

TABLE OF CONTENTS

1. OBJECTIVES OF THIS STUDY	1
2. SUMMARY	3
3. ZUSAMMENFASSUNG	5
4. GENERAL INTRODUCTION	7
4.1. Influenza virus infection	7
4.1.1. Influenza virus A and its life cycle	8
4.2. Immunological resistance to influenza virus infections	10
4.2.1. Recognition of respiratory viruses	11
4.2.2. Dendritic cells: Link between innate and adaptive immunity	12
4.2.3. Adaptive immunity to respiratory virus infections	15
4.3. Plasmacytoid dendritic cells in immunity	17
4.3.1. Phenotype and development of plasmacytoid dendritic cells	18
4.3.2. Activation of plasmacytoid dendritic cells	19
4.3.2.1. Expression of Toll-like receptors and recognition of nucleic acids	19
4.3.2.2. Downstream signaling pathways of TLR7 and TLR9	20
4.3.3. Role of activated plasmacytoid dendritic cells in immune responses	22
4.4. Migratory properties of plasmacytoid dendritic cells	25
4.4.1. Leukocyte migration paradigm	25
4.4.2. Localization and migration of pDCs in the steady-state	26
4.4.3. PDC trafficking to sites of inflammation	27
4.5. Role of plasmacytoid dendritic cells in pathology	28
4.5.1. Plasmacytoid dendritic cells in viral infections	28
4.5.2. Plasmacytoid dendritic cells in autoimmune disorders and other diseases	29

5. MATERIALS AND METHODS	31
5.1. Mice	31
5.2. Reagents	32
5.2.1. Viruses	32
5.2.2. Cell lines	32
5.2.3. Antibodies	33
5.2.3.1. Antibodies for flow cytometry	33
5.2.3.2. Blocking antibodies in homing studies	35
5.2.3.3. Antibodies for IFN- α ELISA	36
5.2.3.4. Antibodies for immunoblotting	36
5.2.4. Chemokines	36
5.2.5. Chemicals	36
5.2.6. Cell culture reagents	38
5.2.7. Buffers and media	39
5.2.8. Peptides	39
5.3. Methods	40
5.3.1. Genotyping and Phenotyping of mice	40
5.3.1.1. Tailbleeds	40
5.3.1.2. PCR (Polymerase chain reaction)	40
5.3.1.2.1. PCR of Ikaros ^{L/L} mice	40
5.3.1.2.2. PCR of DPE ^{GFP} x RAG-1 ^{-/-} mice	41
5.3.2. Cell culture	42
5.3.3. Viral assays	42
5.3.3.1. Propagation and purification of viruses	42
5.3.3.2. Titration of viral preparations and lung homogenates on MDCK cells	43
5.3.3.3. Hemagglutination assay	44
5.3.3.4. Hemagglutination inhibition assay	44
5.3.4. <i>In vivo</i> Flt-3L-treatment	45
5.3.5. Isolation and activation of plasmacytoid dendritic cells and myeloid dendritic cells	45

5.3.5.1. Isolation of DC subsets by flow cytometry activated cell sorting (FACS)	45
5.3.5.2. <i>In vitro</i> activation of pDCs and mDCs	46
5.3.5.3. <i>In vivo</i> activation of pDCs	46
5.3.6. Influenza virus infection	46
5.3.7. Cytospins and Microscopy	46
5.3.8. Supernatant analysis of activated dendritic cell cultures	47
5.3.8.1. ELISA	47
5.3.8.2. Multianalyte profiling	47
5.3.9. Preparation of cell lysates and immunoblotting	48
5.3.10. Migration assays	48
5.3.10.1. Chemotaxis assay	48
5.3.10.2. Homing assays	49
5.3.10.2.1. Adoptive transfer of pDCs	49
5.3.10.2.2. Homing of endogenous pDCs in response to Thioglycollate-induced inflammation	49
5.3.10.2.3. Competitive homing assay in a peritonitis-model	50
5.3.11. Flow cytometry analysis	50
5.3.11.1. Preparation of tissue samples	50
5.3.11.2. Staining procedure for flow cytometry	51
5.3.11.3. Staining procedure for intracellular cytokines	52
5.3.11.4. Staining for intracellular phosphoproteins	52
5.3.12. Gene chip microarrays	52
5.3.12.1. Sample preparation	53
5.3.12.2. Target preparation and gene chip hybridization	53
5.3.12.3. Data Analysis	54
5.3.13. T cell assays	54
5.3.13.1. T cell enrichment by magnetic activated cell sorting (MACS)	54
5.3.13.2. Labeling procedure with CFSE	55
5.3.13.3. <i>In vitro</i> proliferation assay	56
5.3.13.4. <i>In vivo</i> proliferation assay	56
5.3.13.5. Peptide restimulation for cytokine production	57
5.3.14. Statistical Analysis	57

CHAPTER 1

6. INFLUENZA VIRUS PR8 AND CPG 1826 OLIGONUCLEOTIDE INDUCE DIFFERENTIAL ACTIVATION OF PLASMACYTOID DENDRITIC CELLS	58
6.1. Introduction	58
6.2. Results	60
6.2.1. Transgenic DPE ^{GFP} mice harbor pDCs that express high levels of GFP	60
6.2.2. GFP ^{hi} pDCs respond to Flt-3 ligand and can be expanded as the only cell subset that expresses GFP in DPE ^{GFP} xRAG-1 ^{-/-} mice	63
6.2.3. Isolation of pDCs from Flt-3L-treated DPE ^{GFP} xRAG-1 ^{-/-} mice	65
6.2.4. pDCs stimulated with influenza virus A/PR/8 produce higher amounts of IFN- α than pDCs activated by CpG 1826, which express higher levels of costimulatory molecules	67
6.2.5. Maturation of PR8-activated pDCs but not CpG-activated pDCs is type I IFN- dependent	69
6.2.6. pDCs activated by CpG 1826 induce higher proliferation of T cells <i>in vitro</i> and <i>in vivo</i> compared to PR8-stimulated pDCs	70
6.2.7. Distinct transcriptome signatures define differentially activated pDCs	72
6.2.7.1. Change in gene transcripts of pDCs activated by PR8 virus and CpG 1826	72
6.2.7.2. Differential induction of IFN- α genes and transcripts of costimulatory molecules by PR8 virus- and CpG 1826- activated pDCs	75
6.2.7.3. Differential cytokine and chemokine expression profiles by pDCs activated by PR8 virus and CpG 1826	77
6.2.7.4. Induction of distinct signaling pathways by pDCs activated by PR8 virus and CpG 1826	81
6.2.7.5. Comparable upregulation of antiviral and interferon-stimulated genes in pDCs activated by PR8 virus or CpG 1826	83
6.2.8. pDCs acquire discrete phenotypes during influenza virus infection or after injection with CpG 1826 <i>in vivo</i>	85
6.3. Discussion	87

CHAPTER 2

7. MIGRATORY PROPERTIES OF PLASMACYTOID DENDRITIC CELLS	94
7.1. Introduction	94
7.2. Results	96
7.2.1. Expression pattern of adhesion molecules and chemokine receptors by pDCs	96
7.2.2. Differential expression of L-selectin on circulating and splenic immature and activated pDCs	100
7.2.3. Chemotactic response of immature and mature pDCs to CCL21 and CXCL12	103
7.2.4. Homing behavior of immature pDCs	103
7.2.5. Immature blood-borne pDCs home to the inflamed peritoneal cavity in a $\text{G}\alpha_i$ -protein coupled receptor-dependent manner	105
7.2.6. Accumulation of pDCs at sites of inflammation is selectin-dependent	106
7.3. Discussion	109

CHAPTER 3

8. THE ROLE OF PDCS IN IMMUNITY TO INFLUENZA VIRUS INFECTION	113
8.1. Introduction	113
8.2. Results	115
8.2.1. pDC numbers increase in lungs and airways of influenza PR8-infected mice	115
8.2.2. Ikaros ^{L/L} mice lack peripheral pDCs	117
8.2.3. Normal maturation of mDC but impaired IFN- α production of splenic cells from Ikaros ^{L/L} mice following stimulation with PR8 virus <i>in vitro</i>	119
8.2.4. Ikaros ^{L/L} mice mount a normal immune response to influenza virus infection	121

Table of contents

8.2.5. B220 ⁻ CD11c ⁻ mPDCA-1 ⁺ pDC precursors in the bone marrow of Ikaros ^{L/L} mice do not differentiate into conventional pDCs after Flt-3L treatment and influenza virus infection <i>in vivo</i>	122
8.2.6. Ikaros ^{L/L} mice have impaired early T cell recruitment to the airways during influenza virus infection	126
8.2.7. Activation of antigen-specific CD8 ⁺ T cells and differentiation into effector T cells is normal in Ikaros ^{L/L} mice during PR8 infection	127
8.2.8. Generation of memory CD8 ⁺ T cells is normal in Ikaros ^{L/L} mice after PR8 infection	130
8.3. Discussion	132
9. GENERAL DISCUSSION	137
9.1. Differentiation of activated pDCs – a model for two distinctly polarized subsets	138
9.2. Migratory properties of pDCs – cues to their function?	143
9.3. The role of pDCs in viral infections	148
9.4. Conclusions and future outlook	152
10. APPENDIX	154
10.1. Tables of homing molecules	154
10.2. List of Figures	162
10.3. List of Tables	165
11. REFERENCES	166
12. PUBLICATIONS	180
13. CURRICULUM VITAE	181