergent industry. There is a great need to develop human *in vitro* test systems from an ethical point of view. Also, animal tests are poor predictors of allergenicity in humans because of species differences in the immune response to foreign antigens. In contrast, an *in vitro* assay offers numerous advantages such as speed, increased sensitivity and control of test conditions. Since dendritic cells are the first to encounter allergens, which results in the initiation of an allergic immune response, they pose a highly interesting candidate for the *in vitro* test system.

Hulette et al. have approached a similar strategy, and studied the phenotypical changes of HLA-DR, CD54, CD80 and CD86 in DCs differentiated from human PBMC as well as differentiated KG-1 cells, in response to chemical allergens, such as dinitrofluorobenzene (DNFB), methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) and the irritant sodium dodecyl sulfate (SDS) (Hulette et al., 2002). The measurements of these surface marker changes *in vitro* were not sensitive enough to detect any differences in DC responses and the authors conclude that the method was insufficient for assessing the contact sensitization potential of these chemicals. Similarly, the sensitization potential of chemicals has also been evaluated on THP-1 cells, in comparison to KG-1 cells (Yoshida et al., 2003). The known sensitizer dinitrochlorobenzene (DNCB) induced significant upregulation of CD86 and CD54 on naive THP-1 and KG-1 cells. DNCB also augmented HLA-DR and CD80 expression, as well as IL-1β secretion, in naive THP-1 cells. The authors concluded that measuring costimulatory marker expression on naive THP-1 cells was promising as a short-term *in vitro* sensitization test. However, the differences in response to similar chemical allergens, such as DNCB as compared to DNFB, on KG-1 cells, indicate that the cellular responses is not as general as one might desire. Ashikaga et al. tested the influence of several different sensitizing agents and irritants on CD86 expression on THP-1 (Ashikaga et al., 2002). All the sensitizers enhanced CD86 expression, whereas the irritants had little effect. Since very few reports are available, it is difficult to draw any general conclusions about the use of costimulatory molecules as indicators of contact-sensitizing ability. Also, the question remains how significant these mechanisms are in the context of a wider range of allergens.

As determined in Paper V, MUTZ-3 is a superior source of DC precursors, compared to KG-1 and THP-1, and may be a more relevant cell line for deciphering the molecular mechanisms involved in allergic disease. However, measuring the changes in cell surface levels of costimulatory molecules are still most likely inadequate for determining sensitizing capacity of allergens. In my opinion, high-
density transcriptional analysis is by far the most informative approach for these issues. Even though the direct effect of allergens on DCs may provide important clues, the fundamental role that dendritic cells play in the pathogenesis of allergy is due to its ability to process and present allergens to effector T-cells. The decision and outcome of Th2 polarization may instead be a result of the cross-talk between DCs and T cells.
CONCLUDING REMARKS AND FUTURE PERSPECTIVE

DCs play a pivotal role as immune regulators in both health and disease. The powerful adjuvant activity and deterministic ability that DCs possess in stimulating T cells make them interesting targets to study for the development of novel therapeutic approaches in e.g. allergic disease. The recent developments in cancer immunotherapy, using DCs to enhance tumour resistance, as well as the novel approaches with DCs as vaccine vehicles, clearly demonstrate their value for various biomedical applications. The in vitro DC models and manageable isolation procedures of in vivo DCs, in combination with the microarray technology, have unlocked new possibilities of DC research and facilitated rapid collection of information. This thesis is based on six original papers covering different aspects of DC biology, with a focus on characterisation of the transcriptional activity of DCs in allergy and inflammation.

In Paper I and V, we evaluated the transcription induced by inflammatory stimulation in two functional in vitro models of DCs, i.e. MoDCs and MUTZ-3. Paper I showed that DCs are, on a broad front, involved in many important processes in inflammatory conditions, with implications for both innate and adaptive immunity. A global reorganisation of the transcriptome was observed during maturation, and this process was highly time-ordered. Analysis of the kinetics of activation revealed groups of genes that were either transient, sustained, progressively upregulated or downregulated and these genes could be linked to the difference in activities in vivo of immature and mature DCs. In Paper V, we further evaluated this process and compared the transcriptional activity in differentiated MUTZ-3 DCs with MoDCs. MUTZ-3 DCs were shown to mirror MoDCs phenotypically and to display similar transcription in response to identical stimulatory triggers. Therefore, we conclude that MUTZ-3 DCs represent an excellent candidate for in vitro DC studies. Future goals include using MUTZ-3 DCs to test various sensitizing compounds to further analyse its predictive ability in allergy. Also, we will assess the capacity of MUTZ-3 DCs to present allergens to specific T cells from HLA-matched donors.

The finding, as described in Paper II, that FDCs in tonsils express the costimulatory molecule CD137, may have implications for the regulation of germinal center reactions. We also demonstrated that the FDC cell line HK expresses CD137, which may thus be used for future functional studies.

In Paper III and IV, the effect of lipase and grass pollen-allergens on the phenotype and transcriptional activity of DCs was demonstrated. Even though no phenotypical changes were observed in DCs by allergen challenge, we could detect transcriptional changes in allergic individuals as compared to controls, which indicate that DCs indeed do recognize allergens differently in these subjects. Furthermore, in Paper IV, we describe differences in the effect of grass pollen-stimulated DCs on the transcription and phenotype of memory T cells in allergic patients, as compared to healthy individuals. DCs also received signals for
activation by the T cells, which imply that the immune modulation induced by allergen is not a one-way street. Thus, the immunoinstruction may not completely be in the hands of DCs, but rather a result of a fine-tuned cross-talk between DCs and T cells. We are now performing additional studies on the signals involved early in the DC/T cell interaction, which will provide us with information about which signals are essential during effector T cell amplification. The data generated from the transcriptional studies will be further evaluated on a protein level, to facilitate functional studies of the DC/T cell interaction during allergic responses.

Our data in Paper VI highlights the diversity among the DC subpopulations in vivo. Preferentially expressed genes were identified in each blood and tonsillar DC population, with a more pronounced subset-specific transcription in blood DCs. The level of subset-specific gene expression was surprisingly extensive, suggesting that these populations possess innate functional commitments. We also identified gene expression common to the blood and tonsillar BDCA1+ and BDCA3+ DCs, which implies that these populations are related. The pathogenic- or inflammatory environment of tonsils may have accounted for the similar expression levels of interleukins, chemokines, TLRs and c-type lectins in BDCA1+ and BDCA3+ DCs isolated from tonsils. The results in Paper VI also describes the gene expression in PDCs to be quite rigid, as the transcript levels of the assessed markers did not alter in tonsillar PDCs, as compared to blood PDCs. The vast amount of data generated in this study will be useful for future work for the identification of novel DCs markers and further evaluation of the functional specialization of DC subsets.
POPLÄRVERTENSKAPLIG SAMMANFATTNING


Dendritiska celler (DC) är en heterogen grupp av vita blodkroppar som funktionellt befinner sig i gränsen mellan det medfödda och inducerade immunförsvaret. De söker kontinuerligt av omgivningen efter främmande protein och signalerar vid behov till andra immunceller (T- och B-celler) att de ska utveckla sina specifika immunsvär. Vid t.ex. en bakteriainfektion så verkar DC lokalit genom att producera molekyler för att attrahera och aktivera andra immunceller. De tar också upp bakterieproteiner och visar upp dem i form av proteinbilar till T-celler, som i sin tur aktiverar B-celler att producera antikroppar. DC:s selektion av proteiner gör att de har en viktig reglerande roll i immunförsvaret, eftersom de delvis kan bestämma om immunsystemet ska aktiveras. DC är också involverade vid utveckling av autoimmunitet och allergi eftersom de då har aktiverat immunförsvaret på ett felaktigt sätt genom att visa upp antingen kroppsegna proteiner eller annars oskadliga proteiner som t.ex. pollen. I kombination med andra molekylärmechanismer leder detta till en felaktig aktivering av det övriga immunförsvaret.

Den här avhandlingen beskriver DC:s funktion i immunförsvaret, speciellt med avseende på DC:s reglerande roll vid inflammation och allergi. Få primära humana DC i blod och vävnad, i kombination med komplicerade metoder för isolering av dessa celler, har gjort att forskning inom detta område har försvårats. Utveckling av alternativa metoder som nu gör det möjligt att odla fram DC från monocyter har ökat kunskapen om DC. Vi har använt monocytkomponenter DC för att studera hur inflammatoriska signaler och allergiframkallande proteiner (gräspollen och tvättmedelsenzym) påverkar DC. Vi har även studerat en cellinjektion av DC:s potential att bli ”DC-like” och utvecklat ett modellsystem för framtidiga DC studier. Med hjälp av genchiptekniken, som varit central i den här avhandlingen, har vi kunnat studera hur genuttrycket i DC påverkas av olika sorts stimulans som t.ex. inflammation eller allergena proteiner. Teknologin gör det idag möjligt att analysera tiotusentals genuttryck, d.v.s. i princip hela det humana genomet, samtidigt. Våra data visar att inflammatorisk stimulering av DC resulterar i en dramatisk och global omorganisation av DC, s.k. utmognad, som innefattar
hundratals gener. Vi visar också att allergena proteiner påverkar genuttrycket av DC och att detta skiljer sig åt mellan friska och allergiska personer. Vi kunde även påvisa skillnader i genuttryck hos T celler, från friska och allergiska individer, som odlats tillsammans med allergen-stimulerade DC. En stor del av genuttrycket kunde kopplas till ett allergiskt immunssvar och många gener var förut inte beskrivna i allergisammanhang. Detta banar vägen för fortsatt kartläggning av vilka molekylära mekanismer som är involverade i interaktionen mellan DC och T celler i allergi.

Även om dessa s.k. in vitro-deriverade DC fungerar som komplement till humana DC och kan ge oss mycket information om DCs biologi så är det viktigt att studera hur DC fungerar in vivo. Vi har därför isolerat olika populationer av DC från humant blod och tonsiller och studerat skillnader i ytmarkörer och genuttryck mellan dessa subtyper. Med genchipteknik har vi kunnat hitta olika mönster av genuttryck som är specifika för de olika DC populationerna. Denna karaktärisering och identifiering av vilka gener i det humana genomet som är aktiva i respektive DC population ger oss information om olika subtypers specialisering samt hur genuttrycket i DC skiljer sig åt mellan blod och lymfoida organ. Det senare ökar vår förståelse av DCs migrationsmönster och funktionellt engagemang.
ACKNOWLEDGEMENTS

This work would not have been possible without the support of numerous people and I would like to express my gratitude to:

First and foremost, my head supervisor Prof. Carl Borreback – for allowing me the opportunity to be part of your research team. Thanks for always being optimistic and enthusiastic and for putting up with my impatience. These 5 years of PhD studies have been a wonderful experience and I greatly appreciate your encouragement and help.

Bengt - my assistant supervisor during the master project and first years/projects as a PhD student. You introduced me to tonsils, cell sorting, confocal microscopy etc and made me realise the marvels of dendritic cells. Deepest thanks for your enthusiasm, criticism and support throughout my PhD studies, you inspired me.

Collaborators in the allergy projects: Åsa and Kristina La – thanks for always being relaxed and friendly, and giving me perspective on things. Kristina Lu – for enthusiasm and curiosity. You three brighten up the cell lab. Ann-Charlott – you have provided invaluable help throughout the years with microarray research. Also, thanks to all the allergy-experts outside the department for productive collaborations and interesting discussions – Erwin Roggen, Magnus Korsgren and Lennart Greiff. I would also like to express my gratitude to all collaborators in the EU project HuCellAll for stimulating teamwork and organisation of wonderful meetings. It has been a pleasure working with all of you.

Siggi, Mats and Christer - for input, help and/or stimulating teamwork. Tommie and Marianne – for assistance and friendly chats. Importantly, thanks to all people helping me with input and corrections of the manuscripts and thesis.

The European Commission (FP5 project HuCellAll) and the National Science Council (VR-M) for providing financial support for these projects.

I want to thank all the people at Immunotechnology for providing an enjoyable and inspirational atmosphere at work and for all the excellent goose dinners, Christmas parties, dissertations and Friday pub nights. Many people have contributed to this thesis, on and off work hours. Special thanks to Sara, my precious room mate, for invaluable support, help and companionship throughout the years. Lavinia and Helena – for big hearts and open minds. You three make every day brighter and I truly value your friendship.
Carl-Magnus, for always being patient and helpful. I greatly appreciate your genuine interest in people and your calm, which many times in the lab have balanced out my frustration.

Peter, Fredrik and Johan for great laughs and crazy PhD parties.

A great hug to all my friends outside the department for patience and never-ending encouragement. A special thanks to: Sara G – my old “kombo” who know me better than most. You are the reason I contacted the department to start with. Carin and Emily – for friendship since childhood. I wish you would stop being such enthusiastic travellers and come back to Sweden. Sara H and Ola - my soulmates in science – for lunches, discussions and wild times.

My parents Eva and Rolf-Åke – for everyday advise, love and endless support. Elin, Anna and Anton – for laughs and hugs. Elisabeth and Göran for your generosity and open-door mentality.

Finally, thank you Daniel for your love, intelligent calm and for making me happy. We have gone through this process side by side and you beat me by a week.
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