

Aus der Abteilung Medizinische Mikrobiologie und Infektionsimmunologie des
Institut für Infektionsmedizin der
Charité-Universitätsmedizin Berlin
Campus Benjamin Franklin
Abteilungsleiter: Prof. Dr. med Helmut Hahn

**Characterization of a novel subpopulation of autoantibody-producing
B cells in human blood**

Inaugural-Dissertation
zur
Erlangung der medizinischen Doktorwürde
der Charité-Universitätsmedizin Berlin
Campus Benjamin Franklin

vorgelegt von
Anne Schaefer
aus Frankfurt/Main

Referent: Priv. Doz. Dr. Ralf Ignatius

Koreferent: Prof. Dr. T. Blankenstein

Gedruckt mit Genehmigung der Charité-Universitätsmedizin Berlin
Campus Benjamin Franklin

Promoviert am: 3.9.2004

Contents

| | |
|--|-----------|
| 1. Introduction | 5 |
| <i>1.1. Autoimmune diseases</i> | 5 |
| 1.1.1. Autoimmune nature of rheumatoid arthritis | 5 |
| <i>1.2. B cells</i> | 6 |
| <i>1.3. B cell development in the bone marrow</i> | 6 |
| <i>1.4.. Antigen-dependent B cell selection</i> | 7 |
| 1.4.1. Clonal deletion and anergy | 8 |
| 1.4.2. Receptor editing | 9 |
| <i>1.5. Peripheral B cell maturation</i> | 10 |
| <i>1.6. Self-reactive B cells</i> | 11 |
| <i>1.7. Objectives</i> | 13 |
| 2. Materials | 14 |
| <i>2.1. Antibodies</i> | 15 |
| <i>2.2. Enzymes and proteins</i> | 17 |
| <i>2.3. Nucleotides and nucleic acids</i> | 18 |
| <i>2.4. Buffers, chemicals, and solutions</i> | 18 |
| <i>2.5. Commercial kits and special chemicals</i> | 19 |
| <i>2.6. Culture media and additives</i> | 20 |
| <i>2.7. Bacteria and vectors</i> | 21 |
| <i>2.8. Cell lines</i> | 21 |
| <i>2.9. Special instruments and software</i> | 21 |
| 3. Methods | 23 |
| <i>3.1. Antibody production</i> | 23 |
| 3.1.1. B cell preparation and purification | 23 |
| 3.1.2. cDNA synthesis | 24 |
| 3.1.5. PCR amplification of human VH, V κ , and V λ gene rearrangements from individual B cells | 25 |

| | |
|---|----|
| 3.1.6. Sequencing | 26 |
| 3.1.7. Generation of the IgH, Igκ, and Igλ expression vectors | 26 |
| 3.1.8. Antibody production | 28 |
| <i>3.2. Analysis of the antibody specificity</i> | 30 |
| 3.2.1. Anti-nuclear antibody (ANA) analysis | 30 |
| 3.2.2. Polyreactivity assay | 31 |
| <i>3.3. Analysis of the antigenic specificity of the rheumatoid arthritis patient sera</i> | 32 |
| 3.5. Transgenic mice | 32 |
| 3.6. Statistical analysis | 33 |
| 4. Results | 34 |
| 4.1. General strategy for the analysis of the antibody repertoire expressed by V-preB ⁺ L ⁺ B cells | 34 |
| 4.1.1. Sequence analysis of single cell-derived IgH and IgL gene rearrangements | 35 |
| 4.2. Self-reactivity/polyreactivity of antibodies generated by individual V-preB ⁺ L ⁺ cells | 40 |
| 4.2.1. V-preB ⁺ L ⁺ B cell derived antibodies recognize nuclear antigens | 40 |
| 4.2.2. Anti-GPI activity of VpreB ⁺ L ⁺ B cells | 42 |
| 4.2.3. Polyreactivity of antibodies derived from individual V-preB ⁺ L ⁺ B cells | 43 |
| 4.3. Antibodies against GPI or polyreactive antibodies: linkage to human rheumatoid arthritis? | 45 |
| 4.3.1. Anti-GPI activity in sera of rheumatoid arthritis patients | 45 |
| 4.4. Transgenic mice carrying a particular V-preB ⁺ L ⁺ B cell IgH chain gene display a block in B cell development | 46 |
| 5. Discussion | 48 |
| 6. Abstract | 53 |
| 7. References | 54 |
| 8. Abbreviations | 60 |