The antigen receptor in the pathogenesis of B-cell non-Hodgkin's lymphomas
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**General discussion**

Lymphomagenesis is thought to be a multistep process. The most important cause for malignant transformation of B lymphocytes is the gain of genetic aberrations that alter the proliferation or life span of these lymphocytes. In addition to genetic changes, a role for antigen is proposed in the pathogenesis of several lymphomas, especially for the follicular lymphomas (FLs) and the mucosa-associated lymphoid tissue (MALT)-derived lymphomas. As outlined in the introduction (chapter 1), this concept is based on circumstantial evidence only. In the studies described in this thesis we have explored this concept.

An important argument in favor of a role for antigen in the pathogenesis of FLs is their architecture, which resembles that of normal germinal centers. In fact, the tumor cells in FLs grow in close contact with follicular dendritic cells. Similarly, in MALT lymphomas the tumor cells can colonize germinal centers. These features suggest that FDCs might present antigen to the tumor cells, thus providing necessary survival and/or growth signals. Indeed, the contact with the FDCs seems supportive, as Petrasch *et al.* have shown that the proliferation rate of neoplastic B cells within FDC clusters was higher than that of cells not associated with FDCs. However, Lampert *et al.* reported that antibodies against complement factor C3 did not stain FDCs in follicular lymphomas, in contrast to FDCs in reactive tonsils and lymph nodes. In accordance, several investigators had reported an absence of immunoglobulins on FDC networks in lymphomas. This raised the question whether FDCs in FLs are equally efficient in capturing immune complexes as those in normal germinal centers. More importantly, these data suggest that FLs may depend on FDCs for other reasons than stimulation by antigen. Particularly, FDCs, assisted by infiltrating T cells, may provide growth-sustaining signals, such as cytokines or contact-dependent signals.

The fact that most lymphomas retain their membrane Ig receptor (mIg) for many years even after years of disease, is suggestive for the importance of this receptor for lymphoma growth. The retention of the mIg is indeed remarkable, particularly because in many B-cell malignancies somatic hypermutation is thought to be ongoing. However, despite the fact that we found intraclonal variation in IgV genes of follicular lymphomas (chapter 2 and 3), we questioned if this is necessarily a reflection of ongoing somatic hypermutation. This was based on the observation that somatic mutations did not accumulate in time in a number of FLs. Furthermore, we consistently found a lower intraclonal variation in relapse samples of FLs. These phenomena were in accordance with other reports. We hypothesized that FLs, due to the accumulation of genetic alterations, might finally lose the capacity to actively mutate their V genes in time.
(chapter 2). Thus, at early stages of lymphoma growth, many subclones are still present. However, after selection caused by therapy or by other factors over time, only few subclones survive, which would explain the lower intraclonal variation found in relapses of FLs (chapter 2). In this model, the preservation of the B-cell antigen receptor (BCR) in later stages of FL disease is no proof of its significance, as it is not subject to somatic hypermutation. In addition, the need to retain a BCR may serve another purpose than ligand recognition, as was suggested by the data presented by Lam et al, who generated a transgenic mouse in which the BCR of mature B cells could be deleted by inducible gene targeting, resulting in cell death. A transgenic BCR specific for the hapten 4-hydroxy-3-nitrophenyl acetyl (NP), was used to generate the mice. In this artificial model, it is at least questionable whether the antigen receptors have encountered a ligand during B-cell development. Nonetheless, the need to retain this BCR was clearly demonstrated.

Many investigators use the often found high replacement (R) to silent (S) mutation ratios in the complementarity determining regions (CDRs) as indication for antigen selection in lymphomas. Low R/S ratios in the framework regions (FRs) are indicative of selection for preservation of the BCR. However, Chang and Casali reported that the codon composition of FRs and CDRs is different: point mutations in the CDRs have a higher intrinsic chance to result in an amino acid substitution than those in the FRs. In accordance, high R/S ratios are also found in the CDRs of non-productive, and therefore unselected, rearrangements. Also, additional replacement mutations in the CDRs can be unfavorable in already selected Ig with high affinity. Consequently, the R/S values of CDRs can not be used as arguments for or against antigenic selection, indicating that these analyses should be interpreted with caution. Assuming that the patterns of somatic mutations in the V genes of lymphomas are a reflection of antigen selection, it is unclear at what stage in lymphoma development this selection took place. Not unlikely, such patterns are a remainder of antigen selection processes from an early stage, e.g. in cells in which a first hit occurred, but that did not yet become fully transformed. If somatic hypermutation indeed ceases in the course of further transformation, these mutational patterns remain unaltered at later stages of disease. Thus, the patterns found at these late stages are not per se an indication that antigen selection is still taking place.

Based on the observed intraclonal nucleotide differences in IgV genes of B-NHLs, "evolutional trees" have been constructed, which suggested that antigen selection took place during the tumor phase of the lymphomas. However, to confidently construct such a tree, many clones need to be analyzed, as was shown by Levy et al. These investigators observed that genealogical trees for both \( V_H \) and \( V_L \) genes of 6 heterohybridomas could not be
superimposed. Moreover, the data presented in chapters 2 and 3 question BCR-guided clonal evolution in at least some FLs. In one FL we found evidence that a minor clone present at diagnosis dominated the relapse sample without evidence of BCR evolution (chapter 3). Similarly, no BCR evolution was observed in one gastric MALT lymphoma of which we had tissue material of two time points (chapter 4). The amount of somatic mutations was lower in the relapse sample than in the initial biopsy (15 and 16 mutations were found, respectively). Most likely, a subclone had gained growth advantage over time, based on selection criteria other than BCR affinity.

An important observation in favor of the concept of antigen-driven lymphomagenesis, is the association of MALT lymphomas with chronic inflammation, either caused by organ-specific autoimmune diseases or by an infection with for example Helicobacter pylori. Of note, no reactivity with H. pylori was demonstrated of tumor Ig from at least two gastric MALT lymphomas (and chapter 4). Instead, it was claimed that the MALT lymphoma cells recognized various self-antigens. However, the exact ligands of the MALT lymphomas have never been identified. We did not find specific stainings with tumor Ig from MALT lymphomas on corresponding tissue sections, suggesting that the ligands of these MALT lymphomas were not present in their environment (chapter 4). Alternatively, antigen may play an indirect role in the pathogenesis of gastric MALT lymphomas, as the tumor-infiltrating T cells were H. pylori specific.

Also for other lymphomas attempts have been made to elucidate the specificity of their antigen receptors. Only one report describes the reactivity of tumor Ig from FLs: Dighiero et al tested tumor Ig from 31 FLs by ELISA and via immunohistochemical stainings. In 8 cases reactivity with various self-antigens was found, for example actin, tubulin, IgG or DNA. Similarly, the BCRs of 17 of 32 B-CLLS studied reacted with various autoantigens, such as ssDNA, dsDNA or IgG. In two B-CLLS, the neoplastic cells were shown to produce anti-red blood cell antibodies. However, in most patients with B-CLL, accompanied by autoimmune hemolytic anemia (AIHA), the anti-red blood cell antibodies are thought to be of non-neoplastic, polyclonal B-cell origin. Tumor Ig derived from two B-CLLS of patients infected with the human T-lymphotropic virus type I (HTLV-I) were reported to recognize HTLV-I derived antigens. Ng et al reported that IgM produced by 2 Burkitt’s lymphoma cell lines of AIDS patients reacted with either gp160 of human immunodeficiency virus type I (HIV-1) or with human IgG, respectively. However, this was not confirmed by three other studies, in which no HIV reactivity of Ig derived from 9 AIDS-related lymphomas was found. Instead, reactivity with self-antigens was reported in 5 of 9 cases. Virus-associated lymphomagenesis was suggested.
for a murine B-cell lymphoma, BCL₁, that recognized murine leukemia virus (MuLV). This lymphoma grew poorly in vitro. If splenic stromal cells that produced MuLV particles were added to the culture, BCL₁ lymphoma cells could be cultured for up to one month, suggesting a role for this ligand in the growth of this lymphoma. However, it was not reported if splenic stromal cells alone, i.e. without MuLV particles enhanced the proliferation of the lymphoma.

In summary, there is a limited amount of data that supports the concept of antigen-driven lymphomagenesis. Some ligands of various BCRs of B-NHL have been described, indicating that the tumor cells may be derived of (auto)antigen responsive B cells. However, it is unclear if these ligands are still 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.

References
