Chapter 1

Introduction
1.1. Cancer and the concept of immunoediting

The idea that the immune system is involved in the repression of cancer was conceived in the early 20th century and together with the discovery of tumor-associated antigens has led to the hypothesis of cancer immune surveillance, postulated in 1957 by Burnet¹, which stated “In large, long-lived animals, like most of the warm-blooded vertebrates, inheritable genetic changes must be common in somatic cells and a proportion of these changes will represent a step toward malignancy. It is an evolutionary necessity that there should be some mechanism for eliminating or inactivating such potentially dangerous mutant cells and it is postulated that this mechanism is of immunological character.” In recent years this hypothesis has been refined to reflect the interaction between tumors and the immune system, resulting in the concept of immunoediting. This is a dynamic process divided into three phases: elimination, equilibrium and escape.²³

1.1.1 Elimination

The elimination phase of immunoediting is the same process as described in the initial immune surveillance hypothesis. It includes both an innate and an adaptive immune response to tumor cells by contributors including natural killer (NK) cells, T helper 1 (Th1) cells and CD8-positive cytotoxic T cells (CTL).²⁴

A major factor in the elimination process is interferon gamma (IFNγ).⁵ It can induce anti-tumor effector functions by activating cytocidal activity in macrophages and by stimulating NK cells,⁶ leading to perforin-, Fas- and TRAIL-mediated killing of tumor cells and subsequent release of tumor-associated antigens.⁷–⁹ NK cells in turn will produce more proinflammatory cytokines. Another result of IFNγ production and NK cell activation is the maturation of dendritic cells, upregulation of the antigen processing and presentation pathway and subsequent activation of the adaptive immune response.¹⁰

The adaptive immune response is usually targeted to tumor-associated antigens: differentiation antigens, mutated self-antigens, overexpressed antigens, cancer-testis antigens or viral antigens.¹¹¹² B-cell lymphomas can present their own idiootype as antigens.¹³¹⁴ Both CD4-positive Th1 cells and CD8-positive CTL effector cells are needed to raise an effective anti-tumor immune response (Figure 1). Naive CD8-positive T cells have to receive three signals to develop effector functions: recognition of the antigen in the context of human leukocyte antigen (HLA) class I presented on an antigen-presenting cell (APC), costimulation by ligation of CD28 with CD80/86 and the presence of an inflammatory cytokine (IL-12 or type I interferon).¹⁵¹⁶ After initial clonal expansion, the effector T cells go into a state of ‘split anergy’ termed activation-induced non-responsiveness (AINR), where they retain their effector functions but are unable to undergo further clonal expansion. This AINR state can be reversed
Figure 1. Simplified overview of the adaptive immune system cascade in the elimination phase
Antigen-presenting cells (APC) present peptides derived from tumor-associated antigens to T cells. When presented in the context of HLA class I, these peptides will be recognized by the T-cell receptor (TCR) on CD8-positive cytotoxic T cells (CTL). After additional costimulatory signals such as CD28 ligation and the presence of cytokines, an effector phase will develop in which CTL can recognize and lyse the tumor cells. When presented in the context of HLA class II, the peptides will be recognized by CD4-positive T helper cells (Th), and depending on the cytokines present either a Th1 or a Th2 response develops. A Th1 response mainly stimulates APC antigen presentation and CTL proliferation, while a Th2 response mainly causes antibody production and the stimulation of eosinophils.
by IL-2. The necessary cytokines can be provided by activated CD4-positive Th1 cells either directly (IL-2) or through stimulation of APCs to produce IL-12 and IFNα/β.¹⁷

In addition to providing help to CTL, Th1 cells can also directly induce apoptosis of tumor cells through the Fas/FasL pathway. In lymphoma, Th1-expressed CD40L will interact with CD40 on the lymphoma cell, leading to upregulation of Fas on the lymphoma cell and induction of apoptosis by FasL ligation.¹⁸

1.1.2 Equilibrium

If not all the tumor cells are eliminated, the elimination phase will be followed by an equilibrium phase. Tumor cells are often genetically instable and will continue to evolve and accumulate changes during this phase.¹⁹,²⁰ The immune system will eliminate susceptible tumor clones and so exerts a selective pressure. The constant evolution of the tumor cells will eventually give rise to tumor clones that are able to avoid, suppress or resist the immune surveillance. At this point the immune system will no longer be able to control tumor progression and the tumor will enter the escape phase.³

1.1.3 Escape

Tumor cells that survive the equilibrium phase enter the escape phase. In this phase the tumor can progress without being restrained by the immune response. Tumor cells can escape the immune system by avoiding, suppressing or resisting an immune reaction.

Avoiding immune recognition

A well documented immune escape mechanism is downregulation of HLA proteins on the tumor cells (reviewed in ref. 21). HLA class I proteins present antigens to CD8-positive T cells while HLA class II proteins present antigens to CD4-positive T cells. In the absence of HLA proteins, tumor-associated antigens cannot be presented to the immune system. Downregulation or complete loss of HLA expression can be achieved by different mechanisms, which will be discussed in more detail in paragraph 1.2.

Accumulation of mutations in a tumor-associated antigen (antigenic drift) can also help to avoid the immune system because the tumor-specific cytotoxic T cells will no longer recognize it.²²

Suppressing the immune reaction

Some tumors produce immunosuppressive factors (e.g. IL-10, TGFβ, galectin-1, gangliosides or indoleamine 2,3-dioxygenase (IDO)) or express negative costimulatory signals (B7:CTLA, PD-1L:PD-1, FasL:Fas) to inhibit activation of T cells and dendritic cells (reviewed in ref. 23). Specific cell populations can also contribute to the immune-suppressive environment,
including regulatory CD4-positive T cells, myeloid suppressor cells and tumor-associated macrophages (TAM). TAMs are M2 macrophages which have an IL-12\textsubscript{low} IL-23\textsubscript{low} IL-10\textsuperscript{high} phenotype, produce TGFβ and can skew the immune response towards a Th2 profile. In many human tumors TAMs are associated with poor prognosis.

**Resisting apoptosis induced by immune cells**
Tumors that do not avoid immune recognition or suppress the immune reaction can become resistant to the effects of the immune reaction by being resistant to perforin-/Fas-/TRAIL-mediated apoptosis. This can be achieved by mutations in molecules as Fas, \textsuperscript{DR5}\textsuperscript{30} or caspase 8, \textsuperscript{by overexpression of anti-apoptotic proteins such as FLIP, Bcl2, Bcl-Xl, IP3 or survivin, or by the expression of death ligand decoy receptors.}

### 1.2. Human Leukocyte Antigen

#### 1.2.1 The role of HLA in the human immune system
The adaptive immune system is designed to protect the human body from harmful agents. These agents can be pathogens, such as bacteria or viruses, as well as altered self cells expressing abnormal proteins, such as tumor cells.

When cells become infected or transform into tumor cells, foreign antigens will be present in the cytosol or the nucleus. To eradicate the cells, these endogenous foreign antigens will be processed and presented by HLA class I proteins to CD8-positive cytotoxic T cells (Figure 1).\textsuperscript{39} HLA class I is expressed on the surface of almost all nucleated cells, although the levels of expression may vary between tissues.\textsuperscript{40} However, for the initiation phase of the CTL response it is important that a foreign peptide is presented to the naïve CD8-positive T cell by a professional APC such as a dendritic cell, because these cells can deliver the costimulatory signals needed to activate the T cell. If the infected or transformed cell is not a professional APC itself, the antigen can be presented by APCs through a process called cross-presentation (see paragraph 1.2.2). In the subsequent effector phase of the response, the primed CTL will recognize the same peptide-HLA complex expressed on the infected or transformed cells and subsequently destroy these cells.\textsuperscript{41}

In most bacterial infections the pathogens will not enter the cells. Eradication of these extracellular pathogens requires professional APCs that can internalize these exogenous antigens, process them and present them to CD4-positive T helper cells in the context of HLA class II proteins, eventually resulting in the production of antibodies against the extracellular pathogen (Figure 1).\textsuperscript{42} HLA class II is therefore expressed constitutively on professional APCs
such as dendritic cells, macrophages and B cells. Under inflammatory conditions, expression of HLA class II can also be induced on other cell types such as epithelial or endothelial cells by IFNγ and other cytokines. After recognition of a foreign peptide by CD4-positive T helper cells a Th1 or Th2 response will develop, depending on the cytokines present. Both will eventually lead to the production of antibodies against the foreign antigen; in addition the Th1 response can stimulate proliferation and activation of CTLs through ligation of costimulatory molecules and production of cytokines.

To induce an efficient CTL response to an endogenous antigen a Th1 response, and thus presentation of the antigen by HLA class II proteins, is necessary. Presentation of an endogenous antigen by HLA class II could be mediated by cross-presentation or autophagy (see paragraph 1.2.2).

1.2.2 Antigen processing and presentation

Class I

HLA class I molecules are transmembrane heterodimers consisting of the polymorphic class I heavy chain, encoded by the HLA class I genes on chromosome 6p21.3, and the invariant beta-2-microglobulin (β2m) light chain, encoded by a gene on chromosome 15q21. Newly synthesized class I heavy chain is transported to the endoplasmic reticulum (ER) where it is associated with chaperone protein calnexin. When it is properly folded it binds β2m to form an unstable unloaded class I molecule. To help stabilize the molecule it is then incorporated in the peptide binding complex involving calreticulin, Erp57, TAP and tapasin. Endogenous peptides are degraded by the immunoproteosome and aminopeptidases and transported by TAP to the ER lumen. When an appropriate peptide binds and empty HLA class I molecule, the loaded HLA class I molecule dissociates from the peptide binding complex and is transported to the cell surface (reviewed in ref. 46).

In the alternative cross-presentation pathway, peptides are derived from exogenous sources. Examples are antigens derived from apoptotic cells that can enter the endocytic route of dendritic cells, or antigens present in neighboring cells which can be transferred by direct cell-cell contact such as through gap junctions.

Class II

HLA class II molecules are transmembrane heterodimers consisting of the class II α and β chains, both encoded by HLA class II genes on chromosome 6p21.3. Newly synthesized class II heterodimers are transported to the ER where they associate with the invariant chain (Ii) to prevent premature loading of the HLA molecule (Figure 2). The invariant chain also targets the HLA molecule to the endocytic loading compartment, where it is cleaved by cathepsins leaving only a small part of Ii, the class II-associated Ii peptide (CLIP), bound in the peptide binding groove of the HLA molecule. Non-classical HLA class II protein HLA-DM
Figure 2. Classical HLA class I and class II antigen processing and presentation pathways
HLA class I proteins associate with the peptide loading complex in the endoplasmic reticulum (ER). Endogenous antigens are processed by the immunoproteasome and aminopeptidases into small peptides which are transported into the ER by TAP. There they bind to HLA class I molecules, which dissociate from the peptide loading complex and are transported to the cell surface. HLA class II proteins are targeted to an endocytic loading compartment. Exogenous antigens are processed in endosomes by endosomal proteases and the endosomes containing the processed peptides fuse with the endocytic loading compartment. HLA-DM helps select appropriate peptides which then are exchanged with CLIP. HLA-peptide complexes are then transported to the cell surface.
then releases CLIP and stabilizes the empty HLA molecule. The endocytic loading compartment fuses with endosomes containing exogenous antigens or endogenous transmembrane or secreted proteins. These are processed by endocytic proteases to yield peptides appropriate for loading onto HLA class II molecules. HLA-DM acts as a peptide selector, allowing only peptides with high affinity to bind the HLA class II molecule. The loaded HLA molecules are then transported to the cell surface (reviewed in ref 47).

HLA class II proteins can also present peptides derived from endogenous cytosolic proteins, either through cross-presentation of apoptotic cell debris, or through autophagy. In B cells another non-classical HLA class II protein is expressed. HLA-DO modifies the function of HLA-DM and when DO:DM ratio is high presentation of antigens taken up through the B-cell receptor is favored over presentation of non-specific antigens.

The human HLA region at chromosome 6p21.3

The HLA genes are located in a cluster at chromosome 6p21.3, named the HLA region (or MHC region). It spans 3.6 Mb and can be divided in 3 subregions: class I, class III and class II from telomere to centromere (Figure 3). It is the most gene-dense region of the genome and contains 224 genes of which about 128 are expressed. Immune-related genes are overrepresented, comprising roughly 40% of the expressed genes in the region. The class I region contains the genes for the classical class I heavy chain HLA-A, -B and -C proteins and the non-classical class I heavy chain HLA-E, -F and -G proteins. The class III region genes include those involved in the complement cascade, stress response, leukocyte maturation, immune regulation and inflammation. The class II region contains the genes for classical class II HLA-DR, -DP and -DQ proteins, as well as genes encoding components of the antigen processing machinery such as non-classical HLA-DM and -DO, TAP and the immunoproteosome components LMP2 and LMP7.

The HLA class I and class II proteins are highly polymorphic. This polymorphism is present at multiple levels from alleles to the population, and is conserved through evolution to allow immune responses against a vast array of pathogens and to minimalize the possibility that one pathogen can eradicate an entire population. At the moment of writing this thesis, in November 2007, about 2900 HLA class I and II alleles are known and each year several hundred additional alleles are discovered. The combination of alleles for each locus in the HLA region on a single chromosome makes up the HLA haplotype. The maternal and paternal haplotypes form the HLA genotype of an individual. All HLA genes are codominantly expressed, making up the phenotype of an individual. An extra level of polymorphism is provided by the HLA-DRB region, where different haplotype groups exist differing in the number and nature of the HLA-DRB loci expressed.
Figure 3. HLA region at chromosome 6p21.3
Schematic representation of the HLA region and the division into class I, III and II. All classical and non-classical HLA genes are indicated, as well as a selection of genes involved in antigen processing or other immune-related genes.
1.2.4 Regulation of HLA expression

HLA proteins are not all equally expressed, and expression levels also vary between tissue types. Of the HLA class I proteins, HLA-A and HLA-B are more efficiently expressed than HLA-C. Of the class II proteins, HLA-DR is most highly expressed. The activity of the class I promoter is regulated by NFκB and proteins of the IRF-family, as well as by the class II trans-activator (CIITA). CIITA is the master regulator of HLA class II expression. The expression of CIITA itself, and so indirectly also HLA class II expression, is regulated by IFNγ. Both HLA class II and CIITA can also be regulated epigenetically.40

1.2.5 Avoiding the immune response by downregulation of HLA expression

As discussed in the previous paragraph, tumors can avoid recognition by the immune system through downregulation or complete loss of HLA expression. Like almost all normal nucleated cells, tumor cells express HLA class I. Expression can be downregulated on several levels, from loss of one allele to complete loss of all HLA class I proteins (reviewed in ref. 55). Loss of single alleles can be caused by small deletions or mutations,56,57 while loss of a complete haplotype is usually caused by larger hemizygous deletions or mitotic recombination.58 Loss of expression of all class I proteins can be caused by loss or mutation of the β2m gene59 or by regulatory defects such as hypermethylation of the promoter regions of the HLA-A, -B and -C genes.60 Defects in the antigen processing pathway, such as downregulation of TAP or LMP genes, also result in complete loss of HLA class I expression.61,62

HLA class I proteins inhibit NK-cell cytolytic activity by binding to inhibitory receptors on NK cells, such as KIRs63 and CD94.64 Tumors that have lost expression of HLA class I thus need additional mechanisms to prevent lysis by NK cells,65,66 such as expression of inhibitory ligands such as HLA-E or HLA-C67 or expression of soluble MICA or MICB which are ligands for the NK cell activating receptor NKG2D. These soluble ligands induce internalization and degradation of NKG2D and thus prevent activation of NK cells.68

B-cell lymphomas can also downregulate the expression of HLA class II. Mechanisms involved can be hemi- or homozygous deletions,69 defects in CIITA expression,70 or hypermethylation of the class II genes.71 Defects in the IFNγ signaling pathway will result in loss of CIITA and thus loss of class II expression. Although tumor-associated antigens are usually endogenous and presented by HLA class I proteins, HLA class II proteins can present them through cross-presentation or autophagy. Downregulation of HLA class II thus prevents the activation of tumor-specific CD4-positive Th1 cells that are necessary for an efficient immune response.
1.3. Diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma (DLBCL) constitutes 30–40% of B-cell non-Hodgkin lymphoma cases, with involvement of extranodal sites such as the gastro-intestinal tract, skin, testis, central nervous system (CNS) and bone in up to 40% of cases. It is a highly heterogeneous disease regarding clinical and molecular characteristics. According to the current World Health Organization (WHO) classification there are 4 morphological subtypes: centroblastic, immunoblastic, anaplastic and T-cell/histiocyte-rich.\textsuperscript{72} Cases are stratified into risk groups using the International Prognostic Index (IPI) which is based on patient age and performance status, Ann Arbor stage, involvement of extranodal sites and level of lactate dehydrogenase (LDH).\textsuperscript{73} Current treatment protocols consist of CHOP chemotherapy with added rituximab (anti-CD20).\textsuperscript{74}

Recent research has focused on the molecular basis of the clinical heterogeneity in DLBCL. One of the major studies compared gene expression profiles between DLBCL cases and normal B-cell subsets, resulting in the definition of two DLBCL subtypes with different clinical outcome: one with an expression profile resembling germinal center B cells and a favorable clinical outcome (GCB subtype) and another resembling in vitro activated B cells and with an aggressive clinical behavior and worse prognosis (ABC subtype).\textsuperscript{75} Both subtypes can also be distinguished by immunohistochemistry: GCB-DLBCL express one or both of the GC markers CD10 and Bcl6, and lack expression of MUM1 (IRF4), while ABC-DLBCL lack CD10 expression but do express MUM1.\textsuperscript{76,77} Further study of these subtypes revealed differences in chromosomal aberrations and oncogenic mechanisms that might be the underlying cause of the different clinical characteristics of these two subtypes (TABLE 1).\textsuperscript{78} A hallmark feature of ABC-DLBCL is constitutive activation of NFκB, which is essential for proliferation and survival of these lymphomas.\textsuperscript{79}

Apart from GCB and ABC subtype characteristics, some 25 other potential molecular indicators of prognosis have been reported including molecules involved in the cell cycle, apoptosis, B-cell differentiation, adhesion and angiogenesis (reviewed in ref. 80). One of the indicators of poor prognosis is loss of HLA class II expression.\textsuperscript{81,82} DLBCL (and B-cell lymphomas in general) are different from other tumors because the lymphoma cells are derived from antigen-presenting cells. Lymphoma cells themselves are therefore capable of presenting their own tumor-associated antigens to the immune system in the context of both HLA class I and class II. Loss of expression of HLA class II proteins on the lymphoma cells provides the lymphoma with an immune escape mechanism as discussed in paragraph 1.2.
Although the inflammatory immune response usually acts to protect the body from harmful infections, the immune response itself can also be harmful. In certain sites such as the eye, CNS, testis and the human embryo/fetus the tissue damage caused by an immune response could have devastating effects. The first experimental evidence for the absence or limitation of the immune response in these sites came from the observation that grafts that were genetically different from the accepting tissue showed prolonged survival when transplanted into these sites, as compared to other sites. Therefore these sites were ‘immune privileged’. Historically, it was thought that immune privilege was a passive mechanism, in which the immune system had no access to the antigens because of the presence of a blood-organ barrier or the absence of efferent lymphatics. However, in both CNS and testis the blood-organ barrier is not completely impermeable for the cells of the immune system. In addition, immune privilege in the testis extends to the testicular interstitium, which is not protected by the blood-testis barrier. It is now known that immune privilege is actively maintained by multiple mechanisms that suppress immunity and promote tolerance. These include production of anti-inflammatory cytokines and expression of CD95L. Also the resident APC population probably has an immunomodulatory role.

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<th>Table 1. Chromosomal aberrations and possible oncogenic mechanisms in GCB and ABC subtypes</th>
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<tr>
<td><strong>Proposed normal counterpart</strong></td>
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<tr>
<td><strong>Clinical outcome</strong></td>
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<tr>
<td>60% 5-year survival</td>
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<tr>
<td><strong>Chromosomal aberrations</strong></td>
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<tr>
<td>+12q12</td>
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<tr>
<td>amplification of c-REL (2p16)</td>
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<tr>
<td><strong>Oncogenic mechanism</strong></td>
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<td>c-REL overexpression</td>
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<td>Bcl6 expression</td>
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1.4 Immune-privileged site-associated DLBCL

1.4.1. Immune privilege

Although the inflammatory immune response usually acts to protect the body from harmful infections, the immune response itself can also be harmful. In certain sites such as the eye, CNS, testis and the human embryo/fetus the tissue damage caused by an immune response could have devastating effects. The first experimental evidence for the absence or limitation of the immune response in these sites came from the observation that grafts that were genetically different from the accepting tissue showed prolonged survival when transplanted into these sites, as compared to other sites. Therefore these sites were ‘immune privileged’. Historically, it was thought that immune privilege was a passive mechanism, in which the immune system had no access to the antigens because of the presence of a blood-organ barrier or the absence of efferent lymphatics. However, in both CNS and testis the blood-organ barrier is not completely impermeable for the cells of the immune system. In addition, immune privilege in the testis extends to the testicular interstitium, which is not protected by the blood-testis barrier. It is now known that immune privilege is actively maintained by multiple mechanisms that suppress immunity and promote tolerance. These include production of anti-inflammatory cytokines and expression of CD95L. Also the resident APC population probably has an immunomodulatory role.
1.4.2. Primary testicular DLBCL

Epidemiology

Primary testicular lymphoma (PTL) is a rare disease, accounting for only 2% of all non-Hodgkin lymphomas and 9% of testicular neoplasms. PTL encompasses Burkitt lymphoma, follicular lymphoma and lymphoblastic lymphoma but the most frequent type is DLBCL (70–90%). Testicular DLBCL occurs predominantly in males older than 60 years. There are currently no well established predisposing factors for testicular DLBCL.\(^8\)–\(^9\)

Clinical presentation

Testicular DLBCL present as a painless unilateral or in rare occasions bilateral testicular mass.\(^8\) The most common diagnostic procedure is orchiectomy. The majority of cases present with Ann Arbor stage IE or IIE disease. Testicular DLBCL has a propensity to disseminate or relapse to other extranodal sites including the contralateral testis (in up to 35% of cases), CNS, skin, Waldeyer’s ring or lung.\(^9\)

Prognosis and therapy

Prognosis is poor, with reported median survival rates of 12–90 months.\(^9\) Treatment usually consists of orchiectomy and systemic chemotherapy, with prophylactic scrotal radiotherapy and CNS prophylaxis (high dose methotrexate and intrathecal chemotherapy). The relapse rate is high, between 40 and 70%. Most relapses occur within 2 years of diagnosis, but relapses after as much as 10 years have been reported.\(^9\)–\(^9\)

Microscopy and immunohistochemistry

Testicular DLBCL shows a diffuse growth pattern of large cells with pleomorphic nuclei. Often the testicular parenchyma is completely obliterated but in some cases non-productive seminiferous tubules are present. Tumor-infiltrating T cells and macrophages may be seen.\(^9\)–\(^9\) The immunophenotype is comparable to that of other nodal and extranodal DLBCL.\(^9\) The proliferative index, as detected by Ki-67, ranges from 50–90%.\(^9\) Expression of Bcl2 is frequent but not caused by a t(14;18)(q32;q21).\(^9\) It is unknown whether testicular DLBCL correspond to the ABC or GCB subtype.

Molecular pathogenesis

Only a few studies of chromosomal aberrations or aberrant gene expression have been preformed in testicular DLBCL. Sequence analysis of immunoglobulin heavy chain (IgH) VDJ region in 12 testicular DLBCL revealed a high frequency of somatic hypermutations and the presence of intraclonal IgH sequence variation, indicating a possible antigen-driven stimulation, although it is unclear how this process can take place outside of the germinal center environment.\(^9\) The frequent expression of anti-apoptotic factor Bcl2 might be associated with the poor prognosis of testicular DLBCL, as it is in ABC-type nodal DLBCL. Another proposed
explanation for the aggressive behavior is the unique expression pattern of adhesion mole-
cules resulting in a lack of adhesion to the extracellular matrix.\textsuperscript{99,100} Recent studies have shown
that testicular DLBCL very frequently lack expression of HLA class I and II proteins, often
caused by hemi- and homozygous deletions in the HLA region at chromosome 6p21.3, which
could provide the lymphomas with a mechanism of immune escape.\textsuperscript{69}

1.4.3. Primary central nervous system DLBCL

Epidemiology

Primary CNS DLBCL represents less than 1\% of all non-Hodgkin lymphomas and 2–3\%
of all brain tumors. The median age of presentation is approximately 60 years, with a slight
preponderance of male patients.\textsuperscript{101}

Clinical presentation

Focal neurological deficits are observed in 50–80\% of the patients. Neuropsychiatric symp-
toms (20–30\%) and symptoms due to increased intracranial pressure are noticed. Nuclear
magnetic resonance imaging (NMRI) is the most sensitive technique for tumor visualization.
Approximately 60\% of all CNS DLBCL are located supratentorially and in 20–40\% multiple
lesions are present. Approximately 20\% of patients develop intraocular lesions; dissemination
to extraneural sites is very rare.

Prognosis and therapy

Prognosis used to be very poor but has been remarkably ameliorated by novel chemo-
therapeutic protocols. Polychemotherapy, including methotrexate as the most efficient drug,
has achieved a median overall survival of 50 months. Autologous stem cell transplantation
has been successfully applied in patients with recurrence and as salvage therapy in relapsing
or refractory tumor. Radiotherapy is insufficient to control the tumor and may, in combina-
tion with chemotherapy, result in severe adverse side-effects such as leukoencephalopathy
and cortical/subcortical atrophy. Most relapses affect the CNS; systemic relapses relatively
frequently involve the testis and breast.\textsuperscript{102}

Microscopy and immunohistochemistry

CNS DLBCL shows a diffuse intraparenchymal growth pattern of centroblast-like cells,
sometimes forming perivascular cuffs. Tumor cells may be intermingled with reactive T cells,
CD68-positive macrophages, activated microglial cells, and reactive astrocytes. Like in primary
testicular DLBCL, expression of Bcl2 is frequent but not associated with t(14;18)(q32;q21).\textsuperscript{103}
Only a few recent studies have reported on whether primary CNS DLBCL belong to the ABC
or GCB subtype, either by immunohistochemistry\textsuperscript{104} or gene expression analysis,\textsuperscript{105} with con-
flicting results.
Molecular pathogenesis

Studies of IgH genes revealed a preferred usage of VH4-43, which is often detected in auto-immune disease. As in testicular DLBCL, a high frequency of somatic hypermutation of the VDJ genes and the presence of intraclonal variation suggests ongoing antigen-driven stimulation. Aberrant hypermutation of other genes such as BCL6, PIM1, MYC, RhoH/TTF and Pax5 has also been reported. Expression or absence of chemokines and chemokine receptors or cytokines such as CXCL12, CXCL13 and IL-4 might contribute to the specific localization. Lack of expression of HLA class I and II proteins, often caused by the same 6p21.3 deletions as found in testicular DLBCL, might also allow CNS DLBCL to escape an immune attack. Cytogenetic studies using fluorescence in situ hybridization (FISH) or comparative genomic hybridization (CGH) are more frequent for CNS DLBCL than for testicular DLBCL. Similar to systemic DLBCL, approximately 30–40% of CNS DLBCL have BCL6 translocations but t(14;18)(q32;q21) and t(8;14)(q24;q32) are rare. Common deletions are found at 6q and gains at 12q, 22q and 18q21. Amplification of 18q21 includes BCL2 and MALT1, as has been described for extracerebral DLBCL of the ABC type as well. Homozygous or hemizygous deletions at 9p21 frequently affect CDKN2A/p16.

1.5 Scope of this thesis

The immune-privileged site-associated DLBCL (IP-DLBCL) of the testis and the CNS differ from their non-IP DLBCL counterpart in different clinical and molecular aspects, but the most striking characteristic of IP-DLBCL is the lack of expression of HLA class I and II molecules, often associated with hemi- and homozygous deletions of the HLA region on chromosome 6p21.3. Because these deletions are such a hallmark event in IP-DLBCL we studied their significance in relation to downregulation of gene expression by comparing global gene expression patterns between testicular DLBCL with and without deletions (Chapter 2). Since downregulation of HLA-DR gene expression was revealed as an important event in testicular DLBCL, independent from the mechanism that causes it, we also determined the effect of this downregulation on global gene expression and T-cell infiltration.

In Chapter 3 we used immunohistochemistry for CD10, Bcl6 and MUM1, gene expression analysis and IgH somatic hypermutation analysis to investigate whether testicular DLBCL belong to the ABC or GCB subtype of DLBCL. We also proposed a change to the algorithm used in immunohistochemical analysis to accommodate for cases that express both CD10 and MUM1.
We had the unique opportunity to investigate a primary CNS DLBCL with an isolated relapse to the testis which occurred 8 years after initial diagnosis (Chapter 4). Based on the results of HLA protein expression, ABC/GCB typing and IgH somatic hypermutation analysis in this case and those presented in the previous chapters we propose a model for the development of this lymphoma and IP-DLBCL in general.

It is known that different subtypes of DLBCL such as ABC-type, GCB-type and CD5-positive DLBCL can be characterized by different genomic aberrations, but not much is known about genetic aberrations in IP-DLBCL. In Chapter 5 we investigated whether specific genomic aberrations are present in IP-DLBCL using array-CGH. We also determined the minimal common regions of gain and loss in DLBCL in general. Using a new, statistically robust technique we combined array-CGH data with global gene expression data to investigate which biological pathways are deregulated by the genomic aberrations.

Chapter 6 contains a general discussion centered on a model of IP-DLBCL lymphomagenesis in the context of immune privilege and immune escape, and recommendations for further research. Finally a summary of the results presented in this thesis is given in Chapter 7.
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Chapter 1


Introduction


