Expression of human leukocyte antigens in diffuse large B cell lymphomas

Riemersma, Sietske Annette

Chapter 6

General Discussion

In 1970 Burnet proposed the term immune surveillance with the immune system specifically recognizing and eliminating transformed cells [1]. 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 [2-5]. 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 [6] while peptides derived from exogenous proteins are presented by HLA class II molecules, constitutively expressed on antigen presenting cells (APCs) [7]. 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) [8-10] and antigen specific cytolytic CTL clones expressing CD4 have been described as well [11,12].

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 [13,14]. 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 [15,16]. 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 [17,18]. 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) [19,20]. 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 [21-23]. The origin of DC and the cytokine environment during priming determines whether $CD4^+$ T cells differentiate into Th1 or Th2 cells [24], with the latter stimulating a humoral immune response which is often less effective [25].

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 [26] and activated T cells can readily patrol the brain [27]. 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 [28]. 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 [29]. 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 [30,31]. 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 [32]. Moreover, macrophage derived Il-10 and TGFß suppresses Th1 cell and NK cell responses (for review see [33]) 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 [34,35]. 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) [36-38] 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 [39,40] and in clinical trials vaccination with Id induced clinical and molecular remissions in lymphoma patients [41,42].

Both in testicular and CNS lymphomas, we observed high numbers of activated tumour infiltrating CTLs [43]. 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 [44-46] 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[47,48] . 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 [49]. 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 [43]. Moreover, all cases were positive for pro-caspase 3 (unpublished results), one of the targets of GB [50], that when activated, contributes to cytochrome *c* release and loss of mitochondrial membrane potential and eventually DNA fragmentation [51,52]. 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 [53-55]. 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 B_2M/HLA class I complex [56]. Two of those cases showed a mutation in B_2M with concomitant loss of the wild-type allele through loss of heterozygosity [57] which results in loss of HLA surface expression as proper assembly of B_2M with class I chains and peptide within the endoplasmatic reticulum is obligate [58]. In six other cases one copy of B_2M was lost which also results in a significant reduction of class I expression [59]. 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 [60] 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 [43,60,61].

The class I positive tumours on the other hand, might escape through Bcl-2 expression, a strong inhibitor of GB-induced apoptosis [52,62]. 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 [62]. Bcl-2 expression is also associated with chemotherapy resistance [63] which makes this protein an interesting target for new therapeutic interventions including anti-sense Bcl-2 therapy (for

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 [43], 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 B_2M 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

1) Burnet FM. The concept of immunological surveillance. Prog Exp Tumour Res. 1970;13:1-27.
2) Gatti RA, Good RA. Occurrence of malignancy in immunodeficiency diseases. A literature review. Cancer. 1971;28:89-98.
3) Birkeland SA, Storm HH, Lamm LU et al. Cancer risk after renal transplantation in the Nordic countries, 1964-1986. Int J Cancer. 1995;60:183-189.
4) Kaplan DH, Shankaran V, Dighe AS et al. Demonstration of an interferon gamma-dependent tumour surveillance system in immunocompetent mice. Proc Natl Acad Sci U S A. 1998;95:7556-7561.
5) Shankaran V, Ikeda H, Bruce AT et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature. 2001;410:1107-1111.
6) Natali PG, Bigotti A, Nicotra MR et al. Distribution of human Class I (HLA-A,B,C) histocompatibility antigens in normal and malignant tissues of nonlymphoid origin. Cancer Res. 1984;44:4679-4687.
7) Radka SF, Charron DJ, Brodsky FM. Class II molecules of the major histocompatibility complex considered as differentiation markers. Hum Immunol. 1986;16:390-400.

8) Voo KS, Fu T, Heslop HE et al. Identification of HLA-DP3-restricted peptides from EBNA1 recognized by CD4(+) T cells. Cancer Res. 2002;62:7195-7199.
9) Kobayashi H, Omiya R, Ruiz M et al. Identification of an antigenic epitope for helper T lymphocytes from carcinoembryonic antigen. Clin Cancer Res. 2002;8:3219-3225.
10) Slager EH, van der Minne CE, Kruse M et al. Identification of multiple HLA-DR-restricted epitopes of the tumour-associated antigen CAMEL by CD4+ Th1/Th2 lymphocytes. J Immunol. 2004;172:5095-5102.
11) Paludan C, Bickham K, Nikiforow S et al. Epstein-Barr nuclear antigen 1-specific CD4(+) Th1 cells kill Burkitt's lymphoma cells. J Immunol. 2002;169:1593-1603.
12) Brady MS, Lee F, Petrie H et al. CD4(+) T cells kill HLA-class-II-antigen-positive melanoma cells presenting peptide in vitro. Cancer Immunol Immunother. 2000;48:621-626.
13) Berek C, Berger A, Apel M. Maturation of the immune response in germinal centers. Cell. 1991;67:1121-1129.
14) Cory S, Jackson J, Adams JM. Deletions in the constant region locus can account for switches in immunoglobulin heavy chain expression. Nature. 1980;285:450-456.
15) Willis TG, Dyer MJ. The role of immunoglobulin translocations in the pathogenesis of B-cell malignancies. Blood. 2000;96:808-822.
16) Pasqualucci L, Neumeister P, Goossens T et al. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature. 2001;412:341-346.
17) Smith JR, Braziel RM, Paoletti S et al. Expression of B-cell-attracting chemokine 1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma. Blood. 2003;101:815-821.
18) Horstmann WG, Timens W. Lack of adhesion molecules in testicular diffuse centroblastic and immunoblastic B cell lymphomas as a contributory factor in malignant behaviour. Virchows Arch. 1996;429:83-90.
19) Giuntoli RL, Lu J, Kobayashi H et al. Direct costimulation of tumour-reactive CTL by helper T cells potentiate their proliferation, survival, and effector function. Clin Cancer Res. 2002;8:922-931.
20) Ossendorp F, Mengede E, Camps M et al. Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II negative tumours. J Exp Med. 1998;187:693-702.
21) Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature. 1998;393:474-478.
22) Bennett SR, Carbone FR, Karamalis F et al. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature. 1998;393:478-480.
23) Schoenberger SP, Toes RE, van der Voort EI et al. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature. 1998;393:480-483.
24) Rissoan MC, Soumelis V, Kadowaki N et al. Reciprocal control of T helper cell and dendritic cell differentiation. Science. 1999;283:1183-1186.
25) Del Prete GF, De Carli M, Ricci M et al. Helper activity for immunoglobulin synthesis of T helper type 1 (Th1) and Th2 human T cell clones: the help of Th1 clones is limited by their cytolytic capacity. J Exp Med. 1991;174:809-813.
26) Yakirevich E, Yanai O, Sova Y et al. Cytotoxic phenotype of intra-epithelial lymphocytes in normal and cryptorchid human testicular excurrent ducts. Hum Reprod. 2002;17:275-283.
27) Hickey WF, Hsu BL, Kimura H. T-lymphocyte entry into the central nervous system. J Neurosci Res. 1991;28:254-260.
28) Aloisi F, Ria F, Adorini L. Regulation of T-cell responses by CNS antigen-presenting cells: different roles for microglia and astrocytes. Immunol Today. 2000;21:141-147.
29) Segal BM, Dwyer BK, Shevach EM. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. J Exp Med. 1998;187:537-546.
30) Banchereau J, Briere F, Caux C et al. Immunobiology of dendritic cells. Annu Rev Immunol. 2000;18:767-811.
31) Nouri-Shirazi M, Banchereau J, Bell D et al. Dendritic cells capture killed tumour cells and present their antigens to elicit tumour-specific immune responses. J Immunol. 2000;165:3797-3803.
32) Ogden CA, Pound JD, Batth BK et al. Enhanced apoptotic cell clearance capacity and B cell survival factor production by IL-10-activated macrophages: implications for Burkitt's lymphoma. J Immunol. 2005;174:3015-3023.
33) Elgert KD, Alleva DG, Mullins DW. Tumour-induced immune dysfunction: the macrophage connection. J Leukoc Biol. 1998;64:275-290.
34) Shevach EM. Regulatory T cells in autoimmmunity*. Annu Rev Immunol. 2000;18:423-449.
35) Fuss IJ, Boirivant M, Lacy B et al. The interrelated roles of TGF-beta and IL-10 in the regulation of experimental colitis. J Immunol. 2002;168:900-908.
36) Weiss S, Bogen B. MHC class II-restricted presentation of intracellular antigen. Cell. 1991;64:767-776.
37) Weiss S, Bogen B. B-lymphoma cells process and present their endogenous immunoglobulin to major histocompatibility complex-restricted T cells. Proc Natl Acad Sci U S A. 1989;86:282-286.
38) Lauritzsen GF, Weiss S, Dembic Z et al. Naive idiotype-specific CD4+ T cells and immunosurveillance of B-cell tumours. Proc Natl Acad Sci U S A. 1994;91:5700-5704.
39) Sirisinha S, Eisen HN. Autoimmune-like antibodies to the ligand-binding sites of myeloma proteins. Proc Natl Acad Sci U S A. 1971;68:3130-3135.

40) Jorgensen T, Hannestad K. Specificity of T and B lymphocytes for myeloma protein 315. Eur J Immunol. 1977;7:426-431.
41) Bendandi M, Gocke CD, Kobrin CB et al. Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. Nat Med. 1999;5:1171-1177.
42) Hsu FJ, Benike C, Fagnoni F et al. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nat Med. 1996;2:52-58.
43) Riemersma SA, Oudejans JJ, Vonk MJ et al. High numbers of tumour-infiltrating activated cytotoxic T lymphocytes, and frequent loss of HLA class I and II expression, are features of aggressive B cell lymphomas of the brain and testis. J Pathol. 2005;206:328-336.
44) Thompsett AR, Ellison DW, Stevenson FK et al. V(H) gene sequences from primary central nervous system lymphomas indicate derivation from highly mutated germinal center B cells with ongoing mutational activity. Blood. 1999;94:1738-1746.
45) Montesinos-Rongen M, Van Roost D, Schaller C et al. Primary diffuse large B-cell lymphomas of the central nervous system are targeted by aberrant somatic hypermutation. Blood. 2004;103:1869-1875.
46) Hyland J, Lasota J, Jasinski M et al. Molecular pathological analysis of testicular diffuse large cell lymphomas. Hum Pathol. 1998;29:1231-1239.
47) Xie X, Wacker HH, Huang S et al. Differential expression of cancer testis genes in histological subtypes of non-Hodgkin's lymphomas. Clin Cancer Res. 2003;9:167-173.
48) Dyomin VG, Rao PH, Dalla-Favera R et al. BCL8, a novel gene involved in translocations affecting band 15q11-13 in diffuse large-cell lymphoma. Proc Natl Acad Sci U S A. 1997;94:5728-5732.
49) Berke G. The CTL's kiss of death. Cell. 1995;81:9-12.
50) Darmon AJ, Nicholson DW, Bleackley RC. Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B. Nature. 1995;377:446-448.
51) MacDonald G, Shi L, Vande VC et al. Mitochondria-dependent and -independent regulation of Granzyme B-induced apoptosis. J Exp Med. 1999;189:131-144.
52) Metkar SS, Wang B, Ebbs ML et al. Granzyme B activates procaspase-3 which signals a mitochondrial amplification loop for maximal apoptosis. J Cell Biol. 2003;160:875-885.
53) Garrido F, Ruiz-Cabello F, Cabrera T et al. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. Immunol Today. 1997;18:89-95.
54) Amiot L, Onno M, Lamy T et al. Loss of HLA molecules in B lymphomas is associated with an aggressive clinical course. Br J Haematol. 1998;100:655-663.
55) Moller P, Herrmann B, Moldenhauer G et al. Defective expression of MHC class I antigens is frequent in B-cell lymphomas of high-grade malignancy. Int J Cancer. 1987;40:32-39.
56) Barnstable CJ, Bodmer WF, Brown G et al. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens-new tools for genetic analysis. Cell. 1978;14:9-20.
57) Jordanova ES, Riemersma SA, Philippo K et al. Beta2-microglobulin aberrations in diffuse large B-cell lymphoma of the testis and the central nervous system. Int J Cancer. 2003;103:393-398.
58) D'Urso CM, Wang ZG, Cao Y et al. Lack of HLA class I antigen expression by cultured melanoma cells FO-1 due to a defect in B2m gene expression. J Clin Invest. 1991;87:284-292.
59) Bicknell DC, Rowan A, Bodmer WF. Beta 2-microglobulin gene mutations: a study of established colorectal cell lines and fresh tumours. Proc Natl Acad Sci U S A. 1994;91:4751-4755.
60) Ansell SM, Stenson M, Habermann TM et al. Cd4+ T-cell immune response to large B-cell non-Hodgkin's lymphoma predicts patient outcome. J Clin Oncol. 2001;19:720-726.
61) Bashir R, Chamberlain M, Ruby E et al. T-cell infiltration of primary CNS lymphoma. Neurology. 1996;46:440-444.
62) Pinkoski MJ, Waterhouse NJ, Heibein JA et al. Granzyme B-mediated apoptosis proceeds predominantly through a Bcl-2-inhibitable mitochondrial pathway. J Biol Chem. 2001;276:12060-12067.
63) Miyashita T, Reed JC. bcl-2 gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. Cancer Res. 1992;52:5407-5411.
64) Kim R. Recent advances in understanding the cell death pathways activated by anticancer therapy. Cancer. 2005;103:1551-1560.
65) Andersen MH, Svane IM, Kvistborg P et al. Immunogenicity of Bcl-2 in patients with cancer. Blood. 2005;105:728-734.
66) Mounier N, Briere J, Gisselbrecht C et al. Rituximab plus CHOP (R-CHOP) overcomes bcl-2--associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL). Blood. 2003;101:4279-4284.
67) Stam NJ, Vroom TM, Peters PJ et al. HLA-A- and HLA-B-specific monoclonal antibodies reactive with free heavy chains in western blots, in formalin-fixed, paraffin-embedded tissue sections and in cryo-immuno-electron microscopy. Int Immunol. 1990;2:113-125.
68) Riemersma SA, Jordanova ES, Schop RF et al. Extensive genetic alterations of the HLA region, including homozygous deletions of HLA class II genes in B-cell lymphomas arising in immune-privileged sites. Blood. 2000;96:3569-3577.

69) Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A. 1971;68:820-823.
70) Miller TP, Lippman SM, Spier CM et al. HLA-DR (Ia) immune phenotype predicts outcome for patients with diffuse large cell lymphoma. J Clin Invest. 1988;82:370-372.
71) Spier CM, Grogan TM, Lippman SM et al. The aberrancy of immunophenotype and immunoglobulin status as indicators of prognosis in B cell diffuse large cell lymphoma. Am J Pathol. 1988;133:118-126.
72) Momburg F, Herrmann B, Moldenhauer G et al. B-cell lymphomas of high-grade malignancy frequently lack HLA-DR, -DP and -DQ antigens and associated invariant chain. Int J Cancer. 1987;40:598-603.
73) O'Keane JC, Mack C, Lynch E et al. Prognostic correlation of HLA-DR expression in large cell lymphoma as determined by LN3 antibody staining. An Eastern Cooperative Oncology Group (ECOG) study. Cancer. 1990;66:1147-1153.
74) Rosenwald A, Wright G, Chan WC et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:1937-1947.
75) Rimsza LM, Roberts RA, Miller TP et al. Loss of MHC class II gene and protein expression in diffuse large B-cell lymphoma is related to decreased tumour immunosurveillance and poor patient survival regardless of other prognostic factors: a follow-up study from the Leukemia and Lymphoma Molecular Profiling Project. Blood. 2004;103:4251-4258.
76) Zucca E, Conconi A, Mughal TI et al. Patterns of outcome and prognostic factors in primary large-cell lymphoma of the testis in a survey by the International Extranodal Lymphoma Study Group. J Clin Oncol. 2003;21:20-27.
77) Darby S, Hancock BW. Localised non-Hodgkin lymphoma of the testis: the Sheffield Lymphoma Group experience. Int J Oncol. 2005;26:1093-1099.
78) Shenkier TN, Voss N, Chhanabhai M et al. The treatment of primary central nervous system lymphoma in 122 immunocompetent patients: a population-based study of successively treated cohorts from the British Colombia Cancer Agency. Cancer. 2005;103:1008-1017.
79) Pels H, Schmidt-Wolf IG, Glasmacher A et al. Primary central nervous system lymphoma: results of a pilot and phase II study of systemic and intraventricular chemotherapy with deferred radiotherapy. J Clin Oncol. 2003;21:4489-4495.
80) Lane PJ, McConnell FM, Schieven GL et al. The role of class II molecules in human B cell activation. Association with phosphatidyl inositol turnover, protein tyrosine phosphorylation, and proliferation. J Immunol. 1990;144:3684-3692.
81) Mooney NA, Grillot-Courvalin C, Hivroz C et al. Early biochemical events after MHC class II-mediated signalling on human B lymphocytes. J Immunol. 1990;145:2070-2076.
82) Vaickus L, Jones VE, Morton CL et al. Antiproliferative mechanism of anti-class II monoclonal antibodies. Cell Immunol. 1989;119:445-458.
83) Palacios R, Martinez-Maza O, Guy K. Monoclonal antibodies against HLA-DR antigens replace T helper cells in activation of B lymphocytes. Proc Natl Acad Sci U S A. 1983;80:3456-3460.
84) Clement LT, Tedder TF, Gartland GL. Antibodies reactive with class II antigens encoded for by the major histocompatibility complex inhibit human B cell activation. J Immunol. 1986;136:2375-2381.
85) Truman JP, Ericson ML, Choqueux-Seebold CJ et al. Lymphocyte programmed cell death is mediated via HLA class II DR. Int Immunol. 1994;6:887-896.
86) Truman JP, Choqueux C, Tschopp J et al. HLA class II-mediated death is induced via Fas/Fas ligand interactions in human splenic B lymphocytes. Blood. 1997;89:1996-2007.
87) Yoshino T, Cao L, Nishiuchi R et al. Ligation of HLA class II molecules promotes sensitivity to CD95 (Fas antigen, APO-1)-mediated apoptosis. Eur J Immunol. 1995;25:2190-2194.
88) Nagy ZA, Hubner B, Lohning C et al. Fully human, HLA-DR-specific monoclonal antibodies efficiently induce programmed death of malignant lymphoid cells. Nat Med. 2002;8:801-807.
89) Dechant M, Vidarsson G, Stockmeyer B et al. Chimeric IgA antibodies against HLA class II effectively trigger lymphoma cell killing. Blood. 2002;100:4574-4580.
90) Leonard JP, Coleman M, Ketas J et al. Combination Antibody Therapy With Epratuzumab and Rituximab in Relapsed or Refractory Non-Hodgkin's Lymphoma. J Clin Oncol. 2005;23:5044-5051.
91) Sehn LH, Donaldson J, Chhanabhai M et al. Introduction of Combined CHOP Plus Rituximab Therapy Dramatically Improved Outcome of Diffuse Large B-Cell Lymphoma in British Columbia. J Clin Oncol. 2005;23:5027-5033.
92) Schultze JL, Nadler LM. T cell mediated immunotherapy for B cell lymphoma. J Mol Med. 1999;77:322-331.
93) DeNardo GL, DeNardo SJ, Goldstein DS et al. Maximum-tolerated dose, toxicity, and efficacy of (131)I-Lym-1 antibody for fractionated radioimmunotherapy of non-Hodgkin's lymphoma. J Clin Oncol. 1998;16:3246-3256.
94) Multani P. Development of radioimmunotherapy for the treatment of non-Hodgkin's lymphoma. Int J Hematol. 2002;76:401-410.