Chapter 6

General Discussion
In 1970 Burnet proposed the term immune surveillance with the immune system specifically recognizing and eliminating transformed cells. Support for his hypothesis came from observations that humans with a reduced immunological functioning were more prone to develop cancer and from several experimental models with mice mutant for either IFNγ or T cells. The immune system is able to constantly screen for virally infected or transformed cells through presentation to circulating T cells of peptides in the context of HLA class I and II molecules. In principal, HLA class I molecules, expressed on nearly all nucleated cells, present peptides derived from endogenous protein while peptides derived from exogenous proteins are presented by HLA class II molecules, constitutively expressed on antigen presenting cells (APCs). In general, tumour cells present tumour associated antigens (TAA) via HLA class I molecules and the TAA/class I complexes can be recognized by cytotoxic T cells (CTLs) expressing CD8. However, some TAA are presented in the context of HLA class II molecules including Epstein Barr virus derived peptides, carcinoembryonic antigen and testis cancer antigens (TCA) and antigen specific cytolytic CTL clones expressing CD4 have been described as well.

Upon antigen recognition, mature naïve B cells move into the germinal centre where they undergo somatic hypermutation of Ig Variable genes, class switching and receptor editing to generate B cells with high affinity for the encountered antigen. During this refinement of the immune response that involves double-strand DNA breaks and mutations, occasionally chromosomal translocations and activation of oncogenes occur as well. B cells carrying several of such genetic aberrations may subsequently home to different organs including the brain and the testis. The homing of neoplastic B cells has not been studied very extensively to date but either the presence or the lack of certain surface adhesion molecules seems to play a role in this process. If such potentially neoplastic B cells with acquired genetic aberrations, start dividing in an unrestricted and antigen independent manner, a lymphoma may eventually develop. During this process, the host immune system will attempt to eradicate the tumour cells with probably CD8+ CTLs playing a central role in the defense. Activation of tumour-specific CD8+ CTLs requires a Th1 response upon priming of naïve CD4+ Th cells via the peptide/HLA class II complex on APCs such as macrophages and dendritic cells (DC). After antigen uptake, tissue-derived immature DC can migrate to draining lymphoid organs where upon CD40-CD40 ligand interaction with CD4+ T cells, they mature and function as efficient APCs, expressing high levels of HLA class I and II as well as co-stimulatory molecules. The origin of DC and the cytokine environment during priming determines whether CD4+ T cells differentiate into Th1 or Th2 cells, with the latter stimulating a humoral immune response which is often less effective.
The testis and CNS are considered immune privileged sites but this does not imply that immune responses are completely absent, as small numbers of T-cells are present at both locations, even under physiological circumstances. In the normal human testis, CD8 and Granzyme B (GB) expressing T cells have been found and activated T cells can readily patrol the brain. Moreover, we observed high numbers of macrophages infiltrating in the CNS and especially the testicular lymphomas. In addition, microglia cells pre-existing in the CNS can, when activated also function as efficient APCs. Activated microglia cells produce large amounts of IL-12 which will drive the immune response towards a Th1 response, although small amounts of IL-10 are secreted as well, as a sort of counter regulator. Considering the presence of numerous lymphoma infiltrating APCs, one would expect specific immune responses to be elicited when TAA from apoptotic lymphoma cells are captured and subsequently presented to CD4+ T cells. However, macrophages, may play dual roles, when they are actively recruited by tumour cells through chemotactic agents. They were found to reduce cytotoxic activities and even to promote tumour-growth by secreting factors such as BAFFF. Moreover, macrophage derived IL-10 and TGFβ suppresses Th1 cell and NK cell responses (for review see) and deviates differentiation of naïve Th cells towards CD25+ regulatory T cells, a subset of T cells capable of inhibiting cytokine production and proliferation of stimulated naive T cells. Therefore, the presence of tumour infiltrating macrophages might ultimately result in suppression of a strong Th1 mediated anti-tumour immune response in stead of promotion of such a response.

In B cell lymphomas, the initiation of an immune response does not necessarily depend on the presence of macrophages or DC, since lymphoma B cells themselves are in principal excellent APCs. Amongst other tumour derived peptides, they can present their own idiotypes (Id) which are antigenic determinants localised to the variable (V) regions of immunoglobulin (Ig). Because these V regions are extremely diverse, each neoplastic B cell clone will express highly unique monoclonal Ig that may function as tumour specific antigens. Indeed, in mouse models, immunization with Ig resulted in tumour specific CTL clones as well as anti-Ig specific antibodies and in clinical trials vaccination with Id induced clinical and molecular remissions in lymphoma patients.

Both in testicular and CNS lymphomas, we observed high numbers of activated tumour infiltrating CTLs. The majority of CTLs expressed CD8 but in some CNS cases a substantial percentage of CD4+ CTLs was present. The high numbers of activated CTLs indicates that these lymphomas are both highly immunogenic and able to provoke immune responses. This is consistent with the observation that both CNS lymphomas and testicular
show very high rates of somatic hypermutation in Ig and in the 5’ end of several proto-
oncogenes which is likely to result in expression of many antigenic peptides derived
from both idiotypes as well as aberrant proteins. Another explanation might be that
testicular lymphomas express cancer testis antigens, as has been reported in some
DLBCL. We propose that early during lymphomagenesis, TAA in complex with HLA class
I and/or class II molecules are probably presented by the tumour cells themselves thereby
eliciting specific CD8+ CTL responses. Target cell killing by specific CD8+ CTLs is caused by
the concerted action of molecules contained in cytolytic granules, mainly granzyme-B (GB)
and perforin. GB enters the target cell and, together with perforin, triggers the death
pathway, resulting in the induction of apoptosis. The testicular and CNS lymphomas are
in principal sensitive to GB induced apoptosis as all cases we investigated were negative
for PI-9, a GB inhibitor. Moreover, all cases were positive for pro-caspase 3 (unpublished
results), one of the targets of GB, that when activated, contributes to cytochrome c
release and loss of mitochondrial membrane potential and eventually DNA fragmentation.
However, during lymphoma evolution, tumour cells may lose HLA class I expression as
has been described in many solid tumours as well as haematological malignancies. In
our series, at least 15 tumours were completely negative for HLA class I expression as
assessed by negative staining for W6/32 a monoclonal antibody specific for the B2M/HLA
class I complex. Two of those cases showed a mutation in B2M with concomitant loss of
the wild-type allele through loss of heterozygosity which results in loss of HLA surface
expression as proper assembly of B2M with class I chains and peptide within the
endoplasmatic reticulum is obligate. In six other cases one copy of B2M was lost which
also results in a significant reduction of class I expression. Loss of class I expression
renders the tumour cells insensitive to tumour-specific CD8+ CTL attack, which obviously
provides the tumour cells with clonal advantage.
HLA class I negative tumours however, are prone to Natural Killer (NK) cells but the
latter do not seem to play a major role in the testis and the CNS, as we and others did not
find any significant numbers of NK cells infiltrating in lymphomas at those locations.
The class I positive tumours on the other hand, might escape through Bcl-2 expression, a
strong inhibitor of GB-induced apoptosis. All testicular and 70% of the CNS lymphomas
investigated in our studies expressed Bcl-2 (unpublished results). Bcl-2 protects cells from
apoptosis through inhibition of cytochrome c release from the mitochondria. However, in
some Bcl-2 expressing cells, GB-mediated apoptosis can still occur through direct
activation of downstream caspases, although this pathway is by far less efficient. Bcl-2
expression is also associated with chemotherapy resistance which makes this protein an
interesting target for new therapeutic interventions including anti-sense Bcl-2 therapy (for

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review see 64) or adoptive transfer of Bcl-2 reactive T cells as spontaneous anti-Bcl-2 T cell reactivity has been described in several tumours 65. Moreover, in DLBCL patients, Bcl-2 associated resistance to chemotherapy was at least partially overcome by adding Rituximab (anti-CD20-antibody therapy) to standard CHOP chemotherapy 66.

Although 66% of the testicular and 36% of the CNS lymphomas were HLA-A negative, the majority of tumours were still positive for HC10 41, a monoclonal antibody recognizing HLA-B and -C molecules both in complex with peptide and as free heavy chains 67. This indicates that at least one of the two parental genes was still expressed. In addition to B2M mutations, we observed a small homozygous deletion of HLA-A in one case and many hemizygous deletions of the whole HLA class I region. Loss of HLA-DR expression on the other hand, was observed in 76% of the testicular and 50% of the CNS lymphomas and loss of all class II molecules in respectively 53% and 40% of the cases. In contrast to the class I genes, the HLA-DR and HLA-DQ genes were affected by homozygous deletions in nearly 40% of the testicular lymphomas and in two of four CNS lymphomas investigated 43,68. The homozygous deletions of HLA-DR were detected in up to 70% of the cells isolated from frozen and formalin-fixed paraffin-embedded material 68. In addition, in two cases, loss DR or DQ expression was explained by a deleterious mutation in respectively HLA-DRA and HLA-DQB1 with concomitant loss of the wild-type allele through a deletion.

The homozygous deletions were all relatively small and nearly exclusively contained the HLA-DR and HLA-DQ genes. Interestingly, the class III genes were never involved in a homozygous deletion, suggesting that in the latter region genes reside that are crucial for lymphoma cell survival. Altogether, these findings strongly indicate that compared to the class I genes, the HLA class II genes and especially HLA-DR are under much stronger selective pressure. We therefore allege that in line with Knudson’s two-hit model of tumour-suppressor genes (TSG) 69, HLA-DR functions as a TSG in DLBCL of the testis and CNS and accordingly forms a major target for genetic elimination during lymphoma development. Evidence to support the hypothesis that HLA-DR functions as a TSG comes from our own genetic studies, including extensive array-based gene expression studies (M. Booman, unpublished results) clinical studies as well as in-vitro experiments.

Loss of HLA-DR expression has been reported to be an independent prognostic factor for survival in DLBCL, by several authors 54,70-72 although this was not confirmed by others 73. In the latter study though, the HLA-DR negative patients had a shorter median survival than the HLA-DR positive patients. Moreover, loss of HLA class II expression was identified as an independent predictor of survival and strikingly correlated with poor patient outcome in a molecular profiling study analyzing protein expression in a large group of de novo DLBCL patients 74,75. In the group with the lowest expression of HLA-DR, 39% was of extra-nodal
origin although none of the cases was derived from immune privileged sites. In 10 HLA-DR negative tumours, lower numbers of CD8+ CTLs were present compared to 12 positive cases 75. In our group of 76 testicular and CNS DLBCL however, we did not observe significant differences in CTL numbers in class II negative compared to class II positive cases 43. Intriguingly, in a parallel study on cDNA expression for HLA-DR in the same testicular lymphomas as studied by immunohistochemistry, we found a very strong correlation between the number of T cells and HLA-DR cDNA expression in the whole tissue sample containing both neoplastic B cells and professional APCs. This suggests an important role for the concerted action of HLA-DR expression on both the neoplastic cells and APCs (Booman et al., manuscript in preparation). Patients with DLBCL have an extremely variable outcome using standard chemotherapy but patients with a primary CNS or testicular lymphoma have a particularly poor prognosis 76-79. To what extent lack of HLA class II expression contributes to the poor outcome in these particular groups of lymphomas would be an interesting subject for future studies.

The principal role of HLA class II molecules is the presentation of peptides to antigen specific CD4+ T cells leading to activation of peripheral T cells. In B lymphocytes themselves, signal transduction via HLA-DR results in generation of several second messengers including transcription of protein kinase C, activation of tyrosine kinases and phospholipase C and intracellular calcium flux 80,81 which was originally only associated with cellular activation 82,83. Anti-DR antibodies inhibited the proliferation of small, resting B cells induced by T-independent as well as T-dependent stimuli. In contrast, the responses of pre-activated B cells to T cell-derived B cell growth factors were not affected by anti-DR antibodies, indicating that class II signalling was required for cell enlargement and RNA synthesis, only early in the B cell activation cascade 84. Several years later it appeared that HLA-DR signalling also induced apoptosis in B lymphocytes both directly as well as via other effector cells. Monoclonal antibodies against HLA-DR but not against HLA-DQ or -DP or HLA class I, induced programmed cell death in a substantial proportion of class II expressing activated B cells but not in resting B cells 85. HLA class II signalling also increased the sensitivity of activated B cells to Fas mediated apoptosis 86,87. Moreover, by screening the Human Combinatorial Antibody Library, Nagy et al., identified a substantial number of anti-HLA-DR specific human antibodies with tumouricidal activity both in vitro- and in animal models. These antibodies induced caspase-3 independent cell death in activated and tumour-transformed B cells 88. Indirect killing of lymphoma cells was induced by IgA chimeric antibodies via polymorphic mononuclear cells and by IgG1 antibodies via complement 89. Thus, during a peripheral immune response, HLA-DR signalling in B lymphocytes presumably initially results in activation and cell proliferation while later on
HLA-DR mediated apoptosis is induced. This provides the immune system with an efficient and rapid mechanism to eliminate B cells that have completed their role in the peripheral immune response, thereby preventing aberrant B cell proliferation or induction of autoimmune responses.

Based on these data it is conceivable that early during the immune response activated B cells need HLA class II signalling for proliferation and cell growth. During the process of receptor editing and somatic hypermutation, certain B cells may acquire genetic aberrations, become antigen and T cell independent and eventually neoplastic if the normal regulatory mechanisms with elimination of reactive B cells fail. Neoplastic B cells that subsequently lose HLA-class I and HLA-DR expression will subsequently experience major clonal advantage as those cells escape from both CD4+ and CD8+ CTL attack and HLA-class II mediated apoptosis. Furthermore, these lymphoma cells are probably also less sensitive to chemotherapy compared to the class II positive lymphoma cells.

For the treatment of DLBCL, new immunological therapies have been developed over the last decade, as still many DLBCL patients succumbed to their disease despite intensive chemo- and radiotherapy. The use of chimeric monoclonal anti-CD20 antibodies (Rituximab) in combination with chemotherapy has substantially improved the prognosis of DLBCL patients and has become standard therapy now in many institutions 90,91. Therapies using adoptive transfer of tumour-specific CTLs are based on recognition of presented TAA including Id 92. However, for this very specific and individually tailored type of therapy, expression of HLA molecules is a prerequisite and the majority of testicular and CNS lymphomas can therefore be precluded. In aggressive lymphoma, promising results have been obtained using Lym-1, a radiolabelled anti-HLA-DR antibody 93,94. But again, this will not be applicable for most testicular and CNS lymphomas. For the treatment of testicular and CNS DLBCL, other HLA class II negative lymphomas and those patients that do not respond to Rituximab, new immunotherapy’s that target other lymphoma specific molecules need to be developed.

Reference List


