

CONTENTS

ABBREVIATIONS.....	4
1. INTRODUCTION	7
1.1. Chemokines and chemokine receptors.....	7
1.2. Entry of lymphocytes into lymphoid organs	9
1.3. CCR7 and CXCR5 compartmentalize secondary lymphoid organs in homeostasis	10
1.4. Activation of T cells in secondary lymphoid organs	12
1.5. Germinal centers – product of T_{FH} cell - B cell interaction	13
1.6. Molecular interaction of activated T and B cell in lymphoid organs.....	14
1.7. The CD28 family.....	15
1.8. Role of ICOS in regulating activated T and B cells.....	16
1.8.1. ICOS in autoimmunity and infections	17
1.9. TNF/TNFR family	18
1.10. CD4 T cell subsets in peripheral blood	18
2. MATERIALS AND METHODS	22
2.1. Materials	22
2.1.1. Antibodies	22
2.1.2. Recombinant proteins.....	22
2.1.3. Chemicals	23
2.1.4. Primary human material	23
2.1.5. Magnetic cell sorting	23
2.1.6. Flow cytometry and FACS sorting system	25
2.1.7. Transwell plates.....	25
2.1.8. Co-culture medium	25
2.1.9. RNA Isolation.....	25
2.1.10. Gene chips arrays	26
2.1.11. Real-time PCR.....	26
2.1.12. Miscellaneous	28
2.1.13. Programs and software	28
2.2. Methods.....	29
2.2.1. Cell separation strategies	29
2.2.2. Functional assays.....	31
2.2.3. RNA level.....	33

CONTENTS

2.2.4. Secondary validation	36
2.2.5. Buffers	38
3. RESULTS	40
3.1. Isolation of CD4 T cell subsets.....	40
3.1.1. Identification and isolation of tonsillar CD4 T cell subsets based on co-expression of CXCR5 and ICOS	40
3.1.2. Isolation of CD4 T cell subsets from peripheral blood	43
3.2. Varying levels of CXCR5 on CXCR5 ^{lo} ICOS ^{int} and CXCR5 ^{hi} ICOS ^{hi} cells does not confer differential chemotactic potential.....	43
3.3. Spectrum of chemokine receptor expression on tonsillar and peripheral blood CD4 T cell subsets	44
3.4. Follicular B cell help <i>in vitro</i> correlates with CXCR5/ICOS expression	46
3.5. CXCL13 secretion <i>in vitro</i> correlates with CXCR5/ICOS.....	47
3.6. CXCR5 ^{hi} ICOS ^{hi} CD4 T cells have a reduced proliferative potential and enhanced susceptibility to apoptosis	49
3.7. Follicular B helper T cell activity is independent of CD57 expression	53
3.8. Transcript signatures distinguished follicular B helper T cells from central memory and effector memory T cells.....	56
3.8.1. Amount of RNA and quality of the labeled RNA.....	56
3.8.2. Array metrics showed standard data quality	57
3.8.3. Identification of differentially expressed genes	57
3.8.4. Sorting markers displayed expression values as expected	58
3.8.5. Clustering showed distinct gene expression profiles of the CD4 T cell subsets	59
3.8.6. Principal component analysis (PCA) revealed distinctive features of the tonsillar and peripheral blood subsets.....	61
3.8.7. Self-organizing maps on tonsillar subsets indicates CXCR5 ^{hi} ICOS ^{hi} cells might be derived from CXCR5 ⁻ ICOS ^{-lo} cells	62
3.9. Validation of microarray data by RT-PCR.....	63
3.10. Selected genes differentially expressed among tonsillar and peripheral blood subsets	64
3.10.1. Chemokines and chemokine receptors	65
3.10.2. Adhesion molecules	66
3.10.3. Costimulatory/inhibitory molecules	66
3.10.4. Cytokines and Cytokine receptors.....	67
3.10.5. Signaling molecules.....	67
3.10.6. Transcription factors	68

CONTENTS

3.10.7. NOTCH and Frizzled	68
3.10.8. Apoptosis	68
4. DISCUSSION.....	69
4.1. Activation and differentiation of Naïve CD4 T cells in secondary lymphoid organs.....	69
4.2. CD4 T cell subsets in secondary lymphoid organs.....	70
4.2.1. Balanced migration of activated CD4 T cell subsets into B cell follicles	70
4.2.2. Restricted expression of chemokine receptors on tonsillar CD4 T cell subsets	71
4.2.3. CXCR5 ^{hi} ICOS ^{hi} cells cluster readily in spite of poor expression of adhesion molecules	73
4.3. Dynamic control of proliferation and apoptosis in Tonsillar CD4 T cell subsets	74
4.3.1. Expression of positive and negative costimulatory molecules might contribute to the rate of proliferation and apoptosis.....	77
4.4. Potent Follicular B helper activity resides in CXCR5 ^{hi} ICOS ^{hi} cells	79
4.5. Follicular B helper T cells might represent a non-Th1/Th2 cell subset.....	83
4.6. CD57 is coexpressed on a subset of CXCR5 and ICOS expressing cells ...	84
4.7. Gene expression profiles reveal that T _{FH} cells are a distinct subset compared to central memory and effector memory T cells.....	85
5. ZUSAMMENFASSUNG.....	88
6. SUMMARY.....	91
7. REFERENCES.....	93
8. CURRICULUM VITAE.....	104
9. APPENDIX.....	106