The antigen receptor in the pathogenesis of B-cell non-Hodgkin's lymphomas
Aarts, W.M.

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Summary

Normal B cells are continuously tested for the expression and quality of their B-cell antigen receptors (BCRs), or immunoglobulin (Ig) receptors, throughout their development. Similarly, a role for BCR-ligands has been proposed in the growth and/or genesis of some B-cell malignancies. In this thesis we explored the concept of antigen-driven lymphomagenesis by analyses of the BCRs of several B-cell malignancies.

In chapter 1, a general introduction is given on the development of B lymphocytes and the arguments in favor of a role for antigen recognition in B-cell malignancies are reviewed.

In chapter two, the BCRs of a panel of follicular lymphomas (FLs) are analyzed. The variable heavy (V<sub>H</sub>) chain genes were heavily mutated. Similar to normal antigen-experienced B cells, heavy chain isotype-switched FLs contained a higher amount of somatic mutation in their V<sub>H</sub> genes than IgM-expressing FLs. Based on the intraclonal variation is found in their immunoglobulin (Ig) V genes, it is believed that FLs are capable of ongoing somatic mutation. However, in our analysis of 4 FLs of which also relapse samples were available, we found no obvious accumulation in the amount of somatic mutations in 3 of 4 FLs, despite persistent intraclonal variation in the V<sub>H</sub> genes of most of these FLs. In addition, in relapse samples the intraclonal variation was consistently lower than in presentation biopsies. These results caused us to doubt whether somatic hypermutation persists in all FLs. Based on these findings we hypothesized that the hypermutation machinery is shut off after a certain point during an ongoing transformation process. The intraclonal variation found may have remained from an earlier period in tumor cell growth, in which somatic hypermutation did occur. In addition, we found in 3 of 4 FLs no support for the concept of BCR-based clonal evolution. Instead, different subclones with different mutation patterns seemed to be selected over time.

To extend these observations, we analyzed one of these FLs in more detail. This FL consisted of both IgM- and IgG-expressing tumor cells of the same clonal origin. Furthermore, material from different time points was available. We isolated samples of approximately 50 tumor cells by laser microdissection and amplified and sequenced V<sub>H</sub> gene transcripts (chapter 3). Remarkably, although this FL consisted of both IgM- and IgG-expressing cells, we found no evidence for active isotype switching, which suggested that the capacity to switch isotype may have been lost at a certain time point during the growth of this FL. In addition, some of the subclones of the sample of the first time point of this FL, closely resembled the subclone that
dominated the relapse sample. This suggested that the subclone of the relapse sample may have been pre-existent in the presentation biopsy, 9 years earlier. Furthermore, for another FL we proved by PCR and sequencing that the clone that dominated the relapse sample was already present as a minor clone at diagnosis, 2 years earlier. These data strongly suggested that the concept of clonal evolution guided by the BCR is not true for all FLs. Furthermore, our data imply that FLs may have retained fewer functional characteristics of germinal center B cells than generally assumed, in particular the capacities of ongoing somatic hypermutation and isotype switching may be lost.

In chapter 4, the V<sub>H</sub> and the V<sub>k</sub> chain genes of 12 MALT lymphomas are described. Variable numbers of somatic mutation were found. In addition, in one MALT lymphoma both IgM- and IgA-expressing tumor cells were present, indicating that this lymphoma had undergone an isotype switch at some point during its genesis. Furthermore, we tested the specificity of recombinantly produced tumor Ig of 7 MALT lymphomas. With none of these lymphoma-derived Igs, positive stainings were obtained on corresponding tissue sections. Among the MALT lymphomas investigated, one gastric MALT lymphoma was present. Although in the disease history of this patient an infection with Helicobacter pylori was observed, the tumor Ig did not react with H. pylori. Our data suggest that ligands of the tumor Igs are not present in the environment of the MALT lymphomas.

In chapter 5 the variable heavy chain genes of primary cutaneous B-cell lymphomas (PCBCLs) are described. PCBCLs are B-cell lymphomas that arise in the skin. At the moment of this study, virtually no data were present on the configuration of their BCRs. Their V<sub>H</sub> genes were found to contain somatic mutations and also, intraclonal variation was present in some of these PCBCLs. In addition, isotype variants of one PCBCCL were found. These findings indicate that PCBCLs are derived from (post) germinal center B-cells.

In chapter 6 the complex Ig gene of a B-CLL case is described. The B-CLL was accompanied by severe autoimmune hemolytic anemia. The V<sub>H</sub> locus contained a triplification of the JH gene. By consequence, multiple transcripts could be produced. In addition, an illegitimate splice variant was produced, due to the presence of somatic point mutations at critical sites in the expressed V4-34 gene segment. Theoretically, alternative splicing caused by somatic mutation may be an additional mechanism of IgV gene diversification and may potentially have significance for lymphomagenesis. The specificity of the tumor Ig is currently under investigation.

In chapter 7, the concept of antigen-driven lymphomagenesis is discussed. A limited
amount of data supports this concept. Although the tumor cells are derived from antigen-responsive B cells, our data on FLs and MALT lymphomas do not provide evidence that antigen-receptor ligands are essential for the growth of the lymphomas at the time of diagnosis. It is conceivable that despite the configuration of their Ig receptors, the tumor cells have become independent of signals elicited via these receptors. Instead, the tumor cells may be selected by virtue of various other genetic alterations.