The role of antigen in the development of B-cell chronic lymphocytic leukemia
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Summary

Evidence suggests that the B-cell receptor (BCR) plays a pivotal role in the development and expansion of malignant B-cells. In support, activating mutations in the signaling motifs of Igα and Igβ, resulting in constitutive antigen-independent BCR signaling, have been identified in diffuse-large B-cell lymphomas. Studies on the structure of lymphoma-expressed BCRs suggest that antigen-dependent BCR signaling may also play a role in the development of distinct types of B-cell lymphomas. Mantle cell lymphomas (MCL), mucosa-associated lymphoid tissue (MALT)-lymphomas, splenic marginal zone lymphomas and chronic lymphocytic leukemias (CLL) express a restricted BCR repertoire, as compared to normal B-cells. Moreover, MCL, MALT-lymphomas and CLL can be grouped, based on similarities of the highly variable complementary determining region 3 (CDR3) of the immunoglobulin heavy chain variable region (IGHV), strongly suggesting that distinctive antigens are involved in the development of groups of these lymphomas (discussed in detail in Chapter 1). In this thesis, we addressed the specificity of stereotypic BCRs derived from lymphomas, focusing on CLL harboring mutated IGHV (M-CLL). Subsequently, we studied the role cognate antigen stimulation has on primary tumor cells in vitro.

In Chapter 2, we described MALT-lymphomas that express BCRs with specificity for the Fc-tail of IgG, so called rheumatoid factors (RF). We showed that soluble recombinant MALT-lymphoma RF-BCRs are of high-affinity and we provided evidence that these BCRs are not poly-reactive.

In Chapter 3, we identified a group of CLL, expressing somatically mutated IGHV3-7-encoded stereotypic BCRs with CDR3-homology to previously reported RF-BCRs. Soluble recombinant IgM (sIgM) of these CLL indeed bound to IgG, which depended on both the stereotypic IGHV3-7-encoded heavy chain as well as the stereotypic IGKV3-15 light chain. Most importantly, we show that interaction of RF-expressing primary CLL cells with their cognate antigen induces proliferation in vitro.

In Chapter 4, we described a group of four somatically mutated CLL, expressing unusually short stereotypic IGHV-CDR3s. sIgM of these stereotypic CLL were highly specific for the fungal antigen β-(1,6)-glucan. This specificity was shown to depend on the stereotypic IGHV3-7 heavy chain, the stereotypic IGKV2-24 light chain and a stereotypic amino acid in the IGHV-CDR3. Moreover, we provide evidence that these CLL bind to β-(1,6)-glucan with high-affinity and have undergone affinity maturation towards this antigen. Most importantly, we show that primary CLL cells that express
this stereotypic BCR, bind yeast particles \textit{in vitro} and proliferate when cultured in the presence of β-(1,6)-glucan.

In \textbf{Chapter 5}, we studied the specificity of subset #4 (IGHV4-34-M) CLL. \textit{IGHV4-34}-encoded antibodies are known to bind to poly-N-acetyllactosamine epitopes as superantigens, i.e. outside of conventional antigen binding sites. sIgM of subset #4 CLL uniformly bound to a NAL epitope expressed on cells of a distinct B cell line, whereas binding to NAL epitopes on other cell lines varied. Binding to the NAL epitope depended on both the stereotypic \textit{IGHV4-34}-encoded heavy chain as well as the \textit{IGKV2-30}-encoded light chain. Moreover, reversion of (subset-specific) somatic mutations in subset #4 sIgM reduced their NAL-binding capability, indicating that subset #4 CLL are affinity-selected for this epitope.

In \textbf{Chapter 6}, we explored methods to avoid the laborious process of recombinant antibody production by producing CLL-derived soluble Igs conveniently by \textit{in vitro} cell culture. Among the methods studied, stimulation with CD40L and Toll-like receptor ligands most efficiently induced plasmacytoid differentiation and IgM secretion by primary CLL cells. Using this method, we show that four out of seven CLL harboring unmutated \textit{IGHV} express a poly- and/or self-reactive BCRs, whereas none of four \textit{in vitro} produced IgMs of M-CLL were poly-reactive.

Collectively, these studies strongly suggest that MALT-lymphomas and M-CLL in majority are highly selected for single extrinsic antigens and that these antigens can be both self-antigens and exo-antigens. Our finding that primary CLL cells are responsive to stimulation with their cognate antigen suggests that antigen-dependent BCR signaling may drive CLL expansion \textit{in vivo}.