Chapter 8
Mouse Models of Human Mature B-Cell and Plasma Cell Neoplasms

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Contents

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8.1 Introduction

Developing mouse models that accurately reflect features of human B-cell lineage neoplasms has been a daunting but increasingly rewarding task. Studies of spontaneous tumors or those induced by chemicals, irradiation, or retroviruses performed before the 1980s provided remarkable insights into mechanisms and genetics of lymphomagenesis. With the advent of genetic engineering, it became possible to rapidly develop and explore new models and to enhance the value of established systems. Here, we will review past and present accomplishments in modeling mature human B-cell lymphomas and plasmas cell neoplasms (PCN) in mice, examine their strengths and limitations, and discuss obstacles that must be addressed in future work. These systems have accelerated our ability to understand the development of complex disease in vivo and to develop novel therapeutic approaches to diseases, many of which are almost uniformly lethal.

The cellular origins of human mature B cell and PCN defined by the consensus WHO classification (Jaffe, Harris et al. 2001) are presented diagrammatically in Fig. 8.1. The lymphomas reflect various features of normal pregerminal center (pre-GC) and post-GC cells including anatomic location, expression patterns of differentiation markers, and mutational status of immunoglobulin gene variable region (IgV) sequences. Recently, further distinctions have been made based on the results of microarray gene expression profiling of normal B cells and lymphomas.

Most types of human B-cell lymphoma are derived from GC or post-GC B cells. A number of these are recognized as having close parallels among mouse B-lineage tumors classified according to the Bethesda proposals (Morse, Anver et al. 2002) including follicular B-cell lymphoma (FBL) and diffuse large B-cell lymphomas (DLBCL) as well as plasmacytomas (PCT) (Fig. 8.1). Human lymphomas of pre-GC origin, including a subset of chronic lymphocytic leukemia (CLL), termed small B-cell lymphoma (SBL) in mice and splenic marginal zone lymphoma (SMZL), also have parallels among spontaneous and induced tumors of mice. To date, there are no reports of mouse neoplasms with significant similarities to human Hodgkin, Burkitt, primary effusion or post-transplant lymphomas or to hairy cell or prolymphocytic leukemias. There are reports, however, of mouse models of marginal zone lymphomas (MZL) of

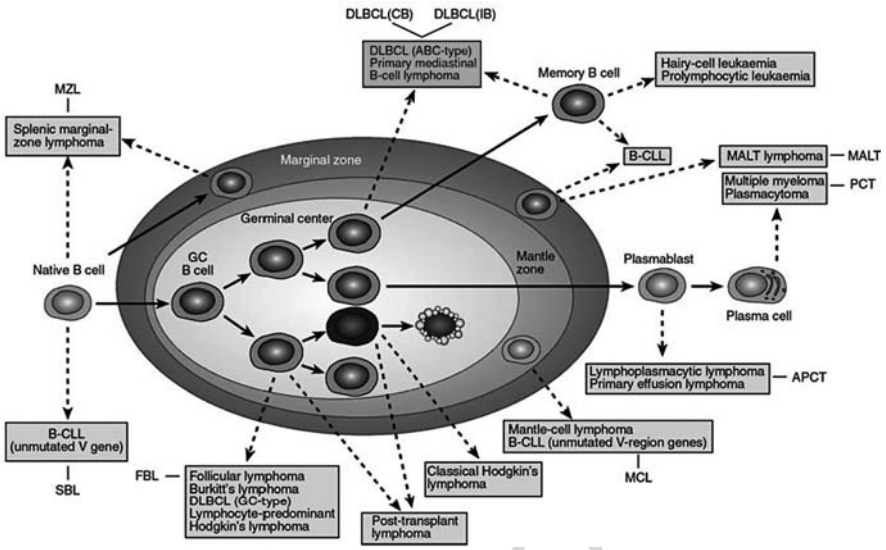

Fig. 8.1 Cellular origins of human and mouse mature B-cell lymphomas and plasma cell neoplasms. After undergoing early maturation in the bone marrow, naïve transitional B cells populate peripheral lymphoid tissues where they are recruited into the follicular and marginal zone B-cell subsets. Following interaction with antigen and helper T cells, follicular B cells establish germinal centers. There they undergo rapid clonal expansion and somatic hypermutation in the dark zone and move to the light zone where they undergo class switch recombination and positive selection on antigen-bearing follicular dendritic cells. Selected cells exit the germinal center to form memory B cells or to become plasma cells. Reciprocal chromosomal translocations involving *IgH* or *IgL* gene and a proto-oncogene, characteristic of most non-Hodgkin lymphomas (NHL), are thought to be generated as unfortunate by-products of the several mechanisms involved in *Ig* gene diversification that involves the generation of double-stranded DNA breaks. Some, such as IgH–BCL2 in follicular lymphoma, appear to occur as a consequence of aberrant V_HDJ_H recombination. Others, located adjacent to rearranged, somatically mutated V_HDJ_H genes, such as IgH–BCL6 in diffuse large B-cell lymphoma, most likely occur as a consequence of somatic hypermutation. A third group features breakpoint in IgH switch regions and appears to reflect aberrant class switch recombination, as in a subset of Burkitt lymphoma. The classes of human lymphomas and the mouse neoplasms most closely related when known are indicated. [Modified from Kuppers (2005).]

mucosa-associated lymphoid tissue (MALT) and mantle cell lymphomas (MCL) as well as lymphoplasmacytic lymphoma, termed anaplastic PCT (APCT) in mice. Systems that help define, explore, and extend these parallels are the subject of this chapter.

An ongoing challenge for developing accurate mouse models ofhuman NHL and PCN is the need to reproduce recurrent somatic mutations in the appropriate B-cell targets. Deciding which B cell to target—pre-GC, GC, or MZ B cell or extrafollicular plasmablast, for example—will be difficult as long as the precise nature and differentiation stage of the human tumor precursors remains unknown. Additional challenges for transgenic (TG) mouse models of NHL

and PCN relate to the recapitulation of tumor progression pathways, mechanisms of stromal interactions, and responses to drugs used in human patients. Although mouse models of NHL and PCN might well be expected to phenocopy the human tumors, engineering the signaling pathways and responses is much more difficult than inducing rapid onset neoplasms such as those using retroviral transfer of classic oncogenes, which have remarkable value in their own right (Hu, Swerdlow et al. 2006).

A variety of TG techniques are now available to address the challenges mentioned above. It is possible to introduce into the mouse germ line gain-of-function mutations such as constitutively expressed oncogenes under control of B cell or plasma cell-specific enhancers and promoters. Conversely, normal mouse germ line genes can be replaced with loss-of-function alleles, which may take the form of classic null alleles ("knock-out" mice) or, of increasing importance, weak-efficiency alleles that retain some gene activity and thus more closely mimic alleles segregating in the human population. A more sophisticated method involves inducible transgenes, which provide for the temporal and spatial control of gene expression or gene attenuation, thereby circumventing potential developmental problems including embryonic toxicity and lethality that may be posed by constitutively expressed TG. Adenoviruses (Strair, Sheay et al. 2002) and retroviruses (Mikkers and Berns 2003) offer additional means for spatial and temporal gene regulation. A lesser known but very attractive example of the latter strategy takes advantage of cell type-specific retroviral binding to specific receptors of the subgroup A avian leucosis virus, affording introduction of several cancer genes into mouse target cells (Du, Podsypanina et al. 2006). Lentivirus-mediated transgenesis (Aronoff and Petersen 2006) represents yet another alternative to conventional technologies for generating the panel of TG mouse strains that may be required to accurately model human plasma cell tumors.

Mouse models of NHL and PCN should not only phenocopy their human counterparts at the histopathologic and molecular level but also exhibit desirable features including high tumor penetrance, short latencies, and predictable stages of tumor progression. Additional expectations, such as means for monitoring the tumor burden in live animals in a repeatable and reproducible manner, need also be considered.

Barriers to using TG mouse models of human mature B-cell lymphomas and PCN more widely are several and include the following:

1. *Problems related to establishment and maintenance of mouse colony*. Differences in genetic background of mice require costly and time-consuming backcrosses of individual TG from donor strains to desired recipient strains. In addition, differences in the microbial floras of mouse colonies frequently results in quarantine and monitoring of microbiological features and health status frequently resulting in rederivation of strains under specific pathogen-free (SPF) conditions in the recipient institution.
2. *Logistical problems*. There are limited financial and space resources for scaling up mouse breeding, husbandry, and genotyping to perform statistically robust

time course analyses of tumor development and drug testing. In addition, there are intellectual property issues that seriously affect time, cost, and mechanisms to negotiate and acquire mouse strains. The complexities of multiparty agreements and differing priorities of academic and commercial outfits lend an additional layer of difficulties. Finally, we are faced with problems related to data acquisition and analysis that include, as one example, a paucity of methods for high-throughput biomedical imaging and high-throughput microscopy.

8.2 Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

8.2.1 CLL/Small Lymphocytic Lymphoma in Humans

CLL and SLL are clonal, accumulative diseases of monomorphic, small, round, slowly proliferating $CD5^+$ B cells in peripheral blood, bone marrow, and lymph nodes admixed with smaller numbers of prolymphocytes and para-immunoblasts. Lymph nodes exhibit a pseudofollicular pattern. The term small lymphocytic lymphoma (SLL) is reserved for non-leukemic cases with similar tissue involvement and cellular phenotype. SLL is not considered to be the precursor to CLL. Rather, tissue involvement is thought to be almost always secondary to an established leukemia. Transformation to clonally related high-grade lymphoma, usually DLBCL, termed Richter syndrome, occurs in about 5% of cases (Jaffe, Harris et al. 2001). Interestingly, CLL may be preceded by a condition, tentatively designated benign monoclonal lymphocytosis (Victor Hoffbrand and Hamblin 2007), that may bear a similar relationship to CLL as monoclonal gammopathy of undetermined significance (MGUS) does to multiple myeloma (MM) (Kyle, Therneau et al. 2002).

Abnormal karyotypes are found by FISH in ~80% of cases with deletions of 13q14 (50%) and 12q trisomy (20%) being among the most common; translocation t(14q32) occurs in 5% of cases (Dohner, Stilgenbauer et al. 2000). The 13q14 deletion encompasses the micro-RNA genes, miR-15a, and miR-16-1 (miR15/16) (Calin, Sevignani et al. 2004). Familial aggregations of CLL are not uncommon. CLL/SLL is usually considered to be incurable with current therapy with an overall 5-year survival rate of around 50%. Molecular and phenotypic characterizations, however, have allowed the definition of subsets with clinically indolent and aggressive forms of the disease (Table 8.1). These are based on studies of IgV regions, cytogenetics, cell surface phenotype, and gene expression. Of particular interest is the observation that cases with mutated IgV genes and particular biases in V gene family utilization, such as V1-69, have a much more favorable course than cases with unmutated IgV genes and biased utilization of a separate set of V regions, such as V4-34 (Chiorazzi and Ferrarini 2003). This suggests that that the ability of BCRs to bind specific antigens might drive

Table 8.1 Features of indolent and aggressive chronic lymphocytic leukemia (CLL)

	Course of disease		
Variable	Indolent	Aggressive	Ref.
IgV regions	Mutated	Not mutated	Chiorazzi and Ferrarini (2003)
IgV preference	V1–69	V4–34	Chiorazzi and Ferrarini (2003)
Chr 11, Chr 17 aberrations	Less common	More common	Dohner, Stilgenbauer et al. (2000)
Chr 13q14 deletion	More common	Less common	Dohner, Stilgenbauer et al. (2000)
CD38 expression	Low	High	Damle, Wasil et al. (1999)
BCL2 expression	Low	High	Faderl, Keating et al. (2002)
ZAP70 expression	Low	High	Crespo, Bosch et al. (2003)
Serum thymidine kinase	Low	High	Hallek, Langenmayer et al. (1999)
Serum CD23	Low	High	Sarfati, Chevret et al. (1996)

expressing cells from a state of normality to the leukemic state. Studies of antigenic specificity have shown these BCRs to be poly- and autoreactive.

8.2.2 Mouse Models of CLL/SLL

8.2.2.1 Spontaneous Small B-Cell Lymphoma/Leukemia in Mice

Old mice of some strains spontaneously develop a clonal, mature B-cell disease termed SBL with many features reminiscent of CLL in humans (Fredrickson and Harris 2000; Hartley, Chattopadhyay et al. 2000). A predominant population of small lymphocytes with low mitotic activity associated with prolymphocytes and proliferation centers originates in the spleen. Advanced cases may exhibit involvement of lymph nodes, liver, and kidneys. A leukemic phase occurs in about 25% of cases and conversion to high-grade immunoblastic (IBL) lymphoma (Richter syndrome) is sometimes seen (Fredrickson and Harris 2000; Morse, Anver et al. 2002). The few cases that were phenotyped by flow cytometry were $CD5^{lo}$. There is no information on V_HDJ_H repertoire for these mice.

8.2.2.2 Models of CLL Based on Studies of New Zealand Mice

The first associations relating CD5 expression on B cells, neoplasia and NZB mice, were made in 1981with the finding that cultured B-lymphoma lines, including several from (BALB/c × NZB)F1 mice, expressed low levels of "Lyt-1" (Lanier, Warner et al. 1981). Later studies demonstrated that a subset of normal spleen cells expressed CD5 with spleens of NZB and (NZB × NZW)F1 mice having the highest frequencies (Manohar, Brown et al. 1982; Hayakawa, Hardy et al. 1983). $CD5^+$ B cells, now termed B1a cells, were later found to comprise a high proportion of peritoneal B cells and to share a distinctive $IgM^{hi}CD11b^+$

phenotype with another subset of functionally similar peritoneal B cells, now designated B1b B cells. B1b cells are not found in spleen, and splenic B1a cells do not express CD11b. Both subsets of peritoneal B1 cells derive from fetal/neonatal progenitors that are distinct from the B-cell progenitors in adult bone marrow that give rise to follicular and MZ B2 cells (Hardy 2006).

In spite of their differing origins, B1 cells share many features with MZ B cells including the ability to act as front-line responders to invading pathogens from the gut or circulating in the blood (Martin and Kearney 2000, 2001). The repertoires of both B1 and MZ B cells are also enriched for poly- or autoreactive antibody specificities. Importantly, the splenomegaly of aging NZB was shown in some cases to be the result of MZ enlargement, and NZB is the strain in which splenic MZL was first described (Yumoto 1980).

CLL cells were first shown to express CD5 in 1980 (Boumsell, Coppin et al. 1980). The fact that NZB mice were known to have high levels of normal splenic $CD5^+$ B cells prompted studies of the possible relationship of these cells to NZB lymphomas (East 1970). Soon, several laboratories reported the identification of clonal populations of $CD5^+$ B cells in aging NZB mice and showed that they were readily transplanted. In some mice, these lymphomas were associated with leukemic phases. This suggested that the $CD5^+$ lymphoma/leukemias of NZB mice might serve as a model for human CLL (Okada, Takiura et al. 1991; Phillips, Mehta et al. 1992; Stall, Farinas et al. 1988).

The clonal B-cell populations were found to develop in a characteristic fashion, first appearing in the peritoneal cavity in mice 2–3months of age; these peritoneal populations were usually oligoclonal. This was followed by successive spread to the spleen at 3–5months, peripheral blood at 5–7months, lymph nodes at 7–10 months, and bone marrow at 10–13 months (Stall, Farinas et al. 1988). The clones identified in spleens or lymph nodes were most often present among the several clones found in the peritoneum of the same animal. A similar pattern was seen in and NZW and (NZB $\times$ NZW)F1 mice (Stall, Farinas et al, 1988). Studies of congenic NZB and NZW and F1 mice showed that animals homozygous for *H-2*^{z} had the highest frequencies of peritoneal $CD5^+$ B cells (Okada, Takiura et al. 1991). Other studies of NZB mice and progeny of crosses with DBA/2 demonstrated that the $CD5^+$ B cells accumulating in the spleens of aging mice were hyperdiploid and readily transplantable (Raveche, Lalor et al. 1988). Interestingly, this group did not identify hyperdiploid cells in the peritoneum of these mice.

Changes in expression of the cytokines IL-5 and IL-10were found to markedly affect the occurrence of $CD5^+$ lymphomas in NZB and (NZB $\times$ NZW)F1 mice. Studies of NZB mice homozygous for a null mutation of IL-10 showed that expansion of $CD5^+$ B cell outside the peritoneum and the development of clonal lymphomas was markedly reduced (Czarneski, Lin et al. 2004). In addition, (NZB $\times$ NZW)F1 mice overexpressing IL-5 from a transgene had greatly increased populations of $CD5^+$ B cells and were at increased risk for lymphoma development (Xiangshu Wen et al. 2004).

Two studies have examined the genetic basis for the lymphoma susceptibility of New Zealand mice. Analyses of backcross progeny from the crosses of (NZW × B10.NZW)F1 × B10.NZW indicted that the frequency of $CD5^+$ B cells in peripheral blood was governed by three susceptibility alleles (Hamano, Hirose et al. 1998). The first, *Bpal-1*, was closely linked to the MHC on Chr 17. This was consistent with earlier studies associating homozygosity for $H\text{-}2^z$ and $CD5^+$ B-cell frequency (Okada, Takiura et al. 1991). The second, *Bpal-2*, was located toward the centromeric end of Chr 13, and the third, *Bpal-3*, was close to the centromere on Chr 17 but not linked to the MHC (Hamano, Hirose et al. 1998). The development of leukemia in older mice was associated with elevated levels of $CD5^+$ B cells in blood at an early age. Each allele functioned independently and in an incompletely dominant fashion.

A second study examined mice from the cross (NZB × DBA/2)F1 × DBA/2, a low lymphoma strain (Raveche, Salerno et al. 2007). Lymphoproliferative disease occurred in 37% of the mice. Histologically, 94% of these cases were diagnosed as splenic MZL. Occurrence of disease was linked to three loci on chromosomes 14, 18, and 19. Sequence studies of Chr 14 identified a single base polymorphism 6 bp downstream of the pre-miR-16-1 sequence. This was associated with reduced expression of miR16 in NZB lymphoid cells and a NZB B-lymphoma cell line. Further studies suggested that miR-16-1normally functions in B cells to retard cell cycle progression and promote apoptosis. These results were very suggestive of an important tie between the lymphomas of NZB mice and human CLL as the miR15/16 locus is frequently deleted in the malignant B cells of CLL.

The IgH V region sequences of lymphomas occurring in New Zealand mice were examined for three cases (Mahboudi, Phillips et al. 1992). All lymphomas expressed unmutated V genes and DFL16.1, a D region gene frequently used by fetal B cells. No N additions were seen. This pattern resembles that of the normal fetal/neonatal B-cell repertoire (Feeney 1990).

Taken together, these studies indicate that the peritoneal and splenic $CD5^+$ B-cell populations of young New Zealand mice are considerably larger than those of most other strains. Over time, the peritoneal and splenic populations exhibit oligo- or monoclonal expansions of $CD5^+$ B cells that can spill into the blood as leukemia, seeding lymph nodes and other tissue but rarely affecting the bone marrow. For mice that have clonal populations in both spleen and peritoneum, the clones are sometimes common to the peritoneal and splenic B-cell populations, while in others they may be restricted to one site or the other (Stall, Farinas et al. 1988; Okada, Takiura et al. 1991). Numbers of $CD5^+$ B-cell numbers in young mice are tied to later development of lymphoma. $CD5^+$ B-cell numbers and lymphoma incidence are both under polygenic control but from genes that appear to differ between NZB and NZW. Little has been done to characterize Ig mutational state, but the few available sequences are very much like those of fetal/neonatal B cells.

Importantly, the lymphomas that develop in these mice have been diagnosed almost uniformly as splenic MZL. Pseudofollicles/proliferation centers,

prolymphocytes, and para-immunoblasts are not features of the lymphomas of New Zealand mice, marking them as histologically quite distinct from human CLL.

8.2.2.3 The TCL1 TG Mouse Model of CLL

The *TCL1* oncogene was initially identified at Chr 14q32.1 as the gene commonly activated by translocations or inversions involving T-cell receptor loci (Fu, Virgilio et al. 1994). The development of T-cell lymphomas in mice with TCL1 expression driven by the *Lck* promoter in T cells established TCL1 as a true proto-oncogene (Virgilio, Lazzeri et al. 1998). TCL1 is also expressed in normal B-lineage cells from the pre-B to follicular B-cell stages and is then downregulated in GC B cells and extinguished in memory and plasma cells (Said, Hoyer et al. 2001; Virgilio, Narducci et al. 1994). High levels of expression have also been seen in a variety of immature and mature B-cell lymphomas (Teitell, Damore et al. 1999; Narducci, Pescarmona et al. 2000; Teitell 2005). The oncogenic effect of TCL1 is thought to be mediated, in part, by its interactions with AKT, enhancing its activation and stimulating downstream pathways that promote proliferation and survival (Teitell 2005).

In 2002, Bichi and her collaborators described features of mice bearing an Eμ–TCL1 TG (Bichi, Shinton et al. 2002) that included the development of late onset clonal $CD5^+$ lymphomas/leukemias. The general picture of this disease is remarkably similar to that described by Stall et al. for NZB mice (Stall, Farinas et al. 1988). Beginning around 2 months of age, the mice exhibited an expanded population of $CD5^+CD11b^+$ B cells in the peritoneum that became evident in the spleen at 4–5 months and then the bone marrow at 8 months. Analyses of Ig gene rearrangements in mice older than 7 months showed that the expanded populations were clonal and that clonal markers were sometimes shared between the peritoneum and spleen. All mice older than 13 months developed marked splenomegaly, hepatomegaly, lymphadenopathy, and leukemia with a mean WBC of 180×10^6/ml.

The B-cell expansion in mice younger than 7 months was polyclonal with a repertoire that was like that of normal $CD5^+$ B cells, including the recurrent use of specific V_HV_L combinations (Bichi, Shinton et al. 2002). The V_H11 sequences examined were essentially unmutated with only low levels of N-region additions, similar to V_H regions that characterize normal $CD5^+$ and fetal B cells to varying extents (Feeney 1990; Li, Hayakawa et al. 1993; Kantor, Merrill et al. 1997).

Histologic studies of mice 8 months of age and older showed a progressive enlargement of the splenic MZ by cells cytologically indistinsguishable from normal MZ B cells. The phenotype of the MZ B cells was atypical, however, as they were $CD21^{lo}CD5^{lo}$ rather than the $CD21^{hi}CD5^-$ phenotype of normal MZ B cells. In older mice, these cells extended into the red pulp in a manner similar to that seen with MZL in NFS.V^+ mice, but with no cytologic progression toward high-grade disease. Even cells from leukemic mice were unchanged.

More detailed studies of Ig utilization by the malignancies of older mice (Yan, Albesiano et al. 2006) revealed several important points: (1) sequences of the expressed V_H and V_L genes were identical to or differed minimally from germline; (2) utilization of V_H families 1, 11, and 12, D segment families, and the J_H segments, J_H1, diverged from that of the normal B-cell repertoire; (3) the HCDR3 and LCDR3 regions of the clones tended to be longer than for normal adult B cells and many contained two or more charged amino acids; (3) the cases exhibited stereotypic V_HDJ_H rearrangements that resembled sequences reported previously for certain autoantibodies and antibodies reactive with microbial antigens; and (4) cloned expressed sequences that were poly- and autoreactive and bound to a variety of polysaccharides. Almost all these features, except for reactivity with non-protein antigens, are similar to those of Ig genes from aggressive, unmutated CLL.

Other studies have forwarded the Eμ-TCL1 TG mouse as a tool for preclinical drug testing for human CLL (Johnson, Lucas et al. 2006). Leukemic mice responded initially to treatment with standard drug used in CLL, fludarabine, but then became resistant, similar to human CLL. Heightened expression of BCl2, MCL1, PDK1, and AKT1 in the transformed lymphocytes suggested that other potential drug targets were available. The importance of AKT signaling in this model was examined in studies of the effects of rapamycin, an mTOR inhibitor, on the survival of mice transplanted with expanded $CD5^+$ B-cell populations from the TG mice. Treated mice began to die significantly later than untreated animals although all animals in both groups were dead by ~200 days after transplantation (Zanesi, Aqeilan et al. 2006).

8.2.2.4 The BLC2/TRAF Model of CLL–the NFkB Connection

CLL and other NHL are characterized by overexpression of BCL2, which contributes to an apoptosis-resistant phenotype. TRAF1 is also overexpressed in a spectrum of NHL and in CLL is associated with aggressive disease (Zapata, Krajewska et al. 2000). TG mice overexpressing BCL2 or a dominant negative form of TRAF2 (TRAF2DN), which mimics the signaling features of TRAF1, develop expanded populations of B cells with occasional BCL2 TG mice developing long-latency, low-grade lymphomas (Strasser, Harris et al. 1993). Mice doubly TG for BCL2 and TRAF2DN were found to die between 6 and 18 months of age with marked splenomegaly, lymphadenopathy infiltration of non-lymphoid tissues, and in many cases, ascites and pleural effusions (Zapata, Krajewska et al. 2004). Leukemias WBC counts around 150×10^6/ml were common. The mice were diagnosed histologically as having SBL with a leukemic phase, and analyses of Ig gene organization showed the disease to be clonal. The tumor cells were $IgM^{hi}CD23^-CD21^{lo/-}CD5^{lo}$, consistent with an origin from B1a cells (Zapata, Krajewska et al. 2004). In addition, the cells were slowly proliferative and exhibited increased resistance to apoptosis due to effects of both BCL2 and TRAF2DN. These combined features were felt to be indicative of a CLL-like disease.

The use of double TG in preclinical drug studies was recently described (Kress, Martinez-Garcia et al. 2007). First, cells from double TG mice and humans with CLL were compared for their responses to treatment in vitro with synthetic triterpenoid derivatives. Both cell types were susceptible to induction of apoptosis. In addition, tests of the same drugs in double TG mice with leukemia resulted in marked decreases in WBC counts and reduced tissue burdens. These results were thought to support the testing of the drugs in patients with CLL.

A fascinating story that relates to that of the double TG mice comes from studies of mice bearing a mutant *NFkB2* gene (Zhang, Wang et al. 2007). Genomic alterations that result in truncation and constitutive activation of NFkB2 occur in a variety of human B- and T-cell malignancies (Zhang , Lombardi et al. 1994). TG mice developed marked splenomegaly, lymphadenopathy, and infiltration of non-lymphoid tissues and died with clonal B cell as well as some T-cell lymphomas between 5 and 18 months of age. The tumors were diagnosed histologically as SBL, but expression of CD5 was not evaluated and a leukemic phase was not described. The cells were non-proliferative and exhibited increased resistance to apoptosis. Interestingly, premalignant B cells and lymphoma exhibited significantly increased levels of TRAF1 and to a lesser extent TRAF2. TRAF1 was shown to be a direct transcriptional target of mutant NFkB2. Remarkably, TRAF1-deficient TG mice did not develop lymphoproliferation or lymphomas. These findings, together with the studies of the double TG mice, suggest a common pathway to development of SBL in mice.

8.2.2.5 APRIL and CLL?

APRIL (a proliferation inducing TNF ligand), also known as TNFSF13, is a secreted member of the TNF superfamily expressed by normal T cells, neutrophils, and dendritic cells as well as by a variety of cancers. APRIL binds to two receptors, BCMA and TACI, on the surface of B-lineage cells and is known to influence plasma cell survival, Ig switching, and the function of B1 B cells (Cancro 2004; Schneider 2005). Previous studies of human NHL showed that APRIL was expressed in association with high-grade DLBCL and Burkitt lymphoma but not with low-grade NHL including mantle cell and MZ lymphoma or CLL (Schwaller, Schneider et al. 2007). In contrast, Planelles, Carvalho-Pinto et al. (2004) found APRIL transcripts to be associated with nearly 50% of the CLL cases tested and demonstrated elevated levels of APRIL in serum from these patients. To investigate a role for APRIL in CLL, they generated TG mice with APRIL expressed from T cells.

Studies of APRIL TG mice older than 9 months revealed an expanded population of peritoneal B1a cells associated with enlargement of the mesenteric LN and Peyer's patches in about 40% of mice and splenomegaly and extralymphoid spread in fewer. The cells were non-proliferative but exhibited increased resistance to apoptosis. Comparisons with non-TG mice suggested that APRIL accelerates the expansion of peritoneal B1a cells seen in NZB and other strains.

Unfortunately, no studies were done to evaluate clonality, the mice were not identified as being leukemic, and none were said to have died of their disease.

8.2.2.6 Conclusions Regarding Mouse Models of CLL

As noted above, SBL, an uncommon spontaneous disease of old mice has many cytologic and histologic features in common with CLL. For mice suggested as models for CLL, some cases with histologic features of SBL are among those seen in BCL2/TRAF2DN mice (Zapata, Krajewska et al. 2004) but other lymphoma classes also develop in these mice, and the occurrence of ascites and pleural effusions is unusual. The description of the disease of mice carrying a mutant NFkB TG is like that of SBL, but the cells were not phenotyped or shown to be clonal. The diseases of NZB and TCL1 TG mice are histologically and cytologically quite distinct from SBL.

Many features of the malignant B-cell diseases of New Zealand mice and TCL1 TG mice are remarkably similar. They originate among oligoclonal populations of B1a cells in the peritoneum and progressively spread to spleen, peripheral blood, lymph nodes, and bone marrow. In the spleen, the histologic appearance is of MZL, but several features weigh against their origin from normal MZ B cells. First, the surface phenotype—$CD5^{lo}CD21^{lo/-}$—is not that of MZ B cells (Martin, Oliver et al. 2001; Bichi, Shinton et al. 2002). Second, the "MZL" often does not show the cytologic progression to high-grade disease seen in $NFS.V^{+}$ congenic mice. Instead, the cytology of cells in spleen, nodes, and even the blood of leukemic mice may differ little from those of mice with early expansion of the MZ. Third, MZL of $NFS.V^{+}$ almost never extend beyond the spleen. Finally, the V_HDJ_H sequences of the lymphomas are closer to those of normal B1a cells than to normal MZ B cells.

The process of peritoneal B1a B-cell transformation and subsequent spread may be accelerated by constitutive expression of TCL1, of the TRAF1 mimic, TRAF2DN, plus BCL2, and possibly of the mutant NFkB2 upstream of TRAF1 since CLL features heightened expression of TCL1, BCL2, and TRAF1. In this regard, it would be of interest to cross the TCL1 TG or mutant NFkB2 mice with NZB. The possible contributions of genetically determined changes in miR15/16 require confirmation but could relate the NZB disease to human CLL in yet another way. Caution is suggested by the understanding that NZW mice are like NZB in the development of clonal B1 populations but do not share the miR16 polymorphism with NZB.

The normal B-cell counterpart of the leukemic cell in CLL is not known but is clearly an issue of significant import. The candidates under consideration include resident or recirculating $CD5^{+}$ mantle zone B cells, MZ B cells, and lastly, the unidentified human equivalent of mouse B1a cells. Mantle cells seem unlikely as they exhibit little auto- or polyreactivity (Herve, Xu et al. 2005) while MZ and B1a cells of mice have this as a prominent feature (Martin, Oliver et al. 2001). A rationale for choosing between these cell subsets for one most like CLL is provided by extensive studies of V_HDJ_H sequences of purified peritoneal B1 and MZ B cells

(Kantor, Merrill et al. 1997; Schelonka, Tanner et al. 2007). Although the conclusions are based solely on analyses of the V_H7183 family, the CDR3s of MZ B cells were considerably shorter than for other B-cell subsets in spleen and bone marrow, D_H usage was biased toward DFL3, and J_H usage toward J_H2, N-region additions were fewer than for other B-cell subsets, and they had an increased proportion with charged amino acids. In contrast, B1a sequences revealed repertoires biased toward V_H1, V_H11 and V_H12, DSP D_H, and J_H1with fewer N-region additions than B1b or conventional B cells. In addition, CDR3 lengths were similar or slightly greater than those for B1b and B2 cells. These observations support a derivation of TCL1 lymphoma/leukemia from peritoneal B1a cells with the greatest discrepancy being the near-germline sequences of the clonal TCL1 TG populations. Human CLL might well derive from a parallel population of B1a cells. Efforts to develop an accelerated model of the NZB or TCL1 TG-based diseases would provide a superior preclinical model of CLL.

8.3 Marginal Zone Lymphomas

8.3.1 MZL in Humans

There are three general categories of MZL in humans: extranodal MZL of MALT lymphoma, nodal MZL, and splenic MZL (Jaffe, Harris et al. 2001). MALT and nodal MZL are characterized by accumulations of a heterogeneous population of small B cells infiltrating the marginal zones of reactive B-cell follicles. These centrocyte-like cells can be associated with occasional centroblasts and immunoblasts, and plasmacytoid differentiation is seen in some cases. In MALT lymphomas, the malignant cells characteristically infiltrate the epithelium-forming lymphoepithelial structures. Nodal MZL morphologically resembles lymph node infiltration by MALT but there is no evidence of extranodal involvement. Patients with MALT often have a history of autoimmune conditions, including Hashimoto's thyroiditis and Sjogren's syndrome, or inflammatory conditions, such as *Helicobacter pylori*-associated chronic gastritis or ocular infections with *Chlamydia psittaci*.

Splenic MZL is a rare disease in which small lymphocytes replace splenic white pulp GCs, infiltrate the surrounding marginal zones, and expand into the red pulp. Extension to splenic nodes and the bone marrow is common but involvement of peripheral nodes is not. These tumors may account for a high proportion of $CD5^-$ chronic lymphoid leukemias, sometimes featuring villous lymphocytes.

Two genes implicated in the development of MALT, *MALT1* and *BCL10*, were originally identified because of their involvement in recurring chromosomal translocations—t(11;18)(q21;q21) and t(14;18)(q32;q21) for MALT1 and t(1:14)(p22;q32) for BCL10—that occur specifically in MALT lymphomas. In normal B cells, MALT1 and BCL10 associate with CARMA1 downstream of the BCR to activate NFkB (Thome 2004).

8.3.2 Mouse Models of MZL

8.3.2.1 Spontaneous MZL in Mice

Splenic MZL is the only type of MZL that occurs spontaneously in mice (Fredrickson, Lennert et al. 1999). The disease, best characterized in NFS.V^{+} mice more than a year of age (Hartley, Chattopadhyay et al. 2000), is a clonal disorder that initiates with expansion of the MZ by cells cytologically indistinguishable from normal MZ B cells and with almost no mitotic activity. Over time, these cells begin to finger into the red pulp and exhibit a more open chromatin pattern with more prominent nucleoli, and mitotic figures are more readily seen. This can progress to a high-grade lymphoma with a high mitotic index and cells cytologically indistinguishable from those of DLBCL of centroblastic (CBL) type. The lymphoma cells compress the white pulp and force out red pulp elements (Fredrickson, Lennert et al. 1999). The disease is almost always confined to the spleen but occasionally spreads to the splenic node and the liver. A leukemia phase is rare. FACS analyses have shown that the lymphomas cells are CD5^{lo}IgM^{hi}B220^{lo} in the majority of cases.

Studies of NFS.V^{+} MZL for somatically acquired proviral insertions of ecotropic MuLV identified a series of common integration sites (CIS) previously identified as candidate cancer genes (Shin, Fredrickson et al. 2004). Seven new CIS unique to MZL were also found including *Gfi1*, *Sox4*, and *Stat6* among others. Heightened expression of *Gfi1* distinguished MZL from other classes of B-cell lymphoma and was characteristic of MZL at all stages of progression suggesting a role in disease initiation.

8.3.2.2 Mouse Model of MALT

Mice infected with *Helicobacter felis* for 22 months or more developed a chronic gastritis associated with the development of lymphoid follicles, the appearance of lymphoepithelial lesions, and glandular destruction (Enno, O'Rourke et al. 1995). The later development of lymphoma was shown to be antigen-dependent since the incidence and severity of disease was significantly reduced in infected mice given anti-microbial therapy (Enno, O'Rourke et al. 1998). In addition, mice immunized against *H. felis* were protected from development of lymphoma (Sutton, O'Rourke et al. 2004). None of the lymphoid lesions were tested for clonality of the expanded B-cell populations, and upregulation of MALT or BCL10 was not described in reports of expression profiling (Mueller, O'Rourke et al. 2003).

8.3.2.3 Mouse Models of Splenic MZL

Aire-deficient mice replicate autoimmune features of patients with autoimmune polyendocrine syndrome type I, an inherited autosomal recessive disorder associated with progressive immune destruction of many tissues (Anderson,

Venanzi et al. 2002; Ramsey, Winqvist et al. 2002). More recent studies showed that mutant mice15–24 months of age exhibited expansion of the MZ (Hassler, Ramsey et al. 2006). Analyses of IgH D–J rearrangements revealed an oligoclonal pattern suggestive of early MZL. Interestingly, the cells populating the MZ were $CD21^{lo}$ and secreted autoantibodies on transfer, consistent with an activated phenotype. Unfortunately, expression of CD5 was not examined. These studies suggest a role for *Aire* as a tumor suppressor gene and stimulation with autoantigens as possibly contributory to development of MZL.

Interestingly, recent studies of mice with TG-induced expression of BCL10 in B cells were found to have significantly expanded populations of splenic MZ B cells, nuclear BCL10, and constitutive activation of the canonical NFkB signaling pathway (Stephen Morris, H. Morse, unpublished observations). Mice older than 18 months have started to develop lymphomas not seen in control littermates.

8.3.2.4 Conclusions Regarding Mouse Models of MZL

Spontaneous splenic MZL in mice has many similarities to splenic MZL in humans, but the latency for disease of greater than a year, the occurrence with other classes of B-cell lymphoma in NFS.V^{+} mice, and the lack of demonstrable involvement of BCL10 or MALT1 make the model impractical for preclinical studies. The *Aire*-deficient mouse model of splenic MZL also suffers from long latency and low penitrance. *Helicobacter*-associated gastric lesions similar to those of gastric MALT never appears to evolve to clonal disease but appears to be useful for understanding the role of antigenic drive in early disease. The fact that there is no unequivocal evidence in mice for MZ B cells other than those in spleen may be responsible for the lack of models for nodal MZL. Mouse disorders with similarities to Sjogren's syndrome or Hashimoto's thyroiditis exhibit B-cell infiltrates of affected tissues but have never shown progression to clonal disease.

8.4 Mantle Cell Lymphoma

8.4.1 MCL in Humans

Human MCL is a mature B-cell neoplasm of small to medium-sized lymphocytes with irregular/cleaved nuclear contours that resemble centrocytes. Synonyms are morphologically descriptive and include intermediate or poorly differentiated lymphocytic lymphoma-diffuse or nodular type centrocytic (mantle cell) lymphoma, and malignant lymphoma diffuses small cleaved cell type. Human MCL typically involves lymph nodes and less frequently the spleen, bone marrow, and GI tract and is an intermediate to aggressive, usually incurable lesion with large cell blastoid variants of ominous prognosis

AQ5 (Jaffe 2001). Until recently, there were no spontaneous or genetic mouse models of MCL, precluding development of a MMHCC classification although two MCL xenotransplant models have been reported (Bryant, Pham et al. 2000; M'Kacher, Farace et al. 2003). A t(11;14)(q13;32) between *IGH* and *CYCLIN D* loci is the hallmark aberration of human MCL, with dysregulated expression of the cyclin CCND1 protein (Williams, Westermann et al. 1990; Williams, Swerdlow et al. 1993). However, *Eμ–cyclin D1* TG mice have usual B-cell development and fail to develop tumors (Bodrug, Warner et al. 1994), indicating that cyclin D1 dysregulation may be necessary but not sufficient for developing a mouse model of MCL. By FACS or IHC, most human MCL are $IgM^+IgD^{+/-}CCND1^+BCL\text{-}2^+CD10^-BCL6^-CD23^-CD43^+FMC\text{-}7^+$ (Jaffe 2001). The cell(s) of origin are unknown although most cases show unmutated *IG* genes, suggesting a naïve or extrafollicular precursor B-cell type.

8.4.2 Mouse Models of MCL

8.4.2.1 MCL in Genetically Engineered Mice

Intraperitoneal injection of the tumor promoter pristane (2,6,10,14-tetramethylpentadecane) for 3 months into *Eμ–CCND1* TG mice >9 months of age resulted in a diffusely infiltrative, clonal, $IgM^+CD5^+CD20^+CD23^-$ B-cell lymphoma expressing the cyclin D1 transgene with intermediately sized, cleaved B cells reminiscent of MCL (Smith, Joshi et al. 2006). CD5 may represent an activation rather than differentiation marker in mouse B-cell tumors, and additional studies of tumor transplantability, aggression, and cell of origin are required to determine this model's resemblance to human MCL. Crossing *Eμ–IL-14α* with *Eμ–Myc* TG mice results in a disseminated, blastoid variant of MCL (MCL–BV) in almost 100% of mice by 3–4 months of age (Ford, Shen et al. 2007). Tumor cells are transplantable into SCID mice and show $sIgM^+CD5^+CD19^+CD21^-CD23^-$ by flow cytometry, with increased expression of endogenous CCND1, BCL2, ATM, RelA, and NF-κB2, and clonal Ig gene rearrangements, providing several molecular features that are observed in human MCL. However, the histology of these tumors is that of diffuse high-grade blastic B-cell lymphoma, frequently seen as a spontaneous disease in many strains of mice as well as in some genetically engineered strains (see below).

8.5 Follicular B-Cell Lymphoma

8.5.1 FBL in Humans

Follicular lymphoma in humans (FBL in mice) is mature B-cell lymphoma of GC origin comprised of a mixture of centrocytes and centroblasts with at least a partially follicular pattern (Jaffe, Harris et al. 2001). The cells are embedded in a

dense network of follicular dendritic cells. The disease appears to originate in lymph nodes, but spleen bone marrow and occasional blood involvement are not uncommon. The cells are usually IgM^+CD5^- and, like normal GC B cells, express BCL6 and have mutated IgV region sequences. Almost all express BCL2, usually as the result of t(14;18)(q32;p21) translocations that bring the BCL2 gene under the control of IgL regulatory sequences (Tsujimoto, Finger et al. 1984; Hockenbery, Nunez et al. 1990). Morphologic transformation to aggressive DLBCL is common and is typically the cause of death. FBL makes up about 35% of adult NHL in the United States.

8.5.2 Mouse Models of FBL

8.5.2.1 Spontaneous FBL in Mice

FBL is a mature B-cell tumor characterized by a varying mixture of neoplastic centrocytes and centroblasts. Synonyms include follicular lymphoma, follicular center cell lymphoma mixed, CBL/centrocytic lymphoma, reticulum cell sarcoma type B, and lymphoma-pleomorphic. FBL is typically a low-grade lesion that resembles human follicular lymphoma and is the most frequent B-cell tumor of aging mice in many inbred strains, including $NFS.V^+$, CFW, and AKXD RI strains (Morse, McCarty et al. 2003). Distinct from human follicular lymphoma, spontaneous FBL is not associated with *Bcl2* gene rearrangements (Morse, Anver et al. 2002). Splenomegaly and variable enlargements of mesenteric lymph nodes and Peyer's patches are typically seen. Histologic examination reveals white pulp expansions that appear as white nodules and coalesce with advancing disease. Centroblasts and centrocytes are the main cell types present, with small follicular B cells pushed to the periphery and the T-cell zone reduced or eliminated. Blast cells should be less than 50% to distinguish FBL from diffuse high-grade blastic B-cell lymphoma/leukemia (DLBCL or DBLL). By FACS, most cases are $IgM^+IgD^-CD5^{dull}CD45R(B220)^{lo/+}$. By IHC, centroblasts and centrocytes are both $IgM^+CD45R(B220)^+CD19^+$. Tumor cells are mono- or oligoclonal for Ig gene rearrangements, with the presumed cells of origin being GC centrocytes and centroblasts.

8.5.2.2 FBL in Genetically Engineered Mice

Two independent lines of *Eμ-BCL2* TG mice develop follicular hyperplasia but rarely develop FBL and then with greatly prolonged latencies (McDonnell and Korsmeyer 1991; Strasser, Harris et al. 1993). In contrast, *VavP–Bcl2* TG mice that do not succumb to autoimmune disease develop follicular hyperplasia, followed by FBL at 10–18 months of age in up to 50% of mice. The disease is characterized by PCNA-positive, class-switched neoplastic B cells containing mutated IgV region genes (Egle, Harris et al. 2004). GC expansion and lymphomagenesis depended upon concurrent expansion of BCL2 transgene-expressing

$CD4^+$ T cells, suggesting that microenvironmental support was required for FBL development.

Eμ–Pim1 TG mice develop pre-T-lymphoblastic lymphoma (pre-T-LBL) at 7–10 months of age. Mice unaffected by pre-T-LBL demonstrating probable FBL and multiple subtypes of DLBCL at older ages using pre-Bethesda nomenclature criteria (van Lohuizen, Verbeek et al. 1989; Repacholi, Basten et al. 1997). A construct using the flanking regulatory elements of human *MCL1* caused lymphoma in 65% of TG mice by 24 months, with about 20% of cases diagnosed as FBL under pre-Bethesda criteria (Zhou, Levy et al. 2001).

Eμ-B29-TCL1 TG mice, encoding the AKT co-activator TCL1 oncoprotein, develop a spectrum of mature GC and non-GC B-cell lymphomas, with relatively rare FBL and more common DLBCL and DBLL generation (Hoyer, French et al. 2002; Shen, Ferguson et al. 2006). Similar to *VavP–Bcl2* TG mice, *Eμ-B29–TCL1* TG mice require concurrent *TCL1*-mediated $CD4^+$ T-cell expansion to transform GC B cells, because mice with only B-lineage *TCL1* transgene expression develop mainly a model of the aggressive form of B-CLL (Bichi, Shinton et al. 2002).

Knock-in of a human *MLL–AF4* fusion gene into the mouse *Mll* locus produces predominantly FBL with clonal $sIgM^+B220^+Pax5^+Bcl6^+CD19^+$ tumor cells arising from follicular centers following a mixed myeloid/lymphoid hyperplasia (Chen, Li et al. 2006). *MLL–AF4* tumor cells metastasized widely without a leukemic phase and are transplantable. Knock-out of the *Ing1* gene, which encodes a nuclear PHD finger-containing protein not yet associated with human lymphoid malignancies, results in 20% of mice developing lymphoma. Tumors originate in the spleen and contain a mixed population of $B220^+$ cells that histologically resemble centroblasts and centrocytes to suggest FBL (Kichina, Zeremski et al. 2006). However, additional marker studies, evaluation of *Ig* mutation status, and clonality and transplantation studies are required to confirm this diagnosis and to exclude a robust follicular hyperplasia instead of malignancy (Kichina, Zeremski et al. 2006).

8.5.2.3 Conclusions Regarding Mouse Models of FBL

Spontaneous FBL in mice and many of the disorders of TG mice models have a number of histologic and particularly cytologic features in common with the human disease. However, the true follicular pattern seen in humans is absent in all these cases including those with extensive lymph node involvement. None of the models exhibit chromosomal translocations affecting the *Bcl2* locus, and the *VavP–Bcl-2* TG is the only one with constitute Bcl2 expression. It may be important that the major breakpoint region (MBR) in the human *BCL2* locus bears is markedly different to the same general region in the mouse locus. These differences may preclude chromosomal rearrangements in the mouse. This possibility is being tested by knocking in 2 kb around the human MBR into the mouse *Bcl2* locus.

8.6 Diffuse Large B-Cell Lymphoma

8.6.1 DLBCL in Humans

DLBCL is characterized by a diffuse proliferation of large neoplastic B cells with nuclear size that exceeds that of normal histiocytes. A number of cytologic variants have been described including CBL, IBL, T-cell/histiocyte rich, plasmablastic, and anaplastic with CBL being the most common. Distinction among these variants suffers from poor interobserver reproducibility, and subsets have not been reliably tied to prognosis. Consequently, a designation simply as DLBCL is felt by pathologists to be most appropriate (Jaffe, Harris et al. 2001).The tumor cells express pan-B-cell markers and surface or cytoplasmic Ig, and about 10% are $CD5^+$. Nuclear BCL6 is expressed in almost all cases, and IgV region genes are mutated. Gene expression profiling by one group using microarrays delineated two major subsets that are related to cell of origin as activated B-cell-like and GC B-cell-like (Alizadeh, Eisen et al. 2000). The distinctions have prognostic significance as the prognosis for patients in the GC B-cell-like subset is considerably better than that of patients with the activated B-cell type. However, array-based studies of DLBCL by another group did not reproduce these associations, defining instead three discreet subgroups designated "oxidataive phosphorylation", "B-cell receptor/proliferation," and "host response" (Monti, Savage et al. 2005).

8.6.2 Mouse Models of DLBCL

8.6.2.1 Spontaneous DLBCL in Mice

DLBCL is an aggressive mature B-cell malignancy that demonstrates a diffuse proliferation of tumor cells with large nuclei and distinct cytologic features. Characteristic DLBCL variants occur spontaneously in aging mice and are classified as CBL, IBL, histiocyte-associated (HA), and primary mediastinal (PM, thymic) subtypes (Morse, Anver et al. 2002). CBL, IBL, and HA variants are common in $NFS.V^+$ mice and usually arise with splenomegaly or lymphadenopathy (Hartley, Chattopadhyay et al. 2000), whereas PM shows mainly thymic enlargement and has been seen only in mice infected with a unique replication-defective retrovirus (Morse, Anver et al. 2002).

8.6.2.2 Spontaneous Variants of DLBCL in Mice

DLBCL–CBL Variant

CBL synonyms include large cleaved follicular center cell lymphoma and CBL lymphoma. About 12% of spontaneous lymphomas in $NFS.V^+$ and 17% in CFW mice are CBL, whereas CBL was not detected in AKXD RI lymphomas

and is not common in other inbred strains (Morse, McCarty et al. 2003). Histologically, the splenic white pulp is greatly expanded by tumor cells with round nuclei, often with one or two prominent nucleoli, basophilic cytoplasm, and numerous mitoses. These cells are admixed with varying amounts of smaller centrocytes. A diagnosis is made when >70% of the cells are blasts. When the proportion of centrocytes to blasts ranges from 40–70% and the ratio varies in different microscopic fields, a distinction between DLBCL and FBL is difficult although CBL may more completely destroy the usual GC architecture than FBL at advanced stages. CBL frequently infiltrates the lung, liver, and kidney and, less frequently, the bone marrow. By FACS, most cases are IgM^+ or IgG^+, $B220^+CD5^{low}CD19^+$. By IHC, they are usually $BCL6^+PAX5^+IRF8^+PU.1^+CD138^-$ $XBP1^-$ $Blimp1^-$. Tumors are clonal for *Ig* gene rearrangements, and oligonucleotide expression microarrays have shown no clear differences between follicular or diffuse CBL subtypes, suggesting that these subtypes may represent earlier and later stages of progression (Morse, unpublished results). By expression microarray analysis, CBL is readily distinguished from the CBL form of MZL and is similar to the CBL variant of human DLBCL.

DLBCL–IBL Variant

IBL lymphoma is the synonym ascribed to IBL. About 8% of spontaneous lymphomas in $NFS.V^+$ and 4% in CFW mice are IBL, whereas IBL was not detected in AKXD RI lymphomas (Morse, McCarty et al. 2003). Histologically, IBLs are highly aggressive and demonstrate large, round nuclei having dispersed chromatin and prominent nucleoli, abundant cytoplasm, and a high mitotic rate. A "starry sky" pattern may be seen with increased apoptotic cells. Tumor cells are clonal for Ig gene rearrangements and often admixed with centroblasts and centrocytes, which may reflect an origin from a FBL or post-GC immunoblast. By FACS, IBLs typically are $sIgM^{low}B220^{dull}$ and by IHC most cases are $BCL6^+PAX5^+IRF8^-PU.1^-XBP1^-IRF4^-Blimp1^-$.

DLBCL–HA variant

DLBCL–HA (histiocyte associated) is the acronym of HA variant. About 20% of spontaneous lymphomas in AKXD RI (and 1% in $NFS.V^+$) strain mice are HA, and HA is not common in other frequently used inbred strains (Fredrickson and Harris 2000; Morse, McCarty et al. 2003). Histologically, all mice show splenomegaly with a marked expansion of histiocytes (macrophages) that may obscure malignant B cells. Histiocytes may occupy the entire white pulp, obliterating the PALS and destroying the usual follicular architecture, thereby pushing B cells to the periphery. Malignant B cells usually have features of FBL or CBL although rare cases show tumor cells with features seen in MZL, SBL, or IBL. Lymphadenopathy is seen in half the cases, and HA may involve the liver early on. The pattern of tumor growth is mainly nodular rather than

diffuse, and this lesion resembles human histiocyte/T-cell-rich DLBCL (Jaffe 2001). It may be difficult to distinguish DLBCL–HA from histiocytic sarcoma. By IHC, the histiocytes of HA are usually EMR1 $(F4/80)^+$LGALS $(Mac\text{-}2)^+$, whereas the malignant B cells express markers consistent with their origin and are typically $Pax5^+$. A clonal *Ig* gene rearrangement with $PAX5^+$ cells in a histologic picture dominated by histiocytes is diagnostic, with the main differential diagnosis being histiocytic sarcoma. The presumed cell of origin for the malignant B-cell component is usually a GC or post-GC B cell, whereas tissue macrophages comprise the non-malignant histiocytic component.

8.6.2.3 DLBCL in Genetically Engineered Mice

DLBCL may arise de novo or by aggressive transformation of FBL and possibly SMZL. Ionizing radiation causes an increased frequency of tumors with histologic features of pre-T-LBL, FBL, and DLBCL–CBL in *Eμ–Pim-1* TG mice (Repacholi, Basten et al. 1997). Targeted deletion of *Riz1*, encoding a Rb-binding zinc finger protein, results in 37% of null and 19% of heterozygous mice developing clonal, $B220^+$ B-cell lymphomas with histologic features of DLBCL–CBL by 18–22 months of age (Steele-Perkins, Fang et al. 2001). *H2-L*^{d}*-Il6* TG mice develop PCT between 6 and 19 months of age, frequently with co-existing FBL or DLBCL–CBL (Kovalchuk, Kim et al. 2002). By IHC, the lymphomas are $IgM^+B220^+CD19^+$ and several contained t(12;15) *IgH/Myc* gene rearrangements.

Eμ-B29–TCL1 TG mice develop clonal $IgM^+B220^+CD5^{low}BCL6^+$ DLBCL, most often HA and occasionally CBL or IBL subtypes with somatically mutated *Ig* genes and widespread dissemination (Hoyer, French et al. 2002). Equally frequent DBLL and rare FBL, SMZL, and T-PLL are also formed in this model (Hoyer, French et al. 2002; Dawson, Hong et al. 2007). B-cell lymphomas were eliminated by crossing the *TCL1* TG with an *OCA-B* null mouse that fare incapable of developing GC structures (Shen, Ferguson et al. 2006).

Knock-out of the proapoptotic BH3-only *Bad* gene results in 20% of mice developing clonal $sIgM^+$ or $sIgG^+$, $B220^+CD19^+CD43^-BCL6^+$ DLBCL of unclear subtype by 18–24 months of age (Ranger, Zha et al. 2003). Knock-in of the murine *Bcl6* gene into the *IgH* locus results in increased GC formation in spleens of non-immunized mice, followed successively by a benign lymphoproliferative disorder with expanded white pulp and then the development of DLBCL and FBL between 13 and 20 months of age (Cattoretti, Pasqualucci et al. 2005). Tumors were clonal and $IgM^+IgD^+B220^+CD43^-CD138^-$ with variable Mum1/IRF4 staining by IHC. They contained mutated *IgV* region genes and frequent trisomy of Chrs 13 and 15. More recent studies showed that mice bearing the *Bcl6* knock-in do not develop DLBCL when crossed onto an AID-deficient background (Pasqualucci and Dalla-Favera, unpublished observations)

Knock-in of the mouse *Myc* gene 5' of the *Eμ* intronic enhancer results in clonal $IgM^+B220^+CD19^+Bcl6^+$ DLBCL of unclear subtype developing between 6 and 21 months of age, along with FBL, DBLL, and PCT formation (Park, Kim

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Table 8.2. Mouse models of human germinal center (GC) tumors[1]

Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
Transfer of human MCL	Peritoneal cavity	MCL	SCID	None	Xenotransplant of leukemic phase MCL cells	Bryant, Pham et al. (2000)
Transfer of human MCL	Lymphoid system	MCL [BV?]	NOD/ SCID	None	Xenotransplant of peritoneal MCL cells	M'Kacher, Farace et al. (2003)
de novo	Peritoneal cavity, lymphoid system, metastases	MCL	C57BL/6	*Eμ–cyclin D1 TG*	Age dependent (>9-months), dependent on peritoneal inflammation (pristane)	Smith, Joshi et al. (2006)
de novo	Lymphoid system, metastases	MCL–BV	C57BL/6	*Eμ–IL–14α X, Eμ–Myc DTG*	Initial leukemia phase with blasts, lymphoma by 3–4 months in 100% DTG mice	Ford, Shen et al. (2007)
de novo	Lymphoid system, metastases	FBL, DLBCL	C57BL/6	*VavP–Bcl2* TG	Other tumors include PCT, LBL, HS	Egle, Harris et al. (2004)
de novo	Lymphoid system, metastases	FBL, DLBCL –CBL	C57BL/6	*Eμ–Pim1* TG	Other tumors include pre-T-LBL, HS	van Lohuizen, Verbeek et al. (1989) and Repacholi, Basten et al. (1997)
de novo	Lymphoid system, metastases	FBL, DLBCL	C57BL/6	*gMCL1* TG		Zhou, Levy et al. (2001)

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Table 8.2. (continued)

Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
de novo	Lymphoid system, metastases	FBL, DLBCL, DBLL	C57BL/6 × C3H	*Eμ-B29–TCL1* TG	Other tumors include MZL, T-PLL	Hoyer, French et al. (2002); Shen, Ferguson et al. (2006); and Dawson, Hong et al. (2007)
de novo	Lymphoid system, metastases	FBL	C57BL/6 × FVB	*MLL–AF4* knock-in	Rare erythroid or myeloid leukemia	Chen, Li et al. (2006)
de novo	Lymphoid system metastases	FBL	C57BL/6	*Ing1* knock-out		Kichina, Zeremski et al. (2006)
de novo	Lymphoid system	DLBCL–CBL	C57BL/6 × 129 Sv	*Riz1* knock-out	Diverse non-lymphoid tumors	Steele-Perkins, Fang et al. (2001)
de novo	Lymphoid system	FBL, DLBCL –CBL	BALB/c	*H2-L*^{d}*-Il6* TG	Occasional *IgH*/*Myc* gene rearrangements, co-existent PCT	Kovalchuk, Kim et al. (2002)
de novo	Lymphoid system, occasional metastases	DLBCL	C57BL/6 or 129/SvJ	*Bad* knock-out		Ranger, Zha et al. (2003)
de novo	Spleen, occasional lymph nodes	DLBCL, FBL	C57BL/6	*Bcl6* knock-in to *IgH* locus	Trisomy 13 and 15 common	Cattoretti, Pasqualucci et al. (2005)

Table 8.2. (continued)

Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
de novo	Lymphoid system	DLBCL, DBLL, FBL	C57BL/6	*Myc* knock-in 5' of *Eμ* locus	Other tumors include LBL, PCT	Park, Kim et al. (2005) and Zhu, Qi et al. (2005)
de novo	Lymphoid system, variable metastases	DLBCL –CBL	C57BL/6	*AF4* invertor knock-in to *Mll* locus		Metzler, Forster et al. (2006)

[1]Mice are classified according to evolving criteria established initially by report of the Mouse Models of Human Cancer Consortium (MMHCC) study (Morse, Anver et al. 2002). Classifications in parentheses are not yet confirmed by MMHCC criteria or there are no MMHCC criteria yet developed for this entity. DBLL, diffuse high-grade blastic B cell lymphoma/leukemia; DLBCL, diffuse large B cell lymphoma; DTG, double transgenic; FBL, follicular B cell lymphoma; HS, histiocytic sarcoma; LBL, lymphoblastic lymphoma; MCL, mantle cell lymphoma; MCL-BV, mantle cell lymphoma-blastoid variant; MZL, marginal zone lymphoma; PCT, plasmacytoma; SCID, severe-combined immunodeficiency; TG, transgenic; T-PLL/CLL, T-prolymphocytic leukemia.

et al. 2005; Zhu, Qi et al. 2005). Using an "invertor" conditional knock-in strategy to bypass embryonic lethality, a Cre-generated *Mll–AF4* fusion gene in the endogenous *Mll* locus results in IgM^+B220^+ CBL with clonal *Ig* gene rearrangements in 60% of cases (Metzler, Forster et al. 2006). Microarray profiling of tumor cells shows strong expression of *Pax5* and *Ebf* and variable expression of *Bcl2* and *Bcl6* differentiation markers (Metzler, Forster et al. 2006). The CBL tumor cells were transplantable into *Rag1*-deficient recipient mice.

8.6.2.4 Conclusions Regarding Mouse Models of DLBCL

The last several years have been marked by striking progress in the generation and validation of mouse DLBCL as shown by studies of *Eμ-B29–TCL1* TG and *Bcl6* knock-in mice. The lymphomas of these mice share histologic features with human DLBCL, carry mutated IgV regions, and are strikingly dependent on normally functioning GC for their development. They provide novel and important in vivo settings for furthering our understanding of the roles played by TCL1 and BCL6 in normal B-cell biology and lymphomagenesis.

8.7 Diffuse High-Grade Blastic B-cell Lymphoma/Leukemia (DBLL) in Mice

The human equivalent or parallel to this disorder is currently not known. DBLL is a highly aggressive lymphoma of medium-sized B cells that exhibit a high mitotic rate, extensive apoptosis, and sometimes a leukemic phase. Synonyms include lymphoblastic lymphoma, Burkitt and Burkitt-like lymphoma, and DLBCL of lymphoblastic lymphoma subtype [DLBCL(LL)]. About 20% of spontaneous lymphomas in $NFS.V^+$ and ~30% in AKXD RI strains and CFW mice are DBLL, but DBLL is not common in other frequently used inbred strains (Morse, McCarty et al. 2003). Cases present with lymphadenopathy, variable involvement of the spleen, and sometimes thymus, with frequent non-hematopoietic organ dissemination. Affected tissues show uniform-appearing lymphoblasts with little cytoplasm, dispersed chromatin, and indistinct nucleoli. Histologic sections show many mitotic figures typically with large numbers of tingible body macrophages ingesting apoptotic cells, leading to a "starry sky" appearance. Infiltration of the deep cortex in lymph nodes progresses to replacement of normal cells and growth outside the capsule into the fat. When involved, the spleen shows diffuse infiltration of both the red and the white pulp. Perivascular and peribronchial infiltrates of the lungs and periportal liver infiltrates are common. Histologically and cytologically, these mature B-cell neoplasms are indistinguishable from precursor T-cell lymphoblastic lymphomas that lack thymic involvement and precursor B-cell lymphoblastic lymphomas. Analyses of *Ig* and *TCR* gene organization and IHC studies provide definitive distinctions among these disorders.

There is a spectrum of IHC phenotypes for DBLL ranging from patterns similar to immature or transitional B cells ($IgM^+IgD^-C1QR1(AA4.1)^+$) to that of GC-experienced B cells that are *Ig* class-switched with *IgV* region mutations. DBLL are clonal for *Ig* gene rearrangements. Structural rearrangements of cellular genes, mostly due to proviral insertions, are seen from pooled studies of $NFS.V^+$ (Hartley, Chattopadhyay et al. 2000) and AKXD RI (Morse, Qi et al. 2001) lymphomas for *Zfp521* (*Evi3*) (11.9%), *Pim1* (5.6%), *Evi1* (4.8%), and *Myc* (0.8%). Lymphomas of *λ-MYC* were characterized by chromosomal instability and frequent biallelic deletions of *Cdkn2a* (p16) (Kovalchuk, Qi et al. 2000). Lymphomas of *Eμ–Myc* TG mice had frequent changes in the $p19^{ARF}$–MDM2–p53 tumor suppressor axis (Park, Kim et al. 2005). Immature or transitional B cells are the presumed cells of origin for *Eμ–Myc*, *IgH/c–myc YAC*, *EμIgH/c–myc YAC*, and *λ–MYC* TG mice. Probable GC or early post-GC B cells are the cells of origin for those with features similar to the DBLL of *Eμ-B29–TCL1* TG mice.

8.7.1 DBLL in Genetically Engineered Mice

An immature or transitional cell immunophenotype is characteristic of most lymphomas of *Eμ–Myc, IgH/c–Myc YAC*, *EμIgH/c–Myc YAC*, and *λ-MYC* TG mice. More mature immunophenotypes occur in spontaneous DBLL of $NFS.V^+$ mice and some lymphomas of *Eμ-B29–TCL1* TG and *Eμ–Myc* knock-in mice, along with many other genetically engineered mice. B-lineage lymphomas with lymphoblastic cytology but distinct from precursor B-lymphoblastic neoplasms are seen at low to high frequency in many strains of genetically engineered mice and a number of conventional inbred strains. The lymphomas of *λ-MYC* TG mice were originally designated Burkitt lymphoma (Morse, Anver et al. 2002), but *Ig* genes are not mutated and tumor cells have an immunophenotype of transitional or immature B cells indicating that they differ from human Burkitt cases. A change in nomenclature is clearly warranted. Mouse cases with similar histology and cytology occurring in mice other than the *λ-MYC* TGs were previously designated Burkitt-like (Morse, Anver et al. 2002). The findings that these tumors rarely have structural alterations in *Myc* and do not overexpress *Myc* distinguish them from human Burkitt-like lymphomas (Jaffe 2001).

8.8 Plasma Cell Neoplasms

8.8.1 Human PCN Including Multiple Myeloma

The evaluation of present mouse models of human PCN, and attempts to devise improved models, should be guided by insights into the natural history of PCN

development in human beings (Mitsiades, Mitsiades et al. 2007) and the biologic and molecular genetic features of frank, untreated PCN at the time of clinical presentation (Carrasco, Tonon et al. 2006). In analogy to cancer development in general (Hanahan and Weinberg 2000), human PCN including MM are thought to be initiated by somatic mutations in oncogenes and/or tumor suppressor genes, followed by the stepwise accumulation of genetic and epigenetic alterations that comprise tumor progression events (Bergsagel and Kuehl 2005; Kuehl and Bergsagel 2005). The later changes alter the phenotype of the incipient tumor cell as well as its interactions with the local microenvironment until fully malignant transformation has occurred. As the acronym suggests, PCN comprise a spectrum of malignancies that share the rather uniform histopathology of the aberrant, neoplastic plasma cell. However, despite their morphologic similarities, PCN demonstrate a great deal of diversity at the molecular level associated with major differences in epidemiology, clinical behavior, and treatment options.

The classification of PCN in the World Health Organization (WHO) nomenclature includes the following:

1. *Plasma cell myeloma*. This is a bone marrow-based PCN, usually multifocal, thus commonly referred to as MM. MM is incurable with a survival rate of ~40% at 5 years after diagnosis. MM is defined by monoclonal Ig protein (M spike) in serum, bone destruction, hypercalcemia, and anemia. The standard of care includes low- and high-dose chemotherapy, bone marrow transplantation, and novel drugs. Tumor variants include non-secretory myeloma (no serum monoclonal Ig), indolent myeloma, smoldering myeloma, and plasma cell leukemia.
2. *PCT*. A solitary, localized, monoclonal PCN that grows either in bone (solitary bone PCT) or soft tissue (solitary extraosseous or extramedullary PCT). PCT is rare but curable with moderate-dose radiotherapy as the preferred treatment. The most common pattern of relapse is systemic, indicating progression to MM.
3. *Ig deposition diseases*. Primary amyloidosis and systemic IgL chain and IgH chain deposition diseases.
4. *Osteosclerotic myeloma (POEMS syndrome)*. POEMS, defined by *p*olyneuropathy, *o*rganomegaly, *e*ndocrinopathy, *m*onoclonal gammopathy, and *s*kin changes is very rare.
5. *Heavy chain diseases*. Distinguished according to the isotype (γ, μ, and α) of the monoclonal IgH chain produced by neoplastic plasma cells.

Among human PCN, MM is by far the most important and well-studied disease. MM is a neoplasm of mature post-GC, Ig-secreting, isotype-switched plasma cells (Zojer, Ludwig et al. 2003) that accumulate in the bone marrow and cause bone destruction (Roodman 2006). Recent molecular and cytogenetic studies have shown that MM is remarkably a heterogeneous disease that can be divided into a number of distinct categories based on global gene expression profiles, detection of reciprocal chromosomal translocations

that recombine Ig loci with oncogenes, and ploidy status of tumor cells (Zhan, Hardin et al. 2002; Shaughnessy and Barlogie 2003). Depending on the criteria applied by different laboratories, it is possible to distinguish 5–8 subcategories of MM. Regardless of how these differences are resolved, the present molecular and cytogenetic subdivision of MM is already of clinical relevance because it predicts significant differences in the prognosis and response to therapy of MM patients, no matter whether treatment relies on standard, high-dose, or novel therapies (Mulligan, Mitsiades et al. 2007).

No definitive cause of MM has been identified. Genetic risk factors include gender (male > female), race (the incidence in African Americans in the United States is twice that of US whites), and age (median age at diagnosis is ~70 years). Familial clustering points to a hereditary predisposition consistent with an autosomal-dominant mode of inheritance, but tumor susceptibility alleles or "MM genes" have not been identified. Ionizing radiation is thought to be the strongest environmental risk factor for MM, but definitive studies have not been described. Although the evidence is not conclusive, there are reports of associations between MM and occupational exposure to various metals (nickel), chemical compounds (aromatic hydrocarbons, silicone, and petrochemical agents), pesticides and animal viruses (farming), protracted infections that can lead to sustained B-cell activation by microbial antigens (*H. pylori*, HHV8), acquired immunodeficiency syndromes, such as HIV/AIDS, that result in reduced immune surveillance by T cells, and autoimmune diseases, such as rheumatoid arthritis.

The neoplastic cell in MM appears to derive from an antigen-experienced isotype-switched post-GC B-lymphocyte that has undergone somatic hypermutation of the expressed *IgH* and *IgL* genes. Pathogenic factors implicated in MM include cytogenetic and molecular genetic alterations that result in the deregulated expression of oncogenes, such as *CCND1* (encoding cyclin D1), *FGFR3* (fibroblast growth factor receptor 3), and *WHSC1* (Wolf–Hirschhorn syndrome candidate 1; also known as *MMSET* or MM SET domain containing protein type III). The interaction of tumor cells with the bone marrow microenvironment is of crucial importance, as it leads to the production of cytokines including IL-6, IGF-1, VEGF, SDF-1α, TNF-α, and TGF-β.

The subgroups of MM are presently distinguished based on recurrent *IGH* translocations, ploidy status of tumor cells, and global gene expression patterns. Hyperdiploid tumors (40% of cases) contain 48–75 chromosomes and are characterized by multiple trisomies of Chr 3, 5, 7, 9, 11, 15, 19, and 21. Non-hyperdiploid tumors (nearly 50% of cases) carry one of seven recurrent chromosomal translocations that recombine *IGH* at 14q32 with seven different oncogenes. These translocations are thought to be very early if not the initiating oncogenic events and are caused by errors in Ig switch recombination or somatic hypermutation during the GC reaction. They fall into one of three groups.

1. D-type cyclins: *CCND1* (cyclin D1) at 11q13 in 15% of cases, *CCND2* (cyclin D2) at 12p13 in <15 of cases, and *CCND3* (cyclin D3) at 6p21 in 2% of cases.

2. MAF family genes: *MAF* (c-Maf) at 16q23 in 5% of cases, *MAFB* (Maf B) at 20q12 in 2% of cases, and *MAFA* (Maf A) at 8q24.3 in 1% of cases.
3. MMSET/FGFR3: *MMSET* (formally designated *WHSC1*) and *FGFR3* at 4p16 in 15% of cases.

Tumor progression events include chromosomal translocations that affect the *MYC* gene at 8q24 (15% of primary MM, 45% of advanced MM, and >90% of MM-derived cell lines) but do not involve aberrant isotype switching or somatic hypermutation and exhibit a similar prevalence in hyperdiploid and non-hyperdiploid tumors. They also include activating mutations of *NRAS*, *KRAS*, or *FGFR3*; amplifications of 1q; deletions of 13q and p53; constitutive activation of NFκB—e.g., via inactivation of TRAF3, constitutive overexpression of NIK (NFκB inducing kinase), or activation of NFKB2—perturbation of the RB pathway—e.g., via methylation of the $p16^{INK4a}$ promoter—and deletion of $p18^{INK4c}$.

MM is preceded in a sizable fraction of cases by a premalignant disorder that is characterized by the abnormal persistence, sometimes for decades, of a clone of Ig-producing plasma cells that are lodged in the bone marrow without causing loss osteoporosis or osteolytic lesions. This disorder is referred to as MGUS (Rajkumar, Lacy et al. 2007). MGUS is defined by a monoclonal serum Ig of <30 g/l, the presence of 10% or fewer plasma cells in the bone marrow, the absence of anemia and lytic bone lesions, and the absence of hypercalcemia and renal insufficiency related to the clonal plasma cell proliferation. The prevalence of MGUS in elderly patients is ~5%. The progression from MGUS to MM occurs at a slow but remarkably steady rate of 1% per year. The etiology of MGUS is not known, but epidemiologic evidence points to age, gender, and race as risk factors. The pathogenesis of MGUS is poorly understood. Approximately 50% of MGUS cell clones carry chromosomal translocations that rearrange *IGH* at 14q32 with oncogenes on one of five partner chromosomes also identified in MM: *CCND1*, *CCND3, FGFR3* and *MMSET*, *MAF*, and *MAFB*. These translocations are thought to play an important role in the initiation of MM. Approximately 40% of the plasma cell clones in MGUS smoldering MM and frank MM are hyperdiploid. This consistency suggests that hyperdiploid MM originates from hyperdiploid MGUS. Likewise, deletions of Chr 13q, which have an adverse prognostic association in MM, are found in similar frequencies in MGUS and MM, indicating a direct precursor–product relationship of $13q^-$ preneoplastic and neoplastic states. Empirical observations of this kind suggest that MGUS occurs as distinct molecular subtypes, which lead, in turn, to different forms of MM.

Evidence suggests that MGUS and MM develop along one of two distinct pathways that result in either non-hyperdiploid tumors that usually carry one of the seven recurrent *IGH* translocations or hyperdiploid tumors that usually are not associated with *IGH* translocations. Despite enormous progress in the past decade in our understanding of MM pathogenesis, many important questions remain. What are the molecular and microenvironmental mechanisms

that drive the transition from MGUS to MM? How does hyperdiploidy contribute to plasma cell transformation? What genetic lesions underlie recurrent cytogenetic changes, such as gain of chromosome 1q or loss of chromosome 13q? Mouse models of human PCN may help to provide answers to these and other important questions.

8.8.2 Established and Newly Emerging Mouse Models of Human PCN

The presently available mouse models of human PCN can be divided into de novo and transplantation models. Although none of the genetically engineered strains recapitulate all features of a particular human PCN, several strains have emerged as useful platforms for mechanistic and therapeutic studies of alterations in signaling pathway found in human PCN (e.g., IL-6, Abl, and Myc). Furthermore, although the succession of oncogenic processes responsible for tumor development that occurs de novo does not completely match those in humans, the incipient tumor cells in mice interact with their microenvironment (immune cells, vascular and lymphatic networks, and extracellular matrix) in ways that mirror the interactions of neoplastic plasma cells in humans with their specific tissue microenvironment. Similarly, mouse models that have been developed to permit the outgrowth of fully transformed transplanted tumor cells in vivo are not suitable for studying mechanisms of tumor development. Nonetheless, they are highly valuable for many other purposes including preclinical drug testing. Table 8.3 shows mouse models of human PCN in chronological order of development, beginning with strains in which tumor development occurs de novo, followed by models that rely on tumor cell transfer.

The first mouse model of de novo PCN, peritoneal PCT in strain BALB/c, was discovered 50 years ago by Dr. Michael Potter of the National Cancer Institute and has been progressively developed and refined by him over the last half century. Salient features of this model include dependency on chronic inflammation (usually induced by intraperitoneal application of pristane), genetic background (BALB/cAnPt is highly susceptible, NZB and BALB/cJ are weakly susceptible, and all other tested strains including DBA/2, C57BL/6, CBA/J, C3H, and 129 are solidly resistant), maintenance of mice in an antigen-rich conventional facility (SPF mice are refractory to tumor development), and the acquisition of *Myc*-deregulating chromosomal translocation in early tumor precursors. The penetrance of peritoneal PCT at 65% is incomplete and the average latency of 220 days is long. However, tumor development can be greatly accelerated by infection of mice with retrovirus expressing any of the series of oncogenes alone or in combination—Abelson-(v-*abl*), RIM (v-Ha-*Ras* and Eμ–c-*Myc*), J3V1 (v-*Raf1* and v-*myc*), and ABL–MYC (v-*abl* and human *MYC*).

Unfortunately, this model of PCN has been largely dismissed by the myeloma community as artificial and irrelevant for human MM, mainly due to

Table 8.3. Mouse models of human plasma cell neoplasms (PCN) including extraosseous plasmactyoma (PCT) and multiple myeloma (MM)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
de novo	Peritoneal cavity	PCT	BALB/c	None	High impact on immunology and cancer research	Potter (2003)
					Dependent on peritoneal inflammation (pristane)	Potter and MacCardle (1964) and Anderson and Potter (1969)
					Dependent on *Myc* translocation	Potter and Wiener (1992) and Janz (2006)
					Accelerated by retroviruses, such as A-MuLV, RIM (c-myc + v-ras), J3V1 (v-myc + v-raf), and ABL–MYC	Potter, Sklar et al. (1973), Ohno, Migita et al. (1984), Clynes, Wax et al. (1988), Troppmair, Huleihel et al. (1988), Weissinger, Mischak et al. (1991)
de novo	Bone marrow	MM	C57BL/Ka	None	Spontaneous tumors Impractical due to long latency and low incidence Source of transplantable 5T tumors (see below) No *Myc* translocations	Radl, Croese et al. (1988), and Radl, Van Arkel et al. (1996)
de novo	Lymphoid system	PCT	BALB/c	Eμ–v-abl	Mice develop peritoneal tumors upon treatment with pristane	Rosenbaum, Harris et al. (1990)
de novo	Lymphoid system, GALT	PCT	BALB/c	H2-L^d-IL-6	Mice develop peritoneal tumors upon treatment with pristane	Kovalchuk, Kim et al. (2002)

Table 8.3. (continued)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
de novo	Lymphoid system	PCT	BALB/c	Eμ–Bcl-2	Mice develop peritoneal tumors upon treatment with pristane	Silva, Kovalchuk et al. (2003)
de novo	Bone marrow, lymphoid system	MM > PCT	C57BL/6 and BALB/c	NPM–ALK	Transgene targeted to T lymphocytes	Chiarle, Gong et al. (2003)
de novo	Peritoneal cavity, lymphoid system, and bone marrow	PCT > MM	Mixed BALB/c	iMyc	Mice develop peritoneal tumors upon treatment with pristane	Park, Kim et al. (2005), Park, Shaffer et al. (2005), and Kim, Han et al. (2006)
de novo	Lymphoid system, bone marrow	PCT > MM	Mixed	iMyc + Bcl–X_L	3'KE–Bcl–X_L transgene	Cheung, Kim et al. (2004)
de novo	Lymphoid system, bone marrow	PCT > MM	FVB/N	Bcl–X_L	Accelerated tumorigenesis upon crossing in the Eμ/c-Myc transgene	Linden, Kirchhof et al. (2004) and Adams et al. (1985)
de novo	Bone marrow and lymphoid system	MM > PCT	C57BL/6	Bcl–X_L + virus		Linden, Kirchhof et al. (2005)
de novo	Bone marrow	MM > PCT	C57BL/6	Xbp1		Carrasco, Sukhdeo et al. (2007)
de novo	Not yet reported	Not yet reported	Not yet reported	TVA/TVB	Unpublished	F. Asimakopoulos and H.E. Varmus[1]
de novo	Bone marrow	MM	Not yet reported	Kappa*Myc	Unpublished	M. Chesi, A.K. Stewart, and P.L. Bergsagel et al.[1]

Table 8.3. (continued)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
Transfer of mouse MM	Bone marrow	MM	C57BL/6	None	Relies on 5T tumor cells homing to mouse bone, extensively studied	Vanderkerken, Goes et al. (1996)
Transfer of human MM	Bone marrow	MM	SCID-hu	None	Human MM cells homing to human fetal bone implanted s.c. in mice, well established	Yaccoby, Barlogie et al. (1998)
Transfer of human MM	Bone marrow	MM	SCID-hu	None	Human MM bone cores implanted i.m. in mice	Campbell, Manyak et al. (2006)
Transfer of human MM	Bone marrow	MM	NOD/SCID-hu	None	Immunodeficiency more pronounced than SCID	Pilarski, Hipperson et al. (2000) and Huang, Tien et al. (2004)
Transfer of human MM	Bone marrow	MM	NOG = NOD/SCID/gammac(null)	None	Immunodeficiency more pronounced than NOD/SCID	Miyakawa, Ohnishi et al. (2004)
Transfer of human MM	Bone marrow	MM	SCID-rab	None	Human MM cells homing to rabbit fetal bone implanted s.c. in mice	Yata and Yaccoby (2004)

[1]Unpublished. Presented at the XI International Workshop on Multiple Myeloma, Kos Island, Greece, June 2007. NPM, nucleophosmin; PCT, plasmacytomas; SCID, severe-combined immunodeficiency.

the lack of bone marrow involvement. The dismissal may be premature, as certain properties of peritoneal PCT may be of great relevance for human MM. For example, peritoneal PCT formation is profoundly inhibited by anti-inflammatory agents, such as corticosteroids, which play an important role in the standard therapy of human MM. BALB/cAnPt mice deficient in IL-6 or treated with the cyclooxygenase inhibitors, indomethacin and sulindac, are also resistant to tumor induction. This suggests an intriguing parallel to the postulated tumor-promoting role of chronic inflammatory processes in the pathogenesis of human MM. Just as MM is preceded by MGUS, peritoneal PCT is preceded by a well defined and easily studied preneoplastic lesion, namely foci of aberrant plasma cells that reside in the inflammatory granulomas of the peritoneum where they can persist for months. Plasma cell foci of this sort may provide a good experimental opportunity to elucidate the enigmatic transition from preneoplastic to neoplastic plasma cell growth in a genetically defined and environmentally controlled study.

The 5T mouse myeloma of strain C57BL/KaLwRij was developed more than a quarter century ago by Dr. Jiri Radl (Radl 1981; Radl, Croese et al. 1988). In a survey of 2-year-old C57BL/Ka mice, he estimated that 0.5% of the mice developed aggressive PCN originating in the bone marrow. These tumors, designated as 5T myelomas, produced copious amounts of monoclonal Ig, were readily transplanted when injected intravenously into syngeneic mice, and, importantly, produced osteolytic lesions in recipient animals. Two serially transplanted neoplasms, called 5T2 and 5T33, are now in common use and are widely considered as the only mouse model that accurately recapitulates key properties of human MM [reviewed in Vanderkerken, Asosingh et al. (2003)]. 5T2 and 5T33 offer the unique advantage of testing new strategies for the treatment of MM in a neoplastic plasma cell that resides in the appropriate microenvironment of the bone marrow in an immunocompetent host. The 5T33 system has recently been adapted to tissue culture in presence of an adherent layer of stromal cells, further enhancing the suitability of this preclinical model system for drug testing. Another enhancement is provided by the continuous improvement of bioimaging methods that allow a more accurate evaluation of myeloma bone disease and tumor burden than was previously possible. Relevant methods include micro-CT of whole bone or bone explants and imaging techniques that detect 5T cells labeled with fluorochromes (GFP), luciferases (bioluminescence), or sodium iodide symporter (SPECT) with great sensitivity in vivo. Unlike BALB/c PCT, 5T myeloma does not harbor a chromosomal c-*Myc* translocation. This defines another intriguing parallel to human MM, in which *MYC* translocations, if they occur at all, appear as so-called secondary translocations involved in tumor progression.

The classic model of BALB/c PCT has been refined and accelerated by the development of a number of TGs that target oncogenes to the B-cell lineage. Among these are the H2-L^d-hu-IL-6, Eμ–Bcl-2, iMyc, and Eμ–v-abl transgenes. The Bcl2 and v-abl TG takes advantage of the intronic IgH enhancer, Eμ, to enforce the expression of the target genes in B-lineage cells. PCT incidence in

Eμ–v-abl mice approaches 100%, does not require treatment with inflammatory agents, is independent of genetic background, and has been reported to extensively involve the bone marrow. Some Eμ–v-abl mice were shown to present with only bone marrow PCT, developing hind limb paralysis as the result of tumor growth in vertebral marrow cavities. If further modification of this mouse were to succeed in reproducing this primary bone marrow manifestation of PCN growth more consistently, strain Eμ–v-abl may evolve into a true counterpart of human MM.

Among many attempts to express the plasma cell growth, differentiation and survival factor, IL-6, in the B cell and other cell lineages of TG mice, the H2-L^d-hu-IL-6 TG has emerged as the most promising for studying PCN. Deregulated expression of IL-6 in young TG mice causes progressive plasma cell hyperplasia in lymphoid tissues, hypergammaglobulinemia, kidney damage, and a histologic picture that resembles human multicentric Castleman's disease (Kovalchuk, Kishimoto et al. 2000). The transition from plasma cell hyperplasia to neoplasia occurs in older mice, usually in enlarged lymph nodes of the gut-associated lymphoid tissue, GALT (Kovalchuk, Kim et al. 2002). Bone marrow infiltration in mice bearing advanced tumors is often extensive. This mouse model may be useful for elucidating the molecular and cellular mechanisms of IL-6-driven plasma cell neoplasia and to test new treatments that target the IL-6 receptor (Yoshio-Hoshino, Adachi et al. 2007) or downstream elements of the IL-6 signaling pathway (Bhutani, Pathak et al. 2007; Hausherr, Tavares et al. 2007; Loffler, Brocke-Heidrich et al. 2007).

The EμSV–Bcl-2-22 TG contains a human *BCL2* cDNA driven by Eμ (Strasser, Whittingham et al. 1991). Transfer of the TG from PCT-resistant C57BL/6 mice onto the PCT-susceptible BALB/c genetic background resulted in a 24-fold increase in tumor incidence and a two-fold reduction in tumor latency. Similar to their IL-6 TG counterparts, *BCL2* TG PCT harbor *Myc*-deregulating T(12;15) translocations (Silva, Kovalchuk et al. 2003). Accelerated plasmacytomagenesis in strain Eμ-Bcl-2may facilitate the design and testing of BCL2 inhibition strategies of potential relevance to BCL2-overexpressing human PCN, such as Waldenström's macroglobulinemia and MM (Kline, Rajkumar et al. 2007).

TG mice, designated iMyc, contain a His_6-tagged mouse c-Myc cDNA, Myc_{His}, inserted head-to-head into different sites of the mouse *IgH* locus in ways that mimic the Myc-activating T(12;15) translocations of BALB/c PCT. A strain carrying the iMyc TG just 5′ of Eμ is the most thoroughly characterized to date (Park, Kim et al. 2005). In analogy to the experience with the IL-6 and Bcl-2 TG mentioned above, the transfer of the iMyc TG onto BALB/c rendered the mice hyper-susceptible to inflammation-induced peritoneal PCT (Park, Shaffer et al. 2005; Kim, Han et al. 2006). As expected, the PCT overexpressed Myc_{His}, produced monoclonal Ig, and exhibited a unique plasma cell signature upon gene expression profiling on mouse lymphochip.

A somewhat surprising observation was made in mice harboring a T-cell-targeted fusion gene joining nucleophosmin (NPM) and anaplastic lymphoma

kinase (ALK). Predictably, these mice developed T-cell lymphomas; however, 20% developed PCN instead of T-cell lymphomas. The PCN arose in peripheral lymphoid tissues or the bone marrow. In the latter case, tumor growth resulted in peripheral neuropathy and hind leg paralysis. NPM–ALK TG mice are currently used primarily for studies on the T-cell neoplasm, anaplastic large cell lymphoma (Amin and Lai 2007). Further modification of this strain for modeling human MM has not been attempted.

With several newly developed TG models at their disposal, researchers began to generate double TG mice in efforts to develop robust models of human PCN. Other modifications, such as infecting TG mice with oncogenic virus or changing the genetic background, were also pursued to further accelerate tumor development and/or shift the tumor pattern from B-cell lymphoma to PCN. PCN formation is dramatically accelerated in double TG mice that carry the H2-L^d-hu–IL-6 TG and a Bcl-2 TG (Janz, unpublished finding), a Bcl-X_L TG (Fang, Mueller et al. 1996; Potter 2003) (Janz, unpublished finding) or iMyc TG (Janz, unpublished finding). The same findings were obtained in iMyc/Bcl-2 double TG mice (Janz, unpublished finding). An interesting alternative approach involved the infection of Bcl-X_L TG mice with ABL–MYC virus (Linden, Kirchhof et al. 2005). This resulted in a unique model of MM that recently was acknowledged by a panel of MM experts as holding promise for the validation of new therapeutics (Dalton and Anderson 2006). Additional research is warranted to better characterize double TG and virally accelerated mouse models of human PCN before recommendations can be made as to which model may be most suitable for elucidating specific aspects of the human disease.

Recent studies have shown that X-box-binding protein-1 (XBP1), a differentiation and unfolded protein/ergoplasmatic reticulum stress response factor essential for normal plasma cell development in mice, may also be implicated in human PCN. This prompted the development of a new model of human MM that relies on enforced expression of Xbp-1s ORF in the B-cell lineage under the control of the IgV_H promoter and Eμ enhancer. The mice are prone to a MGUS-like disorder followed by a type of PCN with many similarities to MM. Like all other TG strains susceptible to PCN, Eμ–Xbp1 TG also develop extraosseous PCN, either together with the MM-like tumors or on their own. Nonetheless, a number of features indicate that the Eμ–Xbp1 mice offer an attractive model of human MM. Among other applications, they may be useful to uncover the elusive genetic changes responsible for the transition of MGUS to MM.

A fresh, unorthodox approach to recapitulating the natural history of MM in a relevant cellular and physiologic milieu is a mouse model system that enables the delivery of stochastic, sequential, somatic mutations to precisely defined plasma cell precursors in vivo. Asimakopoulos and Varmus used BAC TG technology to express two distinct types of avian leukosis virus (ALV) receptors, TVA and TVB, in the expanding centroblasts of the GC dark zone and the committed plasmablasts of the light zone. Mouse cells are refractory to

infection by retroviruses of the ALV family unless they ectopically express the cognate avian-derived receptors. To that end, TG mice were genetically developed that express TVA driven by regulatory elements of *Mybl1* (*A-Myb*), a transcription factor expressed in dividing blasts of the GC dark zone, and TVB under control of Blimp-1, a master regulator of plasma cell differentiation. As a result, the mice express TVA in dividing follicular B cells and TVB in cells of the GC light zone, extrafollicular plasma cells, and mature plasma cells in the bone marrow. Viral vectors have been engineered to carry dominant oncogenes or various inactivators of tumor suppressor genes, permitting the introduction of sequential oncogenic lesions in putative precursors of PCN.

Chesi, Bergsagel, and associates reported recently on a new mouse model of MM designated VK*MYC. This model is based on the VK*MYC TG that contains an inactive, non-coding human *c-MYC* gene under the transcriptional control of the Vκ promoter. TG MYC is activated sporadically in GC B cells undergoing somatic hypermutation. This approach has two potential benefits. First, in contrast to all previously generated MYC TG, expression of the VK*MYC TG occurs only at the GC stage of B-cell development. This circumvents the unwanted transformation of less mature B-lineage cells that occurs when Myc is expressed earlier in differentiation. Second, B cells in which MYC becomes activated are likely to participate in an ongoing T-cell-dependent immune response, because they were part of a GC reaction. This further restricts the pool of MYC target cells to those that define the postulated MM precursor pool in humans. Evidence indicates that virtually all Vk*MYC mice develop MGUS-like disease by 50 weeks of age. Plasma cells are fully differentiated ($CD19^{-}CD138^{+}$), have a very low proliferation index, and are found exclusively in the bone marrow. Vk*MYC mice also develop anemia, bone disease with low trabecular density as well as sporadic lytic bone lesions and hind limb paralysis. Similar to the Xbp1 TG mice, 30% of the Vk*MYC mice exhibit extramedullary disease. Importantly, VK*MYC mice responded to drugs known to be active against MM while demonstrating no response to drugs with little or no clinical activity. These features indicate that strain VK*MYC will be useful in the study of MM biology and the development of new pharmacological and immunological therapies.

8.8.3 Xenograft Models of Human Myeloma in Mice

In contrast to the models of de novo PCN formation in mice, xenograft models use MM cells or cell lines that are transplanted into SCID-Hu, NOD/SCID, or SCID-Rab mice. These systems offer the unique ability to test therapeutics in vivo against true MM (Tassone, Neri et al. 2005). The SCID-Hu mouse model that employs fetal bone permits studies on the interaction of MM cells with the microenvironment of human bone marrow. The NOD/SCID has been adapted to myeloma cells labeled with green fluorescent protein. Intravenous injection

of these cells creates a model in which diffuse PCN dissemination can be visualized using whole-body, real-time fluorescence imaging to reproducibly quantify tumor burden. This allows serial, noninvasive monitoring of drug treatment. The SCID-Rab model avoids the ethical concerns about the use of human fetal bone in the SCID-Hu model by using rabbit bones. This model supports the growth of MM cells in a non-myelomatous, non-human, and non-fetal microenvironment. Although xenograft models are the current work-horses in preclinical testing of efficacy and mechanism of action of novel myeloma drugs, the xenograft implants have their own severe limitations, including the lack of an intact immune system, inability to model premalignant neoplastic stages, and imperfections in recapitulating the interactions between myeloma cells and surrounding stroma.

The SCID-hu mouse, which was originally developed for studies on human hematopoiesis in mice (Shultz, Ishikawa et al. 2007), has been adapted by Dr. J. Epstein, Y. Yaccoby, and their associates to investigate human MM cells in their native microenvironment of the human bone marrow (Yaccoby, Barlogie et al. 1998). In this system, myeloma growth is restricted to and dependent on human bone marrow and leads to osteolytic lesions in the transplanted human bone. The SCID-hu model contributed to our understanding of myeloma biology by demonstrating that myeloma alters the balanced expression of the osteoclast differentiation factor, RANKL/OPG, in the bone marrow (Pearse, Sordillo et al. 2001), depends on osteoclasts for growth and survival (Yaccoby, Pearse et al. 2002), relies, in part, on IL-6 to avoid programmed cell death (Yaccoby, Pearse et al. 2002), abolishes osteoblasts in the course of tumor progression (Yaccoby, Wezeman et al. 2006), and uses the serine phosphatase, fibroblast activation protein, to interact with stromal cells (Ge, Zhan et al. 2006). Importantly, the SCID-hu model was instrumental in showing that the anti-myeloma activity is dependent on its metabolism by liver microsomes (Yaccoby, Wezeman et al. 2006).

Progress in research on humanized mice (Shultz, Ishikawa et al. 2007) led to additional modifications of the SCID-hu model, such as NOD/SCID-hu (Huang, Tien et al. 2004), NOG (NOD/SCID/γ_c^{null}) (Miyakawa, Ohnishi et al. 2004), and SCID-rab (Yata and Yaccoby 2004), which permit the engraftment of human myeloma cells in endogenous human bone or implanted rabbit bone, respectively. The addition of the NOD genetic background to SCID mice enhances the immunodeficiency conferred by the SCID background, resulting in lack of B and T cells, lack of circulating complement, defective macrophage function, and low natural killer cell activity. The γ_c^{null} phenotype causes the complete loss of natural killer cells. Just like the original SCID-hu model, the newer models enhance our understanding of myeloma biology and are useful for evaluating novel drugs and drug candidates in a preclinical setting. The utility of the SCID-rab system to evaluate effects of antibody to DKK1, of bortezomib, and of bone anabolic agents on bone remodeling and myeloma growth illustrates this point (Yaccoby, Ling et al. 2007).

8.8.4 Conclusions Regarding Mouse Models of Human PCN

Recent progress in the design and development of genetically engineered mouse models of human PCN has resulted in two categories of experimental models systems. In the first or de novo category, PCN arise either spontaneously or are induced in inbred or TG mice. Tumor development occurs in predictable stages and is preceded by the expansion of premalignant plasma cells resembling benign monoclonal gammopathy (BMG), MGUS, Castleman's disease, or similar non-malignant human plasma cell disorders. The mouse models in this category are indispensable for mechanistic studies of plasma cell transformation and the design and testing of strategies for tumor prevention. The greatest weakness among these models is their failure to recapitulate the bone marrow manifestations of tumor growth that are typical of MM. Two recent models offer a glimmer of hope along this line (Cheung, Kim et al. 2004; Linden, Kirchhof et al. 2005; Boylan, Gosse et al. 2007; Carrasco, Sukhdeo et al. 2007). In the second or transplantation-based category, fully transformed plasma cells of mouse origin (5T) or human origin (primary MM cells and myeloma cell lines) are transferred into syngeneic, immunocompetent mice (5T) or immunodeficient SCID mice (xenotransplant system) that frequently harbor human or rabbit bone as a nesting ground for the incoming plasma cells. The mouse models in this category have made and continue to make important scientific contributions to the preclinical assessment of myeloma therapeutics and our understanding of myeloma bone disease. Nonetheless, technical and logistic barriers have prevented these models from having a significant economic effect on the process of anti-myeloma drug discovery. They are not extensively used in preclinical trials and have not gained wide acceptance in industry. In addition, transplantation models are not useful for studies on tumor development and prevention.

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Chapter 8

Query No.	Page No.	Line No.	Query
AQ1	180	26	For the citation "Jaffe, Harris et al. 2001," please clarify whether the year is "2001a", "2001b", "2001c," or "2001d."
AQ2	184	9	Damle, Wasil et al. (1999), Faderl, Keating et al. (2002), Crespo, Bosch et al. (2003), Hallek, Langenmayer et al. (1999) and Sarfati, Chevret et al. (1996) are not listed in the reference list. Please provide.
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