The role of immunoediting in lymphomas of immune-privileged sites

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Chapter 7

Summary
Diffuse large B-cell lymphoma (DLBCL) is currently regarded as one disease entity in the WHO classification, but is very heterogeneous in regard to clinical features such as site of presentation, dissemination pattern and prognosis. Based on site of presentation, DLBCL can be subdivided into nodal and extranodal DLBCL. Extranodal DLBCL comprise 40% of all DLBCL cases and present mostly in the gastrointestinal tract, but also in skin, bone and lung, central nervous system (CNS) and testis. The CNS and testicular DLBCL both arise in immune-privileged sites and are therefore referred to as immune-privileged site-associated DLBCL (IP-DLBCL). While both nodal and extranodal DLBCL subgroups are no less heterogeneous than DLBCL in general, the IP-DLBCL are much more homogeneous and share several clinical and molecular characteristics such as a poor prognosis, frequent dissemination to other immune-privileged sites and high expression levels of Bcl2 in the absence of t(14;18) translocations. A very characteristic feature for IP-DLBCL, which is much rarer in nodal DLBCL or non-IP extranodal DLBCL, is the loss of expression of HLA class I and II proteins, often associated with hemi- and homozygous deletion of the HLA genes and surrounding HLA region on chromosome 6p21.3.

In the project presented in this thesis we studied the effects of the loss of HLA expression and 6p21.3 deletions in IP-DLBCL. We also investigated whether IP-DLBCL share additional molecular characteristics that separate them from non-IP DLBCL, which might indicate they should be regarded as a separate entity.

Previous studies in IP-DLBCL revealed two types of deletions in the HLA region on chromosome 6p21.3. Small homozygous deletions only affected the HLA-DR and -DQ genes, whereas hemizygous deletions were larger and affected the whole HLA region. In Chapter 2 we performed cDNA microarray expression analysis on a series of testicular and nodal DLBCL to investigate the impact of the homo- and hemizygous deletions on HLA gene expression as well as the global gene expression patterns of testicular DLBCL. This analysis revealed that HLA-DR and -DQ gene expression was severely downregulated in most testicular DLBCL regardless of the presence of deletions, suggesting that loss of HLA class II expression is a very important feature in testicular DLBCL that is regulated by multiple mechanisms. Indeed, in the cases without deletions the expression levels of HLA-DR and -DQ correlated with the expression levels of the MHC class II transactivator (CIITA) gene. Although neither hemizygous nor homozygous deletions had any major effect on global gene expression patterns, downregulation of HLA-DR gene expression was significantly associated with a strong downregulation of many immune-associated genes and a lower number of infiltrating reactive T cells, indicating a diminished anti-tumor immune response. These results support the idea that loss of HLA-DR expression functions as an immune escape mechanism in testicular DLBCL.

Recent efforts to find a molecular basis of the clinical heterogeneity in DLBCL have led to the division into a subtype with a germinal center B-cell phenotype (GCB) and a subtype with an activated B-cell phenotype (ABC). These subtypes differ in their immunophenotype,
gene expression profile and the presence of ongoing immunoglobulin heavy chain (IgH) somatic hypermutation (SHM). They are also associated with specific genomic aberrations. Most important, DLBCL of the ABC subtype have a worse prognosis than those of the GCB subtype. In Chapter 3 we determined the subtype of testicular DLBCL using immunophenotyping, gene expression analysis, and IgH SHM analysis. There were no testicular DLBCL with a GCB immunophenotype since all cases showed expression of the ABC marker MUM1 (IRF4). Thirty-six percent of these showed additional expression of GCB marker CD10 and were classified as an ‘ambiguous immunophenotype’ since they expressed both GCB and ABC markers. The remaining 64% were negative for CD10 protein expression and thus were classified as the ABC immunophenotype. Subsequent gene expression analysis showed that both ambiguous and ABC immunophenotypes shared the same ABC-like gene expression pattern.

The frequency of somatic hypermutation of IgH genes was very high in testicular DLBCL, higher than had been reported for nodal DLBCL and comparable to frequencies found in CNS DLBCL. Low level intraclonal variation indicating ongoing mutation could only be confirmed in one case. We conclude that testicular DLBCL have uniform ABC subtype characteristics, despite some cases expressing CD10. These findings also indicate that currently used immunophenotyping algorithms to determine the subtype of nodal DLBCL, which classify all CD10 positive cases as GCB subtype regardless of the expression of other markers, might not be fully applicable to IP-DLBCL where cases express both CD10 and MUM1 more often than in nodal DLBCL.

IP-DLBCL have a very characteristic dissemination and relapse pattern, often restricted to other immune-privileged sites. Relapses of primary CNS DLBCL are confined to the CNS in 90-95% of cases. In Chapter 4 we describe a unique patient who was diagnosed with primary CNS DLBCL and had a relapse confined to the testis 8 years after initial diagnosis. This case provided us with the opportunity to investigate the process of immunoediting in IP-DLBCL over time. Both CNS and testicular DLBCL were classified as the ABC subtype by immunophenotyping. There was progressive loss of HLA protein expression: the CNS DLBCL had lost HLA class II expression and the relapse in the testis showed additional loss of HLA class I expression. While very few T cells and macrophages were present in the environment of the CNS DLBCL, the numbers were higher in the testicular DLBCL. Analysis of the mutations in the IgH genes showed a high mutation frequency comparable to those presented in Chapter 3. The intraclonal heterogeneity was consistent with the clinical presentation and indicated that the testicular DLBCL developed from a subclone of the CNS DLBCL, after which both lymphomas independently accumulated further mutations. Based on this case and the results from the previous chapters we proposed a simple model for lymphomagenesis. In this model the high load of somatic hypermutations make the lymphoma cells very immunogenic and susceptible to an anti-idiotypic response, so that they can only survive an immune attack within an immune-privileged site. The presence of the growing lymphoma will then interfere with the actively maintained immune privilege leading to a shift towards an active immune

Summary
response. Under pressure of this immune response the process of immunoediting will start, leading to escape mechanisms such as the ongoing remodeling of the idiotype and progressive loss of HLA expression.

Together with the literature the results presented in the previous chapters suggest that IP-DLBCL form a relatively homogeneous subgroup within the DLBCL in general. To investigate whether they also share characteristic genomic aberrations we analyzed a series of testicular, CNS and nodal DLBCL using array-comparative genomic hybridization (CGH) (Chapter 5). In addition to 30 common regions of loss or gain, we found that some aberrations were associated with site of presentation. Gain of 2p16.1–p25.3 was associated with nodal DLBCL. Loss of 6p21.32–p25.3, including the HLA region, was associated with IP-DLBCL (both CNS and testicular), confirming previous results. We also found gain of 12q15–q21.1 and 12q24.32–q24.33 associated with CNS DLBCL and gain of 19q13.12–q13.43 associated with testicular DLBCL. The presence of genomic differences between nodal DLBCL and IP-DLBCL supports the idea of IP-DLBCL as a specific or separate subgroup of DLBCL, but the presence of site-specific aberrations in CNS and testicular DLBCL also implies that these IP-DLBCL do not form a single entity. By combining the array-CGH data with gene expression microarray data we explored which genes and biological pathways were deregulated by the genomic aberrations. The majority of candidate target genes was involved either in apoptosis or in the modulation of the anti-tumor immune response, emphasizing the importance of deregulation of these pathways in lymphomagenesis.

In the final chapter (Chapter 6) the results presented in this thesis are placed in a broader context of immune privilege and immunoediting, leading to an in-depth discussion of the model for IP-DLBCL-specific lymphomagenesis that was proposed in a simple form in Chapter 4. The discussion concludes with further arguments for a subclassification of DLBCL into IP-DLBCL and non-IP DLBCL, and a recommendation for further research to elucidate the biology of IP-DLBCL.