LYMPHOID MALIGNANCIES: THE DARK SIDE OF B-CELL DIFFERENTIATION

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When the regulation of B-cell differentiation and activation is disrupted, lymphomas and leukaemias can occur. The processes that normally create immunoglobulin diversity might be misdirected, resulting in oncogenic chromosomal translocations that block differentiation, prevent apoptosis and/or promote proliferation. Prolonged or unregulated antigenic stimulation might contribute further to the development and progression of some malignancies. Lymphoid malignancies often resemble normal stages of B-cell differentiation, as shown by molecular techniques such as gene-expression profiling. The similarities and differences between malignant and normal B cells indicate strategies for the treatment of these cancers.

DECISION MAKING IN THE IMMUNE SYSTEM

V(D)J RECOMBINATION
The somatic rearrangement of variable (V), diversity (D) and joining (J) regions of antigen-receptor genes, which leads to the repertoire diversity of both

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Normal lymphocyte differentiation is, in some sense, a disaster waiting to happen. B cells put their genomic integrity in danger during the formation and revision of their antigen receptors. A second potentially dangerous event is the response to antigen. When this response functions normally, the clonal expansion of B cells is regulated tightly by homeostatic controls. However, chronic infections can wreak havoc on lymphocyte homeostasis, as can abnormal responses to self-antigens, and both of these mechanisms might contribute to lymphoid malignancies. Finally, many of the oncogenic events that occur in lymphomas and leukaemias disrupt the molecular pathways that regulate B-cell differentiation, proliferation and apoptosis.

A confounding issue in the study of human lymphoid malignancies has been imprecision of diagnosis. Recent studies using gene-expression profiling and genomic mutational analysis have shown that lymphomas and leukaemias that are difficult to distinguish histologically can nevertheless be molecularly distinct diseases. Using more-precise disease definitions, malignancies can be related often to distinct stages of B-cell differentiation. In this review, we focus on advances in the molecular definition of mature B-cell malignancies and discuss how the relationship between a lymphoma and its normal B-cell counterpart might be exploited to understand and treat these cancers. We discuss also how oncogenic alterations in these cancers subvert homeostatic regulation of lymphocyte responses.

The perils of normal B-cell differentiation

The first dangerous hurdle in B-cell differentiation is rearrangement of the immunoglobulin genes of B-cell precursors in the bone marrow to form a B-cell receptor (BCR). This molecular process, V(D)J RECOMBINATION, involves double-stranded DNA breaks that are initiated by recombination-activating genes (RAG1 and RAG2) and resolved by the non-homologous end-joining repair apparatus[1]. Occasionally, these breaks are resolved aberrantly, leading to chromosomal translocations. In lymphomas, chromosomal translocations typically replace the normal regulatory sequence of a gene with heterologous regulatory elements that drive inappropriate gene expression near the breakpoints. Clear examples of such mistakes in V(D)J recombination are t(14;18) — that is, a translocation between chromosomes 14 and 18 — which involves the *BCL2* gene and the immunoglobulin heavy-chain (IgH) locus in follicular lym-

T- and B-cell receptors.

GERMINAL CENTRE
The structure that is formed by the clonal expansion of antigen-activated B-cell blasts that have migrated to the follicles of lymph nodes. The B cells in these structures proliferate and their immunoglobulin genes undergo somatic hypermutation, before the cells leave as plasma cells or

phoma (BOX 1), and t(11;14), which involves the gene encoding cyclin D1 and the IgH locus in mantle-cell lymphoma (BOX 1). The structures of the recombination breakpoints in these translocations are consistent with RAG-mediated cleavage of the IgH locus, guided by recombination signal sequences (RSSs). However, the recombination sites in the partner genes lack clear RSSs and do not have cryptic sequences that could function as RSSs[2], although they sometimes involve DNA regions with altered structure[3]. The IgH breaks of t(11;14) in mantle-cell lymphoma seem to occur before heavy-chain diversity and joining segment (D_H–J_H) rearrangement, which indicates that this translocation occurs early in B-cell differentiation[4]. As RAGs are not expressed after the immature B-cell stage[5,6], t(14;18) might occur in a pre-GERMINAL CENTRE (GC) B cell as well.

Box 1 | Mature B-cell malignancies

Follicular lymphoma
An often indolent B-cell lymphoma with a follicular growth pattern. Most are characterized by the overexpression of BCL-2, owing to t(14;18). They comprise ~22% of non-Hodgkin lymphomas (NHLs). They cannot be cured by conventional chemotherapy and the survival rate is 73% at 10 years.

Mantle-cell lymphoma
A B-cell lymphoma that localizes to the mantle region of secondary follicles. Mantle-cell lymphoma (MCL) is associated with t(11;14), which results in the overexpression of cyclin D1. MCLs comprise 6% of all NHLs, have a male predominance and occur at a median age of 60. With current chemotherapy regimens, patients with MCL can achieve complete remission, but long-term remission is rare and median survival is 3–5 years.

Burkitt lymphoma
An aggressive B-cell lymphoma of children and young adults that is associated invariably with translocations of *c-MYC*. The endemic form involves Epstein–Barr virus (EBV) infection of malignant cells, whereas the sporadic form is EBV independent. These lymphomas can be cured in more than 80% of cases.

Multiple myeloma
An incurable malignancy of plasma cells with a median survival of three years. Multiple myeloma constitutes ~10% of all haematological malignancies, with a median age at diagnosis of ~65. Neoplastic cells are located in the bone marrow, and osteolytic bone lesions are characteristic. Reciprocal chromosomal translocations between one of the immunoglobulin loci and various other genes, including those that encode cyclin D1, cyclin D3, c-MAF, MMSET (multiple myeloma SET-domain protein) or fibroblast growth factor receptor 3 (FGFR3), are considered to be primary oncogenic events.

Diffuse large B-cell lymphoma
Diffuse large B-cell lymphoma (DLBCL) is the most common type of NHL (30–40% of cases). Up to one third of cases have abnormalities of *BCL6*, and ~20% of cases have translocations of *BCL2*. DLBCLs are clinically, morphologically and molecularly heterogeneous. 40% of patients with DLBCL can be cured by conventional chemotherapy.

Hodgkin lymphoma
This type of lymphoma accounts for ~10% of all lymphoid malignancies, and it usually arises in the lymph nodes of young adults. It can be subdivided into a classical subtype and a less common nodular lymphocyte predominant subtype. Cure rates of more than 80% can be achieved with present therapies.

Lymphoplasmacytic lymphoma
This is a rare form of NHL that comprises ~1.5% of nodal lymphomas. It is usually indolent and frequently involves bone marrow, lymph nodes and spleen. Most patients have monoclonal immunoglobulin M in their serum, and the tumour cells have a plasmacytic morphology. A subset of lymphoplasmacytic lymphomas is characterized by recurrent t(9;14), which involves the *PAX5* (paired box gene 5) and immunoglobulin heavy-chain loci.

Marginal-zone lymphoma
This extranodal lymphoma occurs in organs that normally lack organized lymphoid tissue (such as the stomach, salivary glands, lungs and thyroid glands), and it comprises 7–8% of all B-cell lymphomas. In many cases, chronic inflammation or an autoimmune process precedes development of the lymphoma. Gastric mucosal-associated lymphoid tissue (MALT) lymphoma, the most common type, is associated with *Helicobacter pylori* infection, and 70% of patients at early stages have complete remission after eradication of this bacterium. At later stages, the acquisition of genetic abnormalities might lead to *H. pylori*-independent growth of the tumour cells or to transformation to an aggressive DLBCL.

Chronic lymphocytic leukaemia
The most common type of leukaemia, chronic lymphocytic leukaemia (CLL), is often an indolent disease with a median age of onset of 65. CLL is molecularly and clinically related to a nodal lymphoma known as small lymphocytic lymphoma. Current therapy can reduce symptoms, but it is not curative and does not prolong survival.

memory B cells.

FOLLICULAR DENDRITIC CELLS (FDCs). Cells with a dendritic morphology that are present in lymph nodes, where they present intact antigens held in immune complexes to B cells.

PLASMABLAST
A dividing B cell that is committed to plasma-cell differentiation.

CLASS-SWITCH RECOMBINATION
DNA rearrangement of the VDJ region from immunoglobulin M to any of the IgG, IgA or IgE constant genes at the heavy-chain locus. Recombination occurs in repetitive sequences of DNA that are located upstream of each constant gene.

SOMATIC HYPERMUTATION
The substitution of 'untemplated' nucleotides or small deletions targeted to a rearranged VDJ or VJ segment, which occurs only in B cells. The mutations are found between the promoter and enhancer of the rearranged gene (including non-coding regions), but they are found at the highest frequency in 'hotspots' (RGYW) that are located in the complementarity-determining regions.

This possibility is interesting because follicular lymphomas seem to be arrested at the GC stage of differentiation (see later), which indicates that a naive B cell that has acquired a *BCL2* translocation can nevertheless participate in an antigen-driven GC response.

After antigen encounter, naive B cells follow one of three pathways: they can enter the GC microenvironment, where they interact with T cells, FOLLICULAR DENDRITIC CELLS and antigen[7]; they can differentiate into short-lived PLASMABLASTS outside of the GC[8]; or they can enter an unresponsive state known as anergy (FIG. 1a). In the GC, two molecular processes remodel DNA — immunoglobulin CLASS-SWITCH RECOMBINATION (CSR) and immunoglobulin SOMATIC HYPERMUTATION (SHM).

Both CSR and SHM generate DNA breaks[9–11] and are, therefore, dangerous mechanisms that might predispose to chromosomal translocations. The DNA breaks that are induced by CSR and SHM coincide with the sites of chromosomal translocations that involve the IgH locus in certain lymphoid malignancies. SHM is probably involved in t(8;14) in endemic Burkitt lymphomas (BOX 1) because the *c-MYC* gene is often joined to the IgH locus in a rearranged and somatically mutated IgH variable (V) region[12–14]. SHM can also target non-immunoglobulin loci, such as *BCL6* (REFS 15–17), and the involvement of these genes in translocations is probably a byproduct of this process. CSR is the culprit in many of the translocations that occur in multiple myeloma (BOX 1) and sporadic Burkitt lymphoma, because the translocation breakpoints occur in IgH switch regions[18,19].

Cell of origin

Historically, the relationship between normal B-cell subpopulations and types of lymphoma has been assessed by a combination of microscopic appearance and immunophenotype. By these criteria, most mature B-cell malignancies seem to be 'trapped' at particular stages of normal B-cell development. Follicular lymphomas, for example, have growth patterns that resemble those of normal GC B cells, and they are infiltrated with follicular dendritic cells and T cells. The tumour cells also express the membrane metallo-endopeptidase CD10, which is a hallmark of human GC B cells, leaving little doubt that follicular lymphoma is a disease of GC B cells. However, in some lymphomas, the tumour cells show a spectrum of morphological differentiation, ranging from GC-like cells to plasmacytic cells, which indicates that the block in differentiation is not complete.

When we speak of cell of origin we are, by necessity, referring to the relationship between the phenotype of the tumour on clinical presentation and a normal stage of B-cell differentiation. We cannot observe human lymphoid tumours during their natural evolution from a normal B cell. Therefore, as mentioned earlier, oncogenic translocations might occur at an early stage of B-cell differentiation, after which the transformed B cell might differentiate further and arrest at a later stage of differentiation. The important point is that the phenotype of the tumour at clinical presentation will influence its clinical behaviour and responsiveness to therapy.

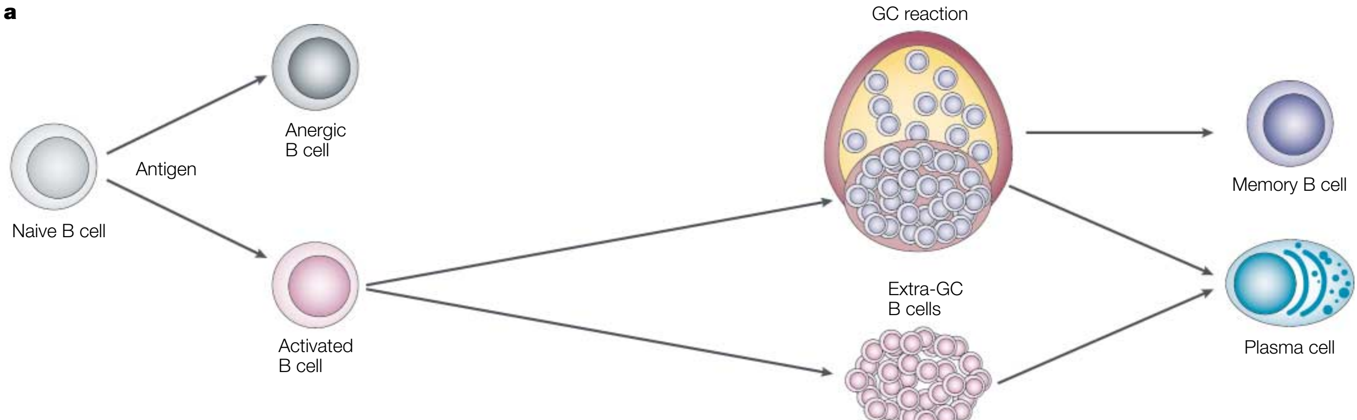
Several possible mechanisms could account for the apparent developmental arrest in many lymphoid malignancies. First, oncogenic alterations could interfere with regulatory networks that control lymphocyte differentiation. As discussed later, translocation of *BCL6* might cause lymphomas, in part, by blocking plasmacytic differentiation. Second, the malignant lymphocyte might lose responsiveness to external cues, such as antigen or other immune cells that regulate normal differentiation. Third, it is conceivable that an oncogenic event might activate pathways that mimic a particular stage of normal differentiation. This possibility seems less likely in some lymphoid malignancies, as described later, that share extensive gene-expression profiles and biological functions with particular stages of B-cell differentiation.

The analysis of somatic mutations in the rearranged immunoglobulin loci of lymphoid malignancies shows that there are clear differences between the diagnostic categories (TABLE 1). Most types of non-Hodgkin lymphoma have highly mutated immunoglobulin genes that bear the hallmarks of SHM. A prominent exception to this rule might be mantle-cell lymphoma, which indicates that this lymphoma might be pre-GC in origin. Of course, the mere presence of immunoglobulin mutations in a lymphoid malignancy only indicates that the cell that gave rise to the tumour had passed through a stage of B-cell differentiation during which SHM occurs. In some lymphomas, however, individual tumour cells in the malignant clone have distinct immunoglobulin sequences, which indicates that the tumour is frozen at a stage of differentiation at which SHM is ongoing (TABLE 1).

The presence of immunoglobulin mutations in lymphoid malignancies is usually taken as evidence that the cell of origin of the tumour passed through the GC microenvironment. Although most SHM takes place in GCs[20], recent work indicates that it can occur also outside of classical GC structures. Signalling through CD40 is required to initiate and maintain the GC reaction[21,22], and studies of hyper-IgM patients with genetic deficiencies in CD40 ligand (CD40L) have shown that some SHM can take place in the absence of CD40 signalling[23]. In particular, a $CD27^+IgM^+IgD^+$ subpopulation of somatically mutated memory B cells is retained in the peripheral blood of these patients, whereas other memory B-cell subpopulations are absent. These studies indicate that SHM can occur outside of the GC, and they are reminiscent of earlier work in lymphotoxin-α-deficient mice, which lack GCs but can initiate SHM after several immunizations[24]. A direct observation of SHM outside of GCs was reported recently using a transgenic mouse engineered to synthesize anti-IgG antibodies (rheumatoid factors)[25]. Clonal expansion of anti-IgG-specific B cells was observed in the T-zone–red-pulp border of the spleen, and the B cells in these proliferative foci were shown to have ongoing SHM at a rate similar to that seen in GC B cells. Given the possibility of extra-GC SHM, the presence of immunoglobulin mutations

in a lymphoid malignancy cannot be taken as definitive evidence for a GC or post-GC cell of origin.

Recently, the relationship of B-cell malignancies to normal stages of B-cell differentiation and activation has been clarified using genomic-scale gene-expression profiling. A unique gene-expression signature distinguishes GC B cells from other stages of B-cell differentiation, including resting naive and memory blood B cells and mitogenically activated blood B cells[26,27] (FIG. 1b). The GC B-cell signature contains several hundred genes, including well-known GC markers (such as the genes encoding CD10, CD77 synthase and BCL-6) and many new genes of unknown function that were identified by high-throughput sequencing of complementary DNA libraries from normal GC B cells[28]. Expression of the GC B-cell signature genes is maintained in some lymphoma cell lines[26], which indicates that this signature is a stable change in gene expression and does not require the cellular interactions that are present in the GC microenvironment to be maintained. So, the GC B cell is at a discrete stage of B-cell differentiation and is not just a specialized type of activated lymphocyte.



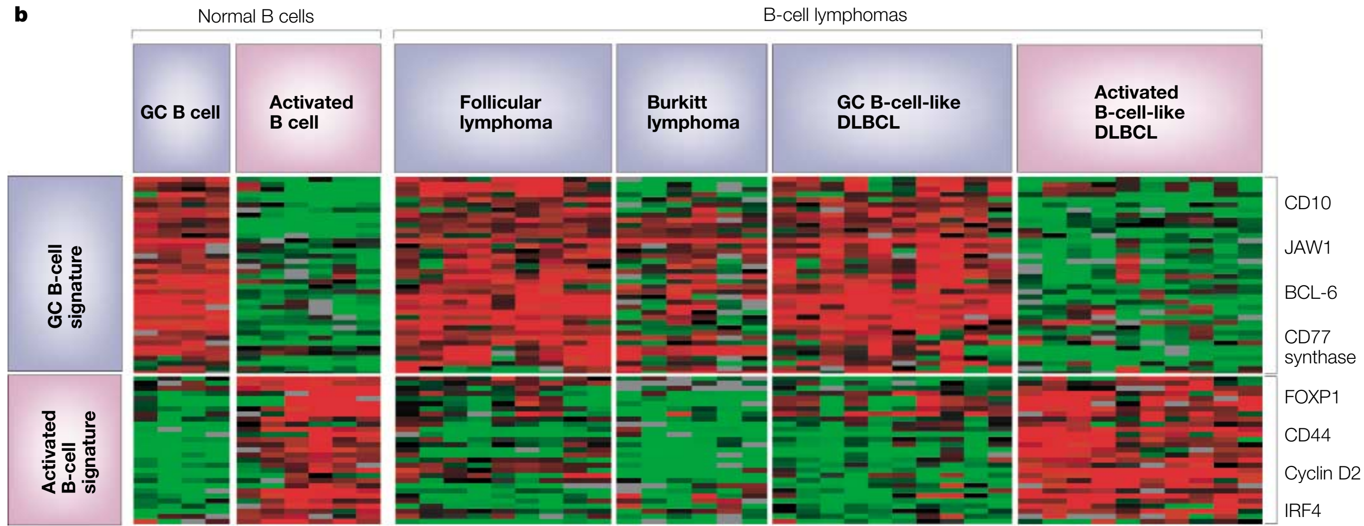


Figure 1 | **Mature B-cell lymphomas: cell of origin. a** | When naive B cells encounter antigen they become activated and face three cell fates: clonal expansion and selection in a germinal centre (GC), clonal expansion and differentiation at extra-GC sites, or anergy. Eventually, B cells either die or differentiate to memory B cells or antibody-secreting plasma cells. **b** | Gene-expression profiling shows a relationship between stages of B-cell differentiation and several types of mature B-cell lymphoma. Each column represents the results of gene-expression profiling from a single messenger RNA sample of normal or malignant B cells. Each row represents the expression of a single gene. Genes were chosen on the basis of their ability to distinguish between diffuse large B-cell lymphomas (DLBCLs) of GC and non-GC phenotype. Samples are compared with a common reference RNA pool, and relative gene expression is shown using a colour scale in which shades of red indicate genes that are expressed at a higher than median level, shades of green indicate genes that are expressed at a lower than median level, and black indicates genes that are expressed at the median level across all samples. A 16-fold range of gene expression is shown. Germinal-centre B-cell signature genes — for example, those that encode CD10, JAW1, BCL-6 and CD77 synthase — relate normal GC B cells to some lymphomas (follicular lymphomas, Burkitt lymphomas and the GC B-cell-like DLBCL subgroup). Genes that are expressed at a higher level in mitogenically activated peripheral-blood B cells than in GC B cells — for example, those that encode FOXP1 (forkhead box P1), CD44, cyclin D2 and IRF4 (interferon-regulatory factor 4) — uniquely identify the activated B-cell-like DLBCL subgroup. Potential cell types of origin for these lymphomas are indicated in pink (activated B cell) and blue (GC B cell) in part **a**.

Table 1 | **Characteristics of mature B-cell malignancies**

Malignancy	SHM	Ongoing SHM	GC B-cell expression profile[27,71,99,130]	Putative cell of origin*
Mantle-cell lymphoma	No (except for a small percentage[131])	No	No	Pre-GC B cell
Chronic lymphocytic leukaemia (CLL)‡	Yes and no	No	No	Antigen-experienced B cell (pre- or post-GC)
Burkitt lymphoma	Yes	No	Yes	GC B cell
Follicular lymphoma	Yes	Yes	Yes	GC B cell
Marginal-zone lymphoma — nodal, extranodal (MALT) and splenic	Yes (except for some splenic variants[132])	Yes (prevalent in MALT lymphomas[133])	No§	GC B cell or post-GC B cell
GC B-cell-like DLBCL	Yes	Yes	Yes	GC B cell
Activated B-cell-like DLBCL	Yes	No	No	GC B-cell subset or extra-GC mutated B cell[23,25]
Lymphoplasmacytic lymphoma (LPL)	Yes	Yes	No¶	Post-GC B cell
Multiple myeloma	Yes	No	No	Post-GC B cell
Hodgkin lymphoma (classical type)	Yes	No	No	GC or post-GC B cell
Hodgkin lymphoma (nodular lymphocyte pre-dominant type)	Yes	Yes	Yes	GC B cell

*Based on the presence or absence of somatic hypermutation (SHM) and the gene-expression profile. ‡CLL consists of two clinically distinct subtypes, one with SHM and one without[134]. In some CLLs, subclones can accumulate additional mutations through SHM or another mutational process. §The gene-expression profile of marginal-zone lymphomas (MZLs) has yet to be determined, but MZL B cells lack germinal-centre (GC) markers (such as CD10 and BCL-6) and express marginal-zone markers (such as CD21 and CD35)[86]. ¶The gene-expression profile has yet to be determined, but LPL has characteristics that relate it to post-GC plasma cells (such as cytoplasmic immunoglobulin)[97]. DLBCL, diffuse large B-cell lymphoma; MALT, mucosal-associated lymphoid tissue.

Several types of B-cell lymphoma express GC B-cell signature genes, including follicular lymphomas, Burkitt lymphomas and a subgroup of diffuse large B-cell lymphomas (DLBCLs)[26,29] (BOX 1 and FIG. 1). This finding establishes that these malignancies are derived from a GC B cell *per se* and not from a post-GC somatically mutated B cell. Although these malignancies retain expression of most of the GC B-cell signature genes, the lymphoma from an individual patient might have lost expression of any one GC B-cell marker. Furthermore, expression of a single gene is usually insufficient to establish the relationship between a malignancy and its normal counterpart, because many of the gene-expression differences between stages of differentiation are quantitative, not qualitative, in nature. Therefore, a 'diagnosis' of a GC B-cell origin must be based on the expression of several GC B-cell signature genes to be accurate. By contrast, other types of lymphoid malignancy fail to express these GC B-cell genes, and they have their own gene-expression signatures that relate them to other stages of B-cell differentiation (TABLE 1).

About half of all DLBCLs fall into a gene-expression subgroup known as GC B-cell-like DLBCLs (GCB DLBCLs), which have a gene-expression profile that closely resembles that of normal GC B cells[26,29] (FIG. 1 and BOX 2). Furthermore, these lymphomas have highly mutated immunoglobulin genes and SHM is ongoing in malignant clones[30]. Gene-expression profiling indicates also that most GCB DLBCLs have undergone immunoglobulin class switching[29] (A.R. and L.M.S., unpublished observations). Together, these observations point to a GC B cell as the cell of origin for GCB DLBCLs, and they show that these tumours are trapped at this stage of differentiation.

Another subgroup of DLBCLs, representing ~30% of cases, are known as activated B-cell-like DLBCLs (ABC DLBCLs), because these lymphomas resemble mitogenically activated peripheral B cells, and not GC B cells, in their gene-expression profile[26,29] (FIG. 1). An important feature of ABC DLBCLs is the high level of expression of nuclear factor-κB (NF-κB) target genes, including those that encode BCL-2, interferon regulatory factor 4 (IRF4), CD44, FLIP (FLICE-like inhibitory protein) and cyclin D2 (see below)[31]. These lymphomas have a high level of immunoglobulin somatic mutations, but they do not have ongoing SHM[30]. Nearly all ABC DLBCLs express a high level of IgM[29] (A.R. and L.M.S., unpublished observations), which indicates that they have not undergone immunoglobulin class-switch recombination, a finding that is unexplained so far.

The cell of origin for ABC DLBCLs is less clear than for GCB DLBCLs, although the absence of the GC B-cell gene-expression signature and the lack of ongoing SHM do not indicate a GC B-cell origin. ABC DLBCLs resemble pre-plasma cells in terms of gene expression in that they have higher levels of expression of immunoglobulin, X-box binding protein 1 (XBP1), IRF4 and other plasma-cell genes than GCB DLBCLs, and a lower level of expression of BCL-6 (REF. 29; A.R. and L.M.S., unpublished observations). GCs contain a subpopulation

of BCL-6⁻BLIMP1⁺IRF4⁺ cells[32,33], which are a possible normal counterpart of ABC DLBCLs. Alternatively, as plasma-cell differentiation and SHM can occur outside of GCs[23,25], it is possible that ABC DLBCLs are derived from B cells that have never entered a GC.

Oncogenic lesions in lymphomas

The various chromosomal translocations, amplifications, mutations and deletions that occur in B-cell lymphomas disrupt normal B-cell homeostasis in three ways: by driving the cells through the cell cycle, by preventing the normal induction of cell death and by blocking terminal differentiation. Analysis of normal GC B-cell gene expression and function indicates how these oncogenic events might perturb mature B-cell differentiation to give a selective advantage to the transformed cells (FIG. 2).

Enhancing cell growth and proliferation. GC B cells are some of the most rapidly proliferating cells in the body, doubling in number every seven hours[7], and gene-expression profiling has shown that they have a correspondingly high level of expression of cell-cycle progression genes that function in the G2/M phase of the cell cycle — for example, the genes that encode CDC2 (cell-division cycle 2), PLK (polo-like kinase) and BUB1 (budding uninhibited by benzimidazoles 1 homologue)[27,29]. Interestingly, however, genes that control cell growth (increase in cell size) are expressed at low levels in GC B cells. These genes encode components of the protein-translation machinery (for example, ribosomal proteins and translation-initiation factors) and intermediary metabolism (for example, glycolytic enzymes), and they are turned on as lymphocytes 'blast' in response to mitogenic stimuli. Many of these genes are targets of the transcription factor c-MYC[34]. Although GC B cells express c-MYC[35,36], they express lower levels of c-MYC messenger RNA than other dividing cells[27,29]. Given the drive for positive and negative selection in the GC, it might be appropriate that cells tip the balance in favour of proliferation rather than cell growth to expand the pool of selectable B cells as rapidly as possible.

The expression of c-MYC is altered by translocations, mutations and/or overexpression in many GC B-cell-derived lymphomas. All Burkitt lymphomas have translocations of *c-MYC* to immunoglobulin loci[37], and these translocations occur also in some DLBCLs[38]. Burkitt lymphomas and DLBCLs can also accumulate somatic mutations of *c-MYC* that might alter its function as a transcription factor[17,39]. These oncogenic lesions of c-MYC could increase cell growth and promote tumour-cell proliferation[34].

Blocking apoptosis. GC B cells seem to be poised for apoptosis unless they are rescued by positive selection[7]. Many anti-apoptotic proteins, such as BCL-2, A1 and BCL-X_L, are expressed at low levels in most GC B cells[27,29,40–42]. The NF-κB signalling pathway transcriptionally activates several of these genes and delivers a potent anti-apoptotic stimulus to cells[43]. NF-κB transcription factors are kept in an inactive state in the cytoplasm by interactions with inhibitor of NF-κB proteins (IκBs). Signalling through various cell-surface receptors activates IκB kinase (IKK), which phosphorylates IκB,

Box 2 | **Diffuse large B-cell lymphoma: many diseases in one diagnostic category**

On the basis of morphological and clinical criteria, the diagnostic framework that is used at present places diffuse large B-cell lymphomas (DLBCLs) in a single category and, consequently, all patients receive the same therapy[97]. However, patients with DLBCL are markedly heterogeneous in their response to multi-agent chemotherapy in that ~40% can be cured, whereas the remainder succumb to the disease[124].

Recent gene-expression profiling of DLBCL has shown that this single diagnostic category includes more than one molecularly and clinically distinct disease. DLBCL consists of at least three gene-expression subgroups, known as germinal-centre B-cell-like (GCB), activated B-cell-like (ABC) and type 3 (REFS 26,29). The gene-expression subgroups differ by the expression of more than 1,000 genes, which makes them as distinct as acute lymphoblastic and acute myelogenous leukaemias. As discussed in detail in this review, these subgroups seem to be derived from different stages of normal B-cell differentiation.

The DLBCL gene-expression subgroups have distinct mechanisms of malignant transformation, which shows that they are pathogenetically distinct diseases. The t(14;18), which involves *BCL2*, is seen exclusively in GCB DLBCLs and is present in ~20% of these cases[29,125]. Similarly, amplification of the *c-REL* locus on chromosome 2p occurs only in GCB DLBCLs[29]. By contrast, activation of the anti-apoptotic nuclear factor-κB (NF-κB) pathway is a feature of ABC DLBCL, but not GCB DLBCL[31].

The molecular distinctions between subgroups of DLBCL are important because the subgroups differ in their ability to be cured by the multi-agent chemotherapy that is used at present[26,29]. Patients with GCB DLBCL have the most favourable cure rate (60% five-year survival), whereas patients with ABC and type 3 DLBCL have five-year survival rates of only 36% and 39%, respectively[29]. These clinical differences might be owing, in part, to the ability of the NF-κB pathway to block many forms of cell death, including that induced by chemotherapy[126].

Further gene-expression differences between DLBCLs can affect the success of chemotherapy[29,127]. The expression of genes that are associated with proliferation predicts poor outcome[29]. By contrast, expression of MHC class II genes by lymphoma cells predicts a favourable outcome, which indicates that DLBCLs might downregulate expression of these genes to evade an immune response[29]. Some patients mount a reactive lymph-node response to DLBCL cells that involves macrophages, natural killer cells and stromal cells, and this innate immune response is associated with survival after chemotherapy[29].

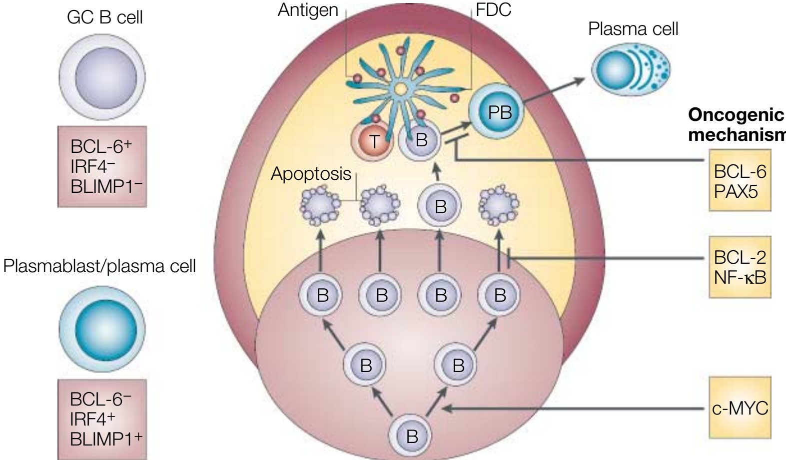


Figure 2 | **Mechanisms of malignant transformation of germinal-centre B cells.** The germinal centre (GC) is a microenvironment in which B cells undergo rapid clonal expansion in the presence of T cells, follicular dendritic cells (FDCs) and antigen. In a process of positive selection, GC B cells mutate their immunoglobulin genes, and those that acquire mutations that maintain or improve the affinity of the B-cell receptor (BCR) for antigen are rescued from programmed cell death and can differentiate further. Most GC B cells express BCL-6 but lack expression of interferon-regulatory factor 4 (IRF4) and B-lymphocyte-induced maturation protein 1 (BLIMP1). Some GC B cells have a plasmablastic (PB) phenotype, have turned off expression of BCL-6 and have turned on expression of IRF4 and BLIMP1. These cells are likely to become plasma cells (PCs). Several oncogenic mechanisms can subvert normal GC B-cell homeostasis in lymphomas. c-MYC, which is not expressed highly by normal GC B cells, is often expressed as a result of translocation by Burkitt lymphoma cells, which promotes clonal expansion. Apoptosis is blocked by oncogenic activation of the nuclear factor-κB (NF-κB) pathway, which occurs in diffuse large B-cell lymphomas (DLBCLs), marginal-zone lymphomas, Hodgkin lymphomas and Epstein–Barr virus-related lymphomas. Apoptosis is also abrogated by translocation, amplification or transcriptional activation of the *BCL2* gene in follicular lymphomas and DLBCLs. Translocation of the *BCL6* gene occurs in various non-Hodgkin lymphomas, which blocks plasmacytic differentiation and promotes the proliferation of GC B cells. Translocation of *PAX5* (paired box gene 5) in lymphoplasmacytic lymphomas is also likely to block plasmacytic differentiation.

promoting its degradation by the proteasome. This leads to the nuclear accumulation of NF-κB and the upregulation of expression of a characteristic set of NF-κB target genes[27,44,45]. In contrast to blood B cells that are stimulated through the BCR or CD40, GC B cells have a low level of expression of many NF-κB target genes[27,29]. The reduced activity of the NF-κB pathway in most GC B cells might contribute to apoptosis and negative selection during the GC reaction. However, CD40 and BCR signalling in some GC B cells (centrocytes) might activate the NF-κB pathway and upregulate expression of BCL-X_L, A1 and/or BCL-2 (REFS 40,42,46), leading to the survival and positive selection of those cells.

GC B-cell-derived lymphomas use several strategies to overcome this propensity for apoptosis. Most follicular lymphomas (~90%) and some DLBCLs have a t(14;18) of *BCL2* to the IgH locus[47,48]. Also, the *BCL2* genomic locus can be amplified in DLBCLs[49], and *BCL2* is transcriptionally upregulated in ABC DLBCLs[31]. In addition, B-cell lymphomas activate the NF-κB pathway by various means. Some DLBCLs have translocations and truncations of the *NFκB2* gene, which encodes a subunit of the dimeric NF-κB factors[50]. In Hodgkin lymphomas (BOX 1), the *IκBα* gene can be inactivated by deletions or point mutations[51,52], which releases NF-κB for translocation to the nucleus. Gastric mucosal-associated lymphoid tissue (MALT) lymphomas frequently acquire a t(11;18) that leads to the overexpression of MALT1 (REF. 53), which is an activator of NF-κB[54,55]. Less commonly, these lymphomas have translocations of the *BCL10* gene[56], which encodes a MALT1-interacting protein[54] that is required for the activation of NF-κB downstream of antigen-receptor signalling[57]. In approximately 50% of Hodgkin lymphomas, the malignant cells are infected with Epstein–Barr virus (EBV) and they express latent membrane protein 1 (LMP1), a viral protein that mimics CD40 signalling and activates NF-κB[58].

Constitutive activation of the IKK complex by unknown mechanisms leads to the nuclear localization of NF-κB in ABC DLBCLs[31] and in some Hodgkin lymphomas[59]. Two cell-line models of ABC DLBCL have constitutive IKK activity, rapid degradation of IκBα and constitutive nuclear localization of NF-κB[31]. One possible explanation for this IKK activity is that ABC DLBCLs are arrested at a stage of B-cell differentiation that involves the upregulation of NF-κB function. Alternatively, unknown oncogenic alterations in ABC DLBCLs might activate the NF-κB pathway. Interference with NF-κB signalling results in the death of ABC DLBCL cells, but it has no effect on GCB DLBCL cells[31]; therefore, drugs that inhibit the NF-κB pathway are attractive candidates for the treatment of patients with ABC DLBCL.

Among the DLBCLs, the *c-REL* gene, which encodes an NF-κB subunit, is amplified and overexpressed exclusively in a subset of GCB DLBCLs[29]. The selective advantage that is conferred by this oncogenic event is unclear, because the GCB DLBCLs that have an amplification of the *c-REL* gene do not have increased expression of NF-κB target genes[29] (A.R. and L.M.S., unpublished observations). One explanation could be that lymphoma cells with amplification of *c-REL* receive enhanced anti-apoptotic and/or proliferative signals when they are triggered through receptors that activate IKK. In this scenario, *c-REL* amplification might have a crucial role in the early stages of the malignant process when the lymphoma cell still receives stimulation from antigen and/or CD40L-bearing activated T cells in the GC. On clinical presentation, these DLBCLs might not be receiving these signals any longer, so that c-REL would remain complexed with IκB in the cytoplasm and be unable to activate NF-κB target genes.

Blocking differentiation. In addition to driving proliferation or preventing cell death, the genetic lesions in some lymphomas seem to arrest differentiation (FIGS 2,3). In the case of GC B cells, developmental arrest at this stage is particularly dangerous as these cells are programmed to divide extremely rapidly. The most common genetic abnormalities in non-Hodgkin lymphomas are translocations and mutations of the *BCL6* gene, which encodes a transcriptional regulator of GC B-cell differentiation and proliferation[60,61]. The *BCL6* gene is the target of promiscuous translocations involving many partner chromosomes, and it might be further dysregulated by SHM of the regulatory regions in

its 5′ end. In all cases, the *BCL6* coding region remains intact and so the lymphomas presumably co-opt the normal function of BCL-6 in the GC. As the expression of BCL-6 is lost during terminal plasmacytic differentiation, it is probable that *BCL6* translocations cause lymphomas by prolonging the expression of BCL-6 beyond its normal developmental limit. To appreciate how this might be oncogenic, it is important to understand the normal function of BCL-6.

BCL-6 is a transcriptional repressor that is expressed at the highest level in GC B cells[62,63]. Mice that are deficient in BCL-6 fail to form GCs during T-cell-dependent immune responses, and they succumb to a fatal inflammatory disease mediated by T helper 2 (T_H2) cells[64–66]. An insight into this complex phenotype was provided by the demonstration that BCL-6 represses genes that are involved in B-cell activation, inflammation and terminal differentiation[67]. One important target of BCL-6 is B-lymphocyte-induced maturation protein 1 (BLIMP1)[67–69], which is a crucial regulator of plasma-cell differentiation[70]. BLIMP1 is a transcriptional repressor that extinguishes the entire mature B-cell gene-expression programme, thereby blocking BCR signalling, CSR, SHM and other mature B-cell functions[71]. BLIMP1 causes this profound change in gene expression by directly repressing the expression of transcription factors that themselves regulate several downstream targets. BLIMP1 also represses *c-MYC*, which contributes to the cell-cycle arrest that is characteristic of plasma cells[71,72]. So, BLIMP1 causes a global change in gene expression, which results in plasma cells that have only minimal resemblance to mature B cells.

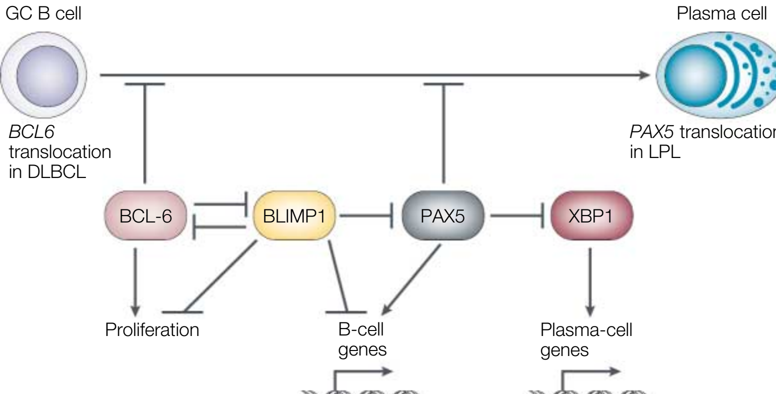


Figure 3 | **The relationship between B-cell differentiation and lymphomagenesis.** The transcription factors BCL-6, BLIMP1 (B-lymphocyte-induced maturation protein 1), PAX5 (paired box gene 5) and XBP1 (X-box protein 1) form a regulatory circuit that controls the progression of germinal-centre (GC) B cells to fully differentiated, immunoglobulin-secreting plasma cells. The GC B-cell repressor, BCL-6, blocks expression of BLIMP1, which is a master regulator of plasma-cell differentiation[67,68]. BCL-6 also promotes proliferation by blocking expression of the cell-cycle inhibitor p27KIP1 (REF. 67). BLIMP1, when expressed, reciprocally inhibits the expression of BCL-6, extinguishes the B-cell gene-expression programme and inhibits the expression of proliferation-inducing genes[72]. BLIMP1 also represses *PAX5*, which induces the expression of B-cell genes and itself represses *XBP1* (REFS 81,83,128,129). XBP1 is a positively acting factor that is required for plasma-cell differentiation[83]. XBP1 probably upregulates the expression of genes that are essential for plasma-cell functions, such as immunoglobulin secretion. This model proposes that translocations of *BCL6* and *PAX5* initiate lymphomas by dysregulating this network, thereby blocking plasmacytic differentiation and promoting proliferation. DLBCL, diffuse large B-cell lymphoma; LPL, lymphoplasmacytic lymphoma.

During normal plasmacytic differentiation, BLIMP1 and BCL-6 are involved in a double-negative regulatory loop (FIG. 3), as the expression of BLIMP1 decreases the expression of BCL-6 (REF. 71). In the GC, most B cells are BCL-6$^+$ and BLIMP1$^-$, but a minority are BCL-6$^-$ and BLIMP1$^+$, and these cells are probably in the process of plasmacytic differentiation and exit from the GC[32]. The BCL-6$^-$ GC B cells also express IRF4, which is a target of NF-κB signalling[33]. This is interesting given that two activators of the NF-κB pathway, BCR and CD40 signalling, cause mRNA levels of BCL-6 to drop markedly[62], and BCR signalling causes BCL-6 protein degradation[73]. These observations might indicate a model in which strong signals through the BCR or CD40 cause the expression of BCL-6 to be reduced, which allows the level of BLIMP1 to rise and plasmacytic differentiation to ensue. In this model, *BCL6* translocations make the *BCL6* gene less responsive to these physiological regulatory influences, thereby blocking the expression of BLIMP1 and plasmacytic differentiation.

In addition to blocking terminal differentiation, BCL-6 might also promote the proliferation of GC B cells. Another gene that is repressed by BCL-6 is *p27KIP1*, which encodes an inhibitor of cyclin-dependent kinases[67]. By inhibiting the expression of p27KIP1, BCL-6 might facilitate the many rounds of cell division that occur during a normal GC reaction. In this regard, it is intriguing that BCL-6 was selected from a genetic screen of fibroblasts for inhibitors of cellular senescence[74]. Senescence is a programmed cellular response that blocks the cell cycle after many rounds of cell division. In fibroblasts, cyclin D1 was found to be required for the inhibition of senescence by BCL-6, but normal GC B cells have low levels of cyclin D1. As p27KIP1 can induce senescence[75], it is possible that the repression of *p27KIP1* could contribute to the ability of BCL-6 to inhibit senescence.

In summary, this model proposes that *BCL6* translocations trap B cells at the GC stage by simultaneously blocking differentiation and promoting unlimited cell division. Secondary oncogenic changes, possibly mediated by errors of SHM or CSR, might then lead to clinically evident lymphomas. DLBCLs, for example, can accumulate mutations in *BCL6*, *c-MYC*, *PIM1*, *PAX5* (paired box gene 5) and other genes as a byproduct of SHM[17]. Therefore, prolonged residence of a B cell at the GC stage of differentiation might allow cells that acquire oncogenic mutations to be selected, leading to malignant progression.

Escape from terminal B-cell differentiation is also a feature of another mature B-cell malignancy, lymphoplasmacytic lymphoma (LPL) (BOX 1), which is a disease of post-GC, immunoglobulin-secreting cells. In 50% of LPL cases, a translocation juxtaposes the IgH and *PAX5* loci[76]. PAX5 is a transcription factor that activates various B-cell-specific genes, and it is required for the commitment of bone-marrow progenitors to the B-cell lineage[77]. PAX5 acts as a master regulator of B-cell identity by blocking differentiation to other haematopoietic lineages[78–80]. PAX5 participates in the complicated regulatory network that guides plasmacytic differentiation by repressing *XBP1* (REF. 81) (FIG. 3), another transcription

factor that is required for plasmacytic differentiation[82]. BLIMP1 represses *PAX5* directly[83], thereby relieving the repression of *XBP1* (REF. 71). The sustained expression of PAX5 in LPLs, owing to chromosomal translocation, is likely to disrupt this regulation, allowing LPL cells to avoid terminal differentiation.

The role of antigen in lymphoid malignancies

Direct and indirect evidence indicates that antigenic stimulation has a role in the pathogenesis of many types of lymphoid malignancy. Despite the frequent occurrence of oncogenic translocations involving the IgH locus, most B-cell malignancies express surface immunoglobulin. Indeed, most IgH translocations involve non-productively rearranged alleles[84], which indicates that, at least early in the history of the malignant clone, the transformed B cell relied on expression of a functional BCR for survival[85]. In most types of lymphoid malignancy, however, it is unclear whether ongoing stimulation of the tumour cells by antigen has a role in the disease.

Direct evidence of a pathogenic role for antigen comes from a study of a type of marginal-zone lymphoma (BOX 1) known as gastric MALT lymphoma[86]. Patients with this type of lymphoma often have gastritis and/or peptic ulcers on presentation, and many of them are infected with *Helicobacter pylori*. Strikingly, 70% of these patients can be cured of their MALT lymphomas by antibiotic treatment targeted at the *H. pylori* infection[87,88]. Even some patients with primary DLBCL of the stomach who are infected with *H. pylori* can be cured by antibiotic treatment[89]. *H. pylori*-specific T cells from these patients can stimulate the proliferation of their MALT lymphoma cells in culture[90]. The BCRs that are expressed by the lymphoma cells have biased usage of certain V_H-family members and they frequently show intraclonal diversification, but it is not clear which antigens they recognize[91,92].

Infection with hepatitis C virus has been associated with lymphoid malignancies in some epidemiological studies[93], but not others[94]. Some patients with a type of marginal-zone lymphoma known as splenic lymphoma with villous lymphocytes are infected with hepatitis C virus, and treatment of this infection with interferon-α (IFN-α) eradicates the lymphoma in most of these patients[95]. IFN-α treatment had no effect on the lymphomas of other patients with this disease who were not infected with hepatitis C virus. Chronic hepatitis C virus infection might, therefore, have a pathogenic role in some lymphomas, and this might involve direct stimulation of the lymphoma by viral antigens[96].

A role for antigen in CLL? Chronic lymphocytic leukaemia (CLL) (BOX 1), the most common type of human leukaemia, involves the clonal expansion of mature B cells that express CD5 and have a low level of expression of surface immunoglobulin[97]. Leukaemic cells from patients with CLL share expression of a characteristic set of genes that distinguishes CLL from other lymphoid malignancies, and so CLL should be viewed as a single disease[98,99]. Nevertheless, two subtypes of CLL were revealed by the analysis of rearranged immunoglobulin V-region sequences from these leukaemias — one subtype has somatically mutated immunoglobulin genes (immunoglobulin-mutated CLL) and the other subtype has immunoglobulin genes that are close or identical to germ-line sequences (immunoglobulin-unmutated CLL)[100–103]. These subtypes can be distinguished easily by gene-expression profiling[98,99]. Patients with immunoglobulin-mutated and -unmutated CLL have markedly different clinical courses: immunoglobulin-unmutated CLL is a progressive disease that often requires early treatment, whereas most immunoglobulin-mutated CLLs are indolent and require late or no treatment[100,102]. In keeping with the generally benign course of immunoglobulin-mutated CLLs, a significant proportion of asymptomatic elderly individuals have clonal populations of B cells that have a CLL surface phenotype and mutated immunoglobulin genes[104].

Several lines of evidence point to a role for antigen in CLL (FIG. 4). CLL B cells use a biased V_H repertoire and have non-random combinations of V, D and J segments that are not characteristic of normal blood B cells[100–102]. Furthermore, certain V_H genes are used differentially by immunoglobulin-unmutated and -mutated forms of CLL. For example, the V_H1-69 gene is associated almost exclusively with immunoglobulin-unmutated CLL, whereas other V_H genes, such as V_H4-34, V_H1-07 and V_H3-21, are over-represented in immunoglobulin-mutated CLL[100–102,105].

Although V_H biases point to a role for antigen in the natural history of CLL, it is unclear still whether continuous antigenic stimulation is required or whether antigen is involved only during early clonal expansion. An ongoing role for antigen is favoured by a recent analysis of a set of immunoglobulin-mutated CLLs with V_H3-21 rearrangements[105]. Unlike patients with other types of immunoglobulin-mutated CLL, these patients have an aggressive disease, with median survival times that are indistinguishable from those of patients with immunoglobulin-unmutated CLL. The complementarity-determining regions 3 (CDR3s) of these V_H segments are restricted in length and sequence and they are associated preferentially in these patients with a single immunoglobulin light-chain V region, Vλ3 (REF. 105). The association of a particular V region with a characteristic clinical behaviour strongly indicates a role for ongoing antigenic stimulation.

The nature of this antigenic stimulus is unknown, but for some CLL B cells it is probably an autoantigen. Most immunoglobulins of CLLs react with self-antigens, such as single- and double-stranded DNA, and IgG, and many are polyreactive[106,107]. Indeed, two of the common CLL V genes, V_H1-69 and V_H3-21, are used frequently to produce rheumatoid factor in other diseases[108,109]. The ubiquitous presence of these self-antigens in the body would allow them to stimulate the malignant clone chronically, even as it accumulates to a large cell number.

In some patients with CLL, the leukaemic cells have features in common with anergic B cells[110]. Anergic B cells have markedly decreased levels of surface IgM[111], which is also characteristic of CLL. The low level of expression of surface IgM by anergic B cells is owing to

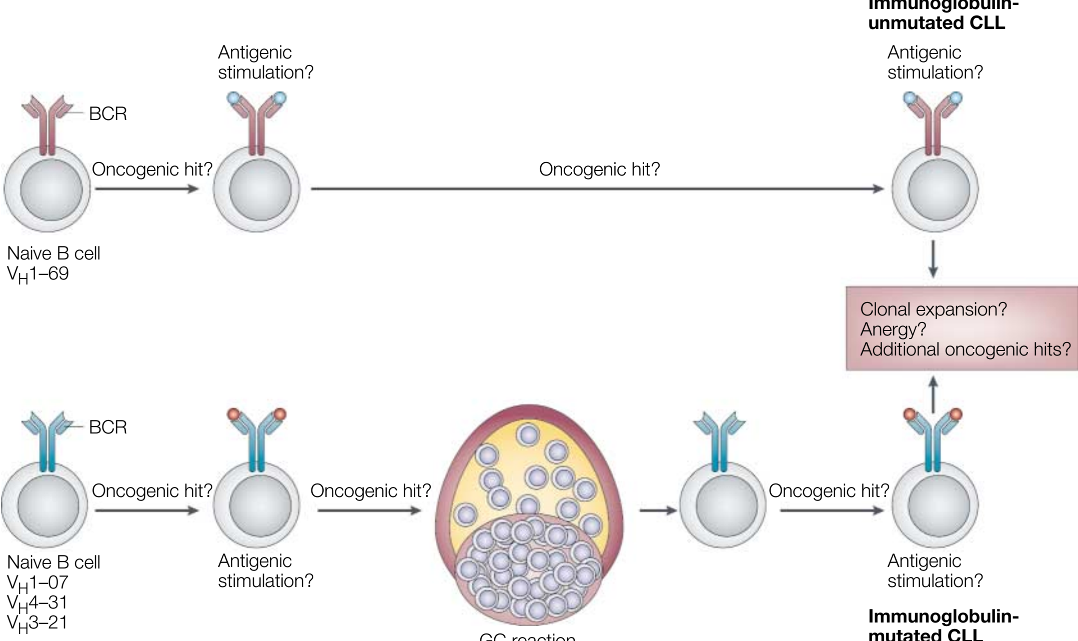


Figure 4 | **Chronic lymphocytic leukaemia: a disease of antigen-experienced B cells.** The two subtypes of chronic lymphocytic leukaemia (CLL) — immunoglobulin-mutated and immunoglobulin-unmutated CLL — are distinguished by the presence or absence of immunoglobulin variable (V)-region mutations, by differences in gene expression and by their clinical courses. This model emphasizes the potential role of antigenic stimulation in the progression of this disease. Immunoglobulin-unmutated and -mutated forms have a different repertoire of heavy-chain V-region (V_H) gene rearrangements, which indicates that the type of CLL that results is dictated by the specificity of the B-cell receptor (BCR). Many studies have implicated an antigen or autoantigen indirectly in the pathogenesis of CLL. Antigenic stimulation might occur before and/or after the B cell acquires a genetic change (oncogenic hit) and becomes a CLL cell. It is also possible that early stages of CLL occur without oncogenic hits to the B cell. Immunoglobulin-unmutated CLL most probably originates from a pre-germinal centre (pre-GC) B cell. Immunoglobulin-mutated CLL might originate from a post-GC B cell. Alternatively, immunoglobulin-mutated CLL might originate from a pre-GC B cell that is nevertheless driven by antigen through a GC reaction. The clinical manifestations of CLL might be related to whether antigen drives continued clonal expansion or induces an anergic state. Disease progression might be influenced by the accumulation of additional oncogenic hits.

a block in transport from the endoplasmic reticulum to the medial golgi[112], and this is a feature of some CLLs[113]. BCR signalling is ineffective in anergic B cells and is characterized by diminished calcium oscillations, as is the case in some CLLs[114–116]. Finally, anergic B cells are characterized by the constitutive nuclear localization of NFATP (pre-existing component of nuclear factor of activated T cells) without nuclear translocation of NF-κB[117,118]. CLL cells can also have constitutive nuclear localization of NFATP[119], and their gene-expression profiles show no evidence of NF-κB activity[99].

FIGURE 4 presents a model of CLL pathogenesis that emphasizes the potential role of antigen. In this model, the biological and clinical behaviours of the leukaemia depend on which immunoglobulin V regions were rearranged in the progenitor of the malignant clone. Immunoglobulin-unmutated CLL is derived presumably from a pre-GC B cell that is nevertheless 'antigen-experienced'. Immunoglobulin-mutated CLL involves presumably a B cell that has participated in a GC reaction, and that might or might not receive ongoing antigenic stimulation. One sixth of all patients with CLL have leukaemic cells at diagnosis that have no clonal chromosomal abnormalities[120]. So, CLL might begin as a defect in normal B-cell homeostasis similar to autoimmunity, without an acquired oncogenic abnormality in the leukaemic cells.

Concluding remarks

It is clearly useful to think like an immunologist when trying to understand lymphoid malignancies. This review has emphasized that many lymphoid malignancies 'inherit' a gene-expression programme and, therefore, part of their biology from a stage of normal B-cell differentiation. However, lymphoid malignancies diverge from their normal counterparts as a result of oncogenic alterations that subvert the homeostatic control of B-cell proliferation, apoptosis and differentiation.

Recent molecular insights into these malignancies provide several potential targets for therapy. Many lymphomas engage the NF-κB pathway — including ABC DLBCLs, gastric MALT lymphomas, EBV-associated lymphomas and Hodgkin lymphomas — which makes it an attractive therapeutic target. Velcade/PS-341 is a proteasome inhibitor that inhibits the NF-κB pathways by stabilizing IκB proteins[121]. In Phase II clinical trials, velcade has been shown to induce remission in patients with multiple myeloma, and it will be an interesting agent to evaluate in patients with lymphoma. BCL-2 is also an attractive therapeutic target given its frequent

overexpression in lymphomas by various mechanisms. Recent studies have indicated that small-molecule inhibitors that target the BCL-2 BH3 domain might be effective as they could allow pro-apoptotic BH3-only proteins to interact with BAX (BCL-2-associated X protein) and/or BAK (BCL-2 antagonist/killer) and initiate apoptosis[122].

Many questions remain. In most lymphomas and leukaemias, we have no idea which signalling pathways drive proliferation. The importance of identifying these pathways is highlighted by the fact that one of the most important factors predicting poor survival after chemotherapy for DLBCL is proliferation[29]. Do oncogenic alterations in the genomes of lymphoma cells drive proliferation? Do B-cell lymphomas depend on T-cell interactions during some phase of their generation and, if so, would inhibition of CD40 signalling be beneficial? Does BCR signalling contribute to the proliferation of malignant lymphocytes, and should this be targeted therapeutically? Lymphomas that occur in the context of chronic stimulation by *H. pylori* or hepatitis C virus provide precedents for these possibilities. Lymphomas might be stimulated also by autoantigens, as might be the case for CLL. In this regard, it is interesting that patients with rheumatoid arthritis, and possibly other autoimmune diseases, have an increased risk of lymphoma in cohort studies[123]. It is conceivable, therefore, that polymorphic alleles of key immunoregulatory genes could be associated with an increased risk of both autoimmune disease and lymphoid malignancies. Although many of these considerations are speculative, recent progress has provided hope that further insights into the pathogenesis of lymphoid malignancies will come from our knowledge of normal immune homeostasis.

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