

# Contents

<b>List of Figures</b>	<b>I</b>
<b>List of Tables</b>	<b>III</b>
<b>List of Abbreviations</b>	<b>IV</b>
<b>1 Summary</b>	<b>1</b>
<b>2 Zusammenfassung</b>	<b>3</b>
<b>3 Introduction</b>	<b>6</b>
3.1 The mammalian immune system . . . . .	6
3.1.1 Peripheral and central tolerance . . . . .	6
3.1.2 CD4+ helper T cells . . . . .	7
3.1.3 Differentiation of naive Th cells into Th subset . . . . .	7
3.1.4 CD4+FoxP3+ regulatory T cells . . . . .	8
3.2 Systemic lupus erythematosus . . . . .	9
3.2.1 The role of B cells in SLE . . . . .	9
3.2.2 The role of CD4+ T cells in SLE . . . . .	9
3.2.3 The role of CD4+FoxP3+ regulatory T cells in SLE . . . . .	10
3.3 Lupus nephritis . . . . .	10
3.3.1 CD4+ T cells in the pathogenesis of LN . . . . .	10
3.3.2 CD8+ T cells in the pathogenesis of LN . . . . .	11
3.4 Current treatment of SLE . . . . .	11
3.5 Interleukin-2 . . . . .	11
3.5.1 Dysregulation of IL-2 production in SLE . . . . .	13
3.5.2 Current therapies using IL-2 . . . . .	13
3.6 Murine models of SLE . . . . .	14
3.7 Aim of this thesis . . . . .	15
<b>4 Material and Methods</b>	<b>16</b>
4.1 Materials . . . . .	16
4.2 Mice . . . . .	19
4.3 Monitoring of disease activity . . . . .	19
4.3.1 Urine dipsticks . . . . .	19
4.3.2 Blood urea nitrogen test . . . . .	20
4.4 IL-2 treatment (recombinant mouse) in NZB/W mice . . . . .	20
4.5 IL-2 treatment (human, Proleukin®) in NZB/W mice . . . . .	21
4.6 RIIBxDEREG mice . . . . .	21
4.6.1 Breeding of RIIBxDEREG . . . . .	21
4.6.2 Depletion of GFP+Treg in RIIBxDEREG mice . . . . .	22
4.6.3 IL-2 treatment in RIIBxDEREG mice . . . . .	22
4.7 Histological analyses . . . . .	22
4.7.1 Hematoxylin and Eosin staining . . . . .	22
4.7.2 Activity index . . . . .	23

4.7.3	Immunofluorescence staining . . . . .	23
4.8	IL-2 neutralization . . . . .	24
4.9	Enzyme-linked immunosorbent assay . . . . .	24
4.10	Collection of samples . . . . .	24
4.10.1	Blood collection . . . . .	24
4.10.2	Organ harvesting . . . . .	25
4.11	Flow cytometry . . . . .	25
4.11.1	Extracellular staining . . . . .	25
4.11.2	Intracellular staining . . . . .	25
4.11.3	General gating strategy to distinguish Treg from Tcon . . . . .	26
4.11.4	Apoptosis analyses by Annexin V staining . . . . .	26
4.12	Fluorescence-activated cell sorting . . . . .	27
4.13	Cell culture . . . . .	27
4.14	Labeling of cells with CFSE . . . . .	27
4.15	<i>In vitro</i> suppression assay . . . . .	28
4.16	Magnetic-activated cell sorting of intrarenal CD4+ T cells . . . . .	28
4.17	Analysis of the <i>in vitro</i> IL-2 and IFN $\gamma$ production . . . . .	28
4.18	RNA expression analyses . . . . .	29
4.18.1	RNA isolation and reverse transcription . . . . .	29
4.18.2	Quantitative real-time PCR . . . . .	29
4.19	Statistics . . . . .	30
<b>5</b>	<b>Results</b> . . . . .	<b>31</b>
5.1	Intrarenal IL-2 deficiency in NZB/W F1 mice . . . . .	31
5.1.1	Progressive homeostatic imbalance between intrarenal Treg and Tcon during development of lupus nephritis . . . . .	31
5.1.2	IL-2 deprived phenotype of intrarenal Treg and progressive hyperactivity of intrarenal Tcon during the development of lupus nephritis . . . . .	34
5.1.3	Organ-related deficiency of IL-2 producing CD4+ T cells in LN . . . . .	36
5.1.4	IL-2 neutralization accelerates intrarenal Tcon hyperactivity and tissue inflammation . . . . .	36
5.1.5	Cytokine production after <i>in vivo</i> IL-2 neutralization . . . . .	39
5.2	Impact of an IL-2 therapy in NZB/W mice . . . . .	39
5.2.1	Short term IL-2 therapy increases the size of Treg pool in the kidney . . . . .	40
5.2.2	Analysis of the suppressive capacity of Treg under short term IL-2 therapy . . . . .	43
5.2.3	Anti-dsDNA antibody levels are not influenced by IL-2 therapy . . . . .	44
5.2.4	Long term IL-2 therapy increases intrarenal nTreg and reduces Tcon hyperactivity . . . . .	44
5.2.5	IL-2 therapy reduces intrarenal PD-1+ memory T cells . . . . .	47
5.3	Clinical and histological effects of IL-2 therapy in NZB/W mice . . . . .	47
5.3.1	Long-term IL-2 therapy reduces renal inflammation . . . . .	47
5.3.2	Anti-dsDNA antibody levels are not influence by long term IL-2 therapy . . . . .	48
5.3.3	Long term IL-2 therapy prolongs the survival of NZB/W mice . . . . .	49
5.3.4	Human IL-2 (Proleukin <sup>®</sup> ) therapy prolongs the survival and delays the disease progression in NZB/W mice . . . . .	49
5.4	Specific depletion of regulatory T cells in the RIIBxDEREG lupus mouse . . . . .	50
5.4.1	RIIBxDEREG mice develop kidney malfunction with advancing age . . . . .	51

5.4.2	Peripheral blood T cells in RIIBxDEREG mice evolve a progressive Tcon hyperactivity and Treg develop an IL-2 deprived phenotype	52
5.4.3	<i>In vivo</i> Treg depletion results in Tcon hyperactivity	54
5.4.4	Treg depletion influences the disease acceleration	57
5.4.5	IL-2 therapy in a Treg deficient environment causes early death of RIIBxDEREG mice	58
5.4.6	In a Treg-deficient environment IL-2 therapy causes Tcon hyperactivity and leads to the consumption of IL-2 by CD8+ T cells	59
<b>6</b>	<b>Discussion</b>	<b>63</b>
6.1	Progressive accumulation of T cell subsets in the inflamed kidney	63
6.2	Intrarenal Treg in lupus prone mice	64
6.3	Intrarenal Tcon hyperactivity	64
6.4	IL-2 deprived phenotype of intrarenal Treg in LN	65
6.5	IL-2 neutralization in young, healthy NZB/W mice results in an acceleration of Tcon activity	66
6.6	IL-2 and its impact to modulate intrarenal T cells	67
6.7	Long term IL-2 treatment effects	68
6.8	Humoral immune responses by IL-2 therapy	69
6.9	Dose-dependent clinical effects by IL-2 therapy	70
6.10	Renal involvement in RIIBxDEREG mice	70
6.11	T cell phenotype of RIIBxDEREG mice with advancing age	70
6.12	Specific <i>in vivo</i> Treg depletion in RIIBxDEREG mice	71
6.13	Effects of an IL-2 therapy in Treg-depleted RIIBxDEREG mice	72
6.13.1	Modulation of CD4 T cell subsets by IL-2 therapy in Treg-depleted RIIBxDEREG mice	72
6.13.2	Effects of an IL-2 therapy on CD8+ T cells Treg-deficient RIIBxDEREG mice	73
6.14	Limitation of the RIIBxDEREG mouse model	74
6.15	Conclusions and Outlook	75
	<b>Bibliography</b>	<b>V</b>
	<b>Appendix</b>	<b>XVI</b>
	Supplemental data	XVII
	Eidesstattliche Erklärung	XXV