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TITLE: Molecular control of B-cell homeostasis in health and malignancy

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ABSTRACT

Altered B-cell homeostasis underlies a wide range of pathologies, from cancers, to autoimmunity, and immunodeficiency. The molecular safeguards against those disorders, which also allow effective immune responses, are therefore particularly critical. Here, we review recent findings detailing the fine control of B-cell homeostasis, during B-cell development, maturation in the periphery and, during activation and differentiation into antibody-producing cells.

INTRODUCTION

Homeostasis is defined as the physiological processes that maintain the equilibrium in our body. The immune system is where homeostasis is best exemplified. Indeed, in health, the size of the lymphocyte pool is remarkably constant. Lymphocyte numbers may augment transiently in response to an infection or activation, but always revert back to a standardised and species-specific set number. This clockwork control of immune cell numbers is made possible through the contribution of many genes and factors, acting with high precision at various stages of lymphocyte development. Equilibrium between production and elimination of lymphocytes is the central element ensuring homeostasis. Changes disturbing this equilibrium may result in autoimmunity or malignancy. Similarly, a defect in lymphocyte development or excessive elimination due to survival defects, may result in immunodeficiency. Here, we will particularly focus on the factors controlling B-cell homeostasis, specifically reviewing recent advances in our understanding of fine mechanisms controlling B-cell numbers and B-cell activation, and how defects in these controls lead to a loss in B-cell homeostasis.

1. Homeostasis of B cells during development and maturation.

B-cell lymphopoiesis is a tightly controlled process which involves the cooperation of diverse molecular components, all required to successfully shape the expression of a functional B-cell receptor (BCR) and its precursor (the pre-BCR). During development and maturation, B cells undergo a series of rigorous positive and negative selection to eliminate self-reactivity. This stringent selection helps generate a diverse naïve mature B-cell repertoire supporting robust host immune defences. This phase of B-cell life is also controlled by selective homeostatic factors.

The early developmental control

In the bone marrow (BM), the progenitor B (pro-B) cells are the earliest cells committed to the B-cell linage. Proliferation of pro-B cells relies on signals transmitted through the interleukin-7 receptor (IL-7R),[1] composed of the common-γ-chain (γc) and the IL7Rα chain. In the BM, IL-7R signals are critical, as deficiency in IL-7Rα prevents B-lymphopoiesis (Fig. 1).[2] Pro-B cells rearrange the heavy chain (HC) locus through the expression of the recombination-activating genes 1 and 2 (*RAG1* and *RAG2*). A productively rearranged HC (μ-chain) associates with the surrogate light chain (LC) Vpre-B and λ5, leading to the expression of the pre-BCR, the signal of which triggers the proliferation and the differentiation of large pre-B cells.[3, 4] Pre-BCR signals drive a proliferative wave followed by cell-cycle arrest, and transition into small-pre-B cells (Fig. 1). At this stage, in-frame rearrangement of the LC leads to the formation of the surface immunoglobulin M (IgM) BCR on immature B cells (Fig. 1). Signalling through the final BCR product further regulates B-cell development by favouring the expression of a safe single HC-LC pair per B cell, away from self-reactivity (Fig. 1). Newly generated BCRs are tested in the BM, productive rearrangements able to transmit a tonic signal of intermediate strength are positively selected. In contrast, excessive signalling to self-antigens leads to negative selection by deletion, receptor editing or anergy of the autoreactive B cells.[5] To secure the formation of a safe repertoire, B cells undergo a series of positive and negative selection mechanisms where 'intermediate' levels of BCR signals are required to properly balance B-cell survival (Fig. 1).[5] Immature B cells leave the BM and complete their development in the periphery where they differentiate into Follicular (FO) and Marginal zone (MZ) B cells.

The program engaged by BCR ligation of mature B cells

Although the strength of BCR ligation to self-antigens is central for B-cell tolerance, it is now evident that signals through the receptor for B-cell Activating Factor from the tumour necrosis factor (TNF) superfamily (BAFF), BAFF-R (also known as TNFRSF13C), Toll-like receptors (TLRs), and CD40 collaborate with BCR signals to establish a healthy B-cell repertoire.[6] Indeed, expression of both BCR and BAFF-R is required to maintain B-cell survival in the periphery (Fig. 1). Interestingly, BAFF-R is expressed at higher levels on non-autoreactive B cells and plays a role in positive selection and differentiation of non-autoreactive immature B cells, following tonic BCR signalling.[7] Through the activation of the Src family kinase (Src), BAFF-R signals induce the phosphorylation of the spleen tyrosine kinase (Syk) and Ig-α, both being proximal components of the BCR signalosome. Although it is established that BAFF-R/BCR cross-talk leads to B-cell survival,[8] the mechanism behind BAFF-dependent Ig-α phosphorylation remains to be determined.

Likewise, BAFF induces CD19 phosphorylation, which supports phosphoinositide 3-kinase (PI3K)-dependent B-cell survival.[9] The PI3K pathway is essential for B-cell development and is involved in both positive and negative B-cell selection. Indeed, altered PI3K function leads to either autoimmunity or immunodeficiency. PI3K also mediates the activation of Akt, which phosphorylates the transcriptional factor forkhead box protein O1 (FOXO1), thereby allowing its exclusion from the nucleus.[10] FOXO1 controls the expression of genes implicated in cell cycle, apoptosis, oxidative stress and DNA damage repair.[11]

Arginine methylation of FOXO1 by the Protein Arginine Methyltransferase 1 (PRMT1) prevents its phosphorylation and induces its accumulation in the nuclei.[10] PRMT1 activity does not only regulate FOXO1 but also controls BCR signalling and supports B-cell survival, proliferation and differentiation by methylating the signalling subunit Ig-α.[12, 13] While PRMT1 is ubiquitously expressed in all B-cell subsets, the methylation status of Ig-α differs between BCR and pre-BCR, a difference key for B lymphopoiesis.[13] Indeed, methylation of Ig-α, defined as a negative mark, is absent in the constitutively active pre-BCR.[13] The PRMT1 and B-cell translocation gene 2 (BTG2) module constrain pre-B-cell expansion by methylating the cycling-dependent kinase 4 (CDK4). In addition, deficiency in PRMT1 induces a partial block in pre-B-cell differentiation.[12] ~~During maturation in the periphery, immature B-cells undergo a series of rigorous positive and negative selection to eliminate self-reactivity. This stringent selection helps generate a diverse naïve mature B-cell repertoire supporting robust host immune defences. This phase of B-cell life is also controlled by selective homeostatic factors.~~

Following antigen recognition, B cells differentiate into antibody secreting cells (ASC) which are comprised of short-lived plasmablasts, and long-lived plasma cells. Short-lived plasmablasts are generated in rapid bursts from activated MZ B cells in response to TLR activation. However, resulting plasma cells produce low affinity antibodies.[14] In contrast, FO B cells, activated in a T-dependent manner, proliferate extensively and expand into germinal centre (GC) B cells with help from specialised T follicular helper (T_{FH}) cells. Differentiation into GC B cell is mediated by activation of the BCR and CD40 co-stimulation (Fig. 1), which induces translation of the transcription factor (TF) B-cell lymphoma 6 (BCL6). BCL6 is pivotal for the formation of GC due to its role as repressor of p53 signalling, which prevents class switch recombination (CSR) and somatic hypermutation (SHM)-induced DNA damage.[15] Furthermore, IL-21 signalling is critical for GC B-cell responses, isotype switching and the generation of memory B cells.[16] T-bet and RORα are

TFs associated with IgG2a/2c and IgA isotype class-switching, respectively, and are vital for the formation and maintenance of some memory B-cell subsets.[17]

Activated GC B cells differentiate into high affinity, long-lived plasma cells and memory B cells. Plasmablasts are cycling and are expanded early during an immune response, whereas plasma cells are post-mitotic. Transcriptional differences between these two subsets and the core B-cell program have now been defined from whole transcriptome sequencing.[18] B-cell maturation antigen (BCMA, also known as TNFRSF17) is expressed on plasma cells and is required for their survival (Fig. 1), as demonstrated in $BCMA^{-/-}$ mice, lacking subsets of long-lived plasma cells.[19] BCMA signalling strongly up-regulates the expression of the anti-apoptotic factor myeloid cell leukemia-1 (Mcl-1), in plasma cells from the BM.[20] Induced genetic deletion of Mcl-1 in adult mice, caused a rapid depletion of plasma cells, indicating that Mcl-1 is essential for maintaining plasma cell survival. The requirement for Mcl-1 in plasma cell survival precedes that of TF B-lymphocyte-induced maturation protein 1 (Blimp-1).[20]

Subsequent plasma cell development hinges on the expression of Blimp-1, which induces cell cycle arrest and commits B lymphocytes to a plasma cell fate by inhibiting genes involved in cellular proliferation, including BCL6 and Myc.[21] Blimp-1 also suppresses genes encoding several TFs such as PAX-5, Spi-B and the inhibitor of DNA binding-3 (Id3), driving terminal differentiation into plasma cells, while allowing expression of key plasma cell genes such as X-box binding protein 1 (XBP1).[21] In addition, plasma cell migration and maintenance in the BM is dependent on the C-X-C chemokine receptor type 4 (CXCR4) (Fig. 1).[22]

BCR crosslinking or CD40 stimulation protect B lymphocytes from Fas-induced apoptosis (Fig. 1). However, reduction in the expression of CD40L on T-helper cells, following resolution of the immune response, lifts the inhibition on Fas signalling in B cells.[23] Indeed, Fas expression, which is induced on activated B cells, is then activated by FasL expressed on T-helper cells, which leads to B-cell apoptosis. This process is referred to as activation-induced cell death (AICD).

Together, past and recent findings illuminate the complexity of B-cell development and maturation, orchestrated by finely tuned transcriptional and post-transcriptional events. Each individual event is key and any alteration of steps within this process has consequences, driving either immunodeficiency, autoimmunity or cancer as detailed below.

2. Loss of B-cell homeostasis: causes and consequences

Primary Immunodeficiency

Several genetic defects in genes controlling B-cell homeostasis are associated with B-cell deficiency or loss of B-cell function. Some cause severe symptoms and are easily diagnosed in infants, while others only become apparent during adulthood.[24] The most severe defects occur early and impair T- and/or B-cell development, whereas the less severe defects normally affect B-cell maturation/activation.[24] Here, we provide a comprehensive list of gene mutations in primary immunodeficiencies, linked to defect in B-cell homeostasis.

Mutations in genes essential for T and B-cell development, such as those necessary for VDJ recombination (e.g. *RAG1, RAG2* and *ARTEMIS),* or encoding enzymes essential for functional purine metabolism (*ADA)* cause T^-B^- Severe Combined Immunodeficiency (SCID).[25] Patients are diagnosed early in life with markedly decreased T and B-cell numbers, which leads to impaired cellular and humoral immunity. Genetic defects exclusively affecting the T-cell compartment (e.g. *CD3D, CD3E* and *IL-2RG*) lead to B-cell dysfunction and defective humoral immunity due to impaired T-cell help.[25] These defects are characterised by absent circulating T cells and normal B-cell numbers (T^-B^+ SCID), together with decreased serum immunoglobulin levels. Infants with SCID present with opportunistic infections and failure to thrive, and require prophylactic treatment and intravenous immunoglobulin. However, SCID is incurable and life-threatening. Therefore, the preferred treatment is hematopoietic stem cell transplantation or gene therapy to correct the underlying genetic defect.[24]

As discussed above, signalling through the BCR is critical for the survival of developing B cells. Accordingly, mutations in components of the pre-BCR or BCR, or in molecules downstream of the BCR, lead to defects in B-cell development and typically result in primary antibody deficiency with absence of circulating B cells. The most common of these conditions is X-linked agammaglobulinaemia (XLA) which is caused by mutations in the Bruton's tyrosine kinase (*BTK*), a cytoplasmic kinase of the BCR signalling pathway. XLA patients suffer from severe bacterial infections.[26]

Other genetic defects may cause less profound immunodeficiency. For example, mutations in *CD19, CD20, CD21* or *CD81,* which amplify signalling through the BCR and cause primary antibody deficiency with normal B-cell numbers. Mutations in genes encoding for proteins important for T-dependent B-cell responses cause Hyper IgM Syndrome (HIGM). This syndrome is characterised by defects in immunoglobulin CSR and leads to decreased serum levels of IgG, IgA and IgE, but high levels of IgM together with normal circulating B-cell numbers. The most common genetic defect associated with this syndrome

is mutations in the gene encoding for CD40L (*CD40LG*), which is expressed on activated T cells.[27] Other less common mutations include, among others, those in genes coding for activation-induced cytidine deaminase (AID) and uracil-DNA glycosylase (UNG).[28]

Dysregulation on some TFs may also impair B-cell homeostasis. For example, dominant mutations in the gene encoding for signal transducer and activator of transcription 3 (STAT3) result in Hyper-IgE syndrome. Patients present with severe eczema, recurrent candidiasis, *Staphylococcal* abscesses, poor antibody responses, and elevated serum IgE, together with other non-immunological features such as hyper extensible joints and osteoporosis.[29] STAT3 is an important TF downstream of many cytokine receptors, which signalling pathways regulate T-cell and B-cell function. For many years, it has been unclear whether elevated serum IgE levels were caused by an intrinsic B-cell defect or by defective T-cell help. Recently, Kane *et al.* have demonstrated, using a B-cell specific *Stat3*-deficient mouse model, that the observed elevated IgE is the result of aberrant isotype class-switching to IgE, caused by B cell-intrinsic STAT3 signalling, and therefore, suggesting that STAT3 is a negative regulator of IgE class-switching.[30]

Lastly, the commonest symptomatic primary immunodeficiency, characterised by recurrent infections, poor responses to vaccines and late onset, is a heterogeneous group termed Common Variable Immunodeficiency Disorders (CVID). It is caused by defective B-cell function, which leads to reduced isotype-switched B cells and depletion of plasma cells. The defect can be B cell-intrinsic or -extrinsic, due to insufficient T-cell help, or attributable to monocyte/dendritic cell defects or loss of NK cells. In most cases, the genetic cause of CVID is unknown.[31] Aside from immunodeficiency, patients suffering from CVID show increased risk of autoimmunity, malignancy and systemic granulomatous disease. Mutations/polymorphisms in the gene encoding for transmembrane activator and cyclophilin ligand interactor (TACI, also named TNFRSF13B) have been associated with CVID.[32] However, they are considered not disease-causing mutations on their own, but rather modifying mutations, as the same defects can be found in healthy individuals, probably indicating that CVID may be caused by a combination of genetic (polygenic) and environmental factors.[31] In summary, numerous genetic defects affect B-cell development and/or function and cause primary immunodeficiency. However rare, the discovery of the causative gene mutations, has shed light on the function of the proteins encoded by these genes.

Autoimmunity

Another example of loss of homeostatic control of B-cell function is autoimmunity. Prolonged disturbance of B-cell homeostasis is often causing loss of tolerance to self-tissues, with the emergence of self-reactive B cells, contributing to autoimmune pathologies. These pathologies can involve aberrant B cells excessively differentiating into plasma cells and secreting pro-inflammatory autoantibodies or cytokines, or B cells acting as antigen-presenting cells to autoreactive T cells. This section will discuss disrupted B-cell homeostasis in the context of systemic autoimmune rheumatic diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), as well as non-systemic autoimmune diseases such as multiple sclerosis (MS).

Dysregulation of B-cell survival factors supporting B-cell mediated diseases

Excessive activation of B cells in an autoimmune disease state is often driven by excessive production of survival factors, which would normally be available in limiting amounts. A well-established example of this is overexpression of the B-cell survival factor BAFF, as detected in the serum of a subset of patients with SLE, RA or Sjögren's syndrome.[33]

Experimentally, mice overexpressing BAFF develop an autoimmune condition associated with the expansion of the B cell compartment and resembling SLE.[34] This discovery was followed by an intensive race for the development of BAFF inhibitors, which resulted in several candidate therapies tested in the clinic, with one, belimumab, approved for use in the clinic (Table 1). Belimumab was approved as a therapy for a subset of seropositive patients with SLE, validating BAFF as an important therapeutic target in autoimmunity. Interestingly, it has been assumed that excessive self-reactive B-cell survival was the underlying cause for B-cell dysregulation in SLE. However, recent work showed that deletion of TACI, which does not support B-cell survival, was protective against excess BAFF-driven SLE in mice,[35, 36] and TACI deletion was also protective in the MRL-Fas/lpr model.[37] As such, TACI is emerging as a novel therapeutic target in SLE, and these results suggest that dysregulation of innate immunity, rather than a catastrophic breakdown in B-cell tolerance, is driving excessive pro-inflammatory autoantibody production, responsible for disease symptoms. Indeed, past work has demonstrated that alteration of B-cell tolerance is limited when BAFF is overexpressed,[38] supporting an alternative mechanism via TACI, as the underlying cause of SLE in mice.

Protein kinase C-associated kinase (PKK, also known as RIP4) is another factor, which supports the survival of B1 B cells, mature recirculating B2 conventional B cells, and B lymphoma cells. Oleksyn *et al.*[45] made a B cell-specific PKK knockout of the SLE-prone

congenic mouse strain B6.*Sle1*.*Sle3*, and showed that PKK deficiency protected mice against splenomegaly, autoantibody production, and kidney nephritis. PKK-deficient mice were also protected from an expansion of the plasma cell compartment and GCs. PKK is therefore critical for disease progression in this model, in which disease is underpinned by excessive B-cell survival and activation. PKK phosphorylates inhibitor of kappa B kinase (IKK)α and IKKβ, thereby supporting nuclear factor κB (NF-κB) signalling, such as what is observed downstream from BAFF signalling.[46]

MS is a progressive autoimmune disease in the central nervous system (CNS) mediated by autoreactive T cells, with $CD4^+$ T cells driving CNS inflammation and $CD8^+$ T cells directly damaging axons. There is also evidence of humoral autoimmunity and B-cell involvement, both in pathogenesis,[39, 43, 47] and in protection via regulatory B cells (B_{regs}).[48] Clinical trials testing B-cell depleting therapy with anti-CD20 monoclonal antibodies have shown a significant improvement of lesions in MS patients.[49] Another clinical trial with atacicept (TACI-Fc fusion protein) which partially reduces B-cell numbers by neutralizing the B-cell survival factors BAFF and a proliferation-inducing ligand (APRIL, also known as TNFSF13), was conducted with the rationale that excess BAFF production from astrocytes in the CNS supports survival of pathogenic B cells recruited into MS lesions. However, atacicept led to worsening MS symptoms and lesions, and the trial was halted.[50] These surprising trial outcomes highlighted the complexity of the role of B cells in MS. As mentioned above B cells can be active pathogenic players in MS, however, recent work has demonstrated that B_{regs} produce immuno-suppressing factors such as IL-10 and IL-35 which have a protective role in MS.[51, 52] Recent results for atacicept were more promising with SLE in a phase IIb trial showing reduced flare activity and more acceptable safety profile in patients compared to patients with MS.[44]

Impaired B-cell homeostasis via interactions with other immune cells

B cells can contribute to autoimmune indirectly or cooperatively via cell-cell interactions. In the RA setting, a combination of mass cytometry by time-of-flight (CyTOF) and transcriptomics approaches was used to characterise a pathogenically expanded population of PD-1^{hi} $CXCR5^-$ peripheral helper T (T_{PH}) cells in the synovium. T_{PH} cells express factors promoting B-cell help, including: IL-21, C-X-C ligand 13 (CXCL13), inducible T-cell costimulatory (ICOS), and MAF (a transcription factor that promotes IL21 production). By providing these factors, T_{PH} cells represent a specialised cell population promoting B-cell responses in inflamed non-lymphoid tissues.[53] An excessive amount of T_{PH} cells would thereby drive additional B cells to contribute to auto-inflammatory pathologies.

T_{PH} cells were transcriptionally distinct from T_{FH} cells, which may be analogous cells but residing within lymphoid tissues in GC.

Particularly in SLE, plasmacytoid dendritic cells (pDC) are implicated as major producers of type-I interferon. *In vitro* co-culture experiments suggest interactions between pDC and B cells isolated from patients with SLE, and, in the presence of RNA-containing immune complexes, presence of pDC expanded the proportions of $CD27^{-}IgD^{-}$ B cells.[54] B cells with these surface marker were previously noted to be substantially elevated in circulation in SLE patients.[55] Increased expression of IL-21R, ICOS ligand (ICOSL), IL4R, and signalling lymphocytic activation molecule family 1 (SLAMF1) on the $CD27^{-}IgD^{-}$ B cells was suggested to increase sensitivity to costimulatory signals, such as those from T_{FH} cells.[54]

OX40L is a TNF superfamily ligand expressed on B cells and T cells. *TNFSF4* (the gene encoding OX40L) is a risk-associated gene for SLE.[56] Cortini *et al.*[57] recently reported that OX40L expression on B cells supports T_{FH} cell development, contributing to SLE pathogenesis; B-cell-conditional $OX40L^{fl/fl}$CD19-cre mice crossed to the B6.*Sle16* lupus-prone mouse model were protected from SLE, with protection likely involving a major reduction in T_{FH} cells.

Cancer (B-cell lymphomas/leukaemias)

As mentioned above, B-cell numbers are tightly controlled at each stage of their development and differentiation. Indeed, a breakdown in B-cell homeostasis can lead to an uncontrolled accumulation of B cells, which may result in cancer. B-cell malignancies are the most common lymphoid cancer and originate as the result of genetic and epigenetic changes in B-cell genes that lead to excessive proliferation and/or survival or insufficient apoptosis.

Oncogenic transformation can occur during B-cell differentiation, for example Acute Lymphoblastic Leukaemia (B-ALL) arises from pre-B cells, while B Chronic Lymphocytic Leukaemia (B-CLL), lymphomas and Multiple Myeloma (MM) originate from mature and terminally differentiated B cells,[58] respectively (see Table 2 for a summarised list of B-cell malignancies).

~~B-cells intrinsically possess a powerful proliferative capacity to respond effectively to pathogens, which may illustrate why B-cell malignancies are the commonest type of lymphoid cancer. In addition~~ During GC reaction, activated B cells undergo affinity maturation via hypermutation of their Ig genes. However, when this process is misdirected, it

can lead to chromosomal translocations or target non-Ig genes critical for B-cell control. As a result, a number of B-cell malignancies derive from GC B cells.

In recent years and with the advancement and affordability of high-throughput sequencing technologies, the genetic defects underlying B-cell malignancies are being identified. Genetic abnormalities commonly associate with oncogene activation (e.g., *MYC*, *NOTCH1*, *BCL2*) or tumour suppressor gene repression (*TP53*, *ATM*). Chromosomal aberrations are common, probably as a result of aberrant SHM, for example virtually all Burkitt's lymphoma (BL) cases show c-Myc overexpression as a result of a chromosomal translocation of the *MYC* oncogene into one of the immunoglobulin loci.[59] Chromosomal deletions are also frequently found in B-cell malignancies, for example, around 60% of B-CLL patients show deletions at chromosome 13q,[60] this defect correlates with overexpression of BCL2, an important oncogene that inhibits apoptosis. Importantly, this observation led to the development of BCL2 inhibitors,[61] ABT-199 (Venetoclax), which was approved to treat B-CLL in the United States in 2016 and in Australia and the European Union in 2017, and is also showing promising results in other B-cell malignancies. The commonly observed 13q deletion in B-CLL patients is the first example of changes in microRNAs (miRNAs or miRs) expression profiles in B-cell malignancies. miRNAs are non-coding RNAs involved in post-transcriptionally regulating gene expression. miR-15/16 is lost in patients with 13q deletion, it acts as tumour suppressor as it negatively regulates BCL2 expression, hence its deletion leads to BCL2 overexpression.[60] Since the discovery of miR15/16 in B-CLL, many other miRNAs relevant for B-cell malignancies have been identified. Overexpression of miRNAs targeting oncogenes (miR-21 in ABC-DLBCL and B-CLL; miR-155 in MCL, DLBCL, FL and B-CLL; miR-17–92 cluster in DLBCL and B-CLL) or loss of those targeting tumour suppressor genes (miR-34a in B-CLL, DLBCL and MCL; miR-150 in DLBCL, MCL, B-CLL and BL) have been described.[62]

In addition to changes in oncogenes or tumour suppressor genes, alterations in several signalling pathways may also result in the development of B-cell malignancies. For example, transient activation of various types of NF-κB is needed to control the survival, proliferation, activation and differentiation of normal B cells. By contrast, enhanced activity of the NF-κB signalling pathway causes uncontrolled proliferation and survival.[63] Enhanced NF-κB activation is a hallmark of ABC-DLBCL, HL and PMBL-DLBCL, and inhibition of the NF-κB pathway *in vitro* promotes apoptosis in cell lines derived from these malignancies. Mutations in genes encoding NF-κB proteins, gain of function mutations in positive

regulators, and loss of function mutations in negative regulators of this signalling pathway have been identified in a small proportion of cancer patients.[64] Other mechanisms contributing to the enhanced activation of this pathway may be signalling through NF-κB activator surface receptors (BCR, BAFF receptors, TLRs, CD40, CD30, IL-6) and may be responsible for the NF-κB signature observed in B-CLL and MM.[65, 66] Lastly, lymphoma-associated viruses such as Epstein-Barr virus (EBV) can induce constitutive NF-κB signalling. The principal EBV viral oncoprotein, Latent Membrane Protein 1 (LMP1) mimics CD40 signalling, thereby activating the NF-κB signalling pathway. Remarkably, LMP1 is frequently expressed in a variety of B-cell lymphomas.[67]

Normal B cells require tonic BCR signalling for their survival. Likewise, activation of this pathway has been identified as a main contributor to the pathogenesis of several B-cell malignancies.[68] In B-CLL, a third of patients express stereotyped BCRs, meaning near identical immunoglobulin variable regions, hence suggesting that antigen driven activation of the BCR has an important role in pathogenesis. The cognate antigen of some B-CLL BCRs have been identified, unmutated-*IGHV* show polyreactivity to several auto-antigens,[69] some stereotyped BCRs have been shown to react to bacterial and fungal antigens,[70] whereas others recognise and internal epitope in the BCR itself and therefore are able of autonomous antigen independent signalling.[71] Pathogen derived antigens can also engage the BCR of some malignant B cells, for example, antigen from hepatitis C virus (HCV) has been shown to drive SMZL and antiviral therapy in HCV infected patients correlates with complete remission.[72] Similarly, MALT lymphomas very frequently correlate with *Helicobacter pylori* infection, eradication with antibiotic treatment has proven efficacious, leading to a complete remission for 60-80% of patients. However, a direct correlation with antigen driven lymphoma expansion has not been proven yet, and the association may be due to the inflammatory milieu created by the infection.[73]

In addition to antigenic activation, the BCR signalling pathway can be intrinsically activated by mutations in *CD79B* or *CARD11*, as observed in some ABC-DLBCL cases. All this evidence highlights the importance of BCR signalling in the pathology of B-cell malignancies and has led to the development of novel therapies targeting key kinases in this pathway (BTK, SYK and PI3K inhibitors), which are proving to be remarkably efficacious, irrespective of markers of poor prognosis,[68] a major advance in the treatment of these cancers.

3. Concluding Remarks

Precise control of B-cell numbers is achieved by a multitude of molecular mechanisms throughout the development, maturation, and activation of B cells. Genes alone are only one aspect of these controls. Indeed, epigenetic, post-translational protein modifications, regulated cytokine production, interaction with other immune cells and the anatomical localisation of B cells, are other factors contributing to the regulation of B-cell homeostasis. As such, there are many elements of importance which if defective alter the B-cell compartment and/or its function and regulation. The study of human primary immunodeficiencies clearly demonstrated the specific importance of B cells in some infections. In addition, the use of therapies targeting B cells in the clinic have shed light on their central pathogenic role, in a number of autoimmune diseases. However, current therapies targeting B cells remain very broad and unspecific. New knowledge and advanced technologies, such as single cell analysis, may in the future better identify subsets of human B cells contributing to diseases, and unique markers of these cells may allow for the development of more targeted and safer therapies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1: An update on selected B-cell targeted treatments for autoimmune diseases (SLE, RA, and MS). Abbreviations: APRIL, a proliferation inducing ligand; BAFF, B-cell activating factor of the TNF family; FDA, federal drug administration (USA); mAb, monoclonal antibody; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Biologic	Trial status and mechanistic commentary
Rituximab (anti-CD20)	Potent depletion of B-cells. Phase III trials failed to show efficacy for lupus nephritis, although anecdotal case studies report efficacy in some patients. FDA approved for RA in 2006, subsequent trials with anti-CD20 and anti-BAFF agents below did not show an advantage over rituximab.
Ocrelizumab (anti-CD20)	Targets a different CD20 epitope to rituximab, but also depletes B-cells. Phase III (MS): reduced lesions and reduced disease progression compared to placebo;[39] FDA approved for progressive MS in March 2017. Phase III (SLE): failed to meet primary endpoint for lupus nephritis

	Phase III (RA): terminated
Ofatumumab (anti-CD20)	As above, depletes B-cells. Development for RA was discontinued since no advantage was seen over rituximab. Phase II (MS) demonstrated reduced formation of new brain lesions.[40] Phase III (MS) recruiting.
Belimumab (anti-BAFF)	Partial depletion of B-cells by loss of BAFF:BAFF-R pro-survival signalling (not required for memory, or plasma cells). Mechanism of therapy in SLE may actually be via reducing TACI signalling rather than B-cell depletion, based on studies showing absence of TACI expression completely protects BAFF-Tg mice from disease.[35, 36] Statistically significant but modest efficacy may stem from not blocking signalling through TACI by APRIL. FDA approved for seropositive lupus in March 2011.
Tabalumab (anti-BAFF)	Phase III (SLE, RA); only modest efficacy in SLE[41] – discontinued development. Phase II (MS)
Blisibimod (BAFF-binding peptibody)	Phase III (SLE: "CHABLIS-SC1") – lack of efficacy. Phase III (SLE: "CHABLIS-7.5") – stopped based on results above. The tetravalent peptibody modality offers potentially better anti-BAFF potency, compared to the bivalent mAbs. A potential drawback may be increased potential for immunogenicity against the peptibody preventing long-term use.[42] Further development is not expected to go ahead for SLE, although the FDA granted orphan drug status for the treatment of IgA nephropathy.
Atacicept (TACI-Ig)	Blockade of BAFF and APRIL, preventing signalling through three receptors (BAFF-R, TACI, BCMA), expected to cause a loss of B-cell survival. Phase II trials in MS were stopped due to safety issues and increased disease activity.[43] Phase IIb (SLE: "ADDRESS II") showed evidence of efficacy, mainly in high-disease-activity and seropositive patients, and reduced flare activity.[44]

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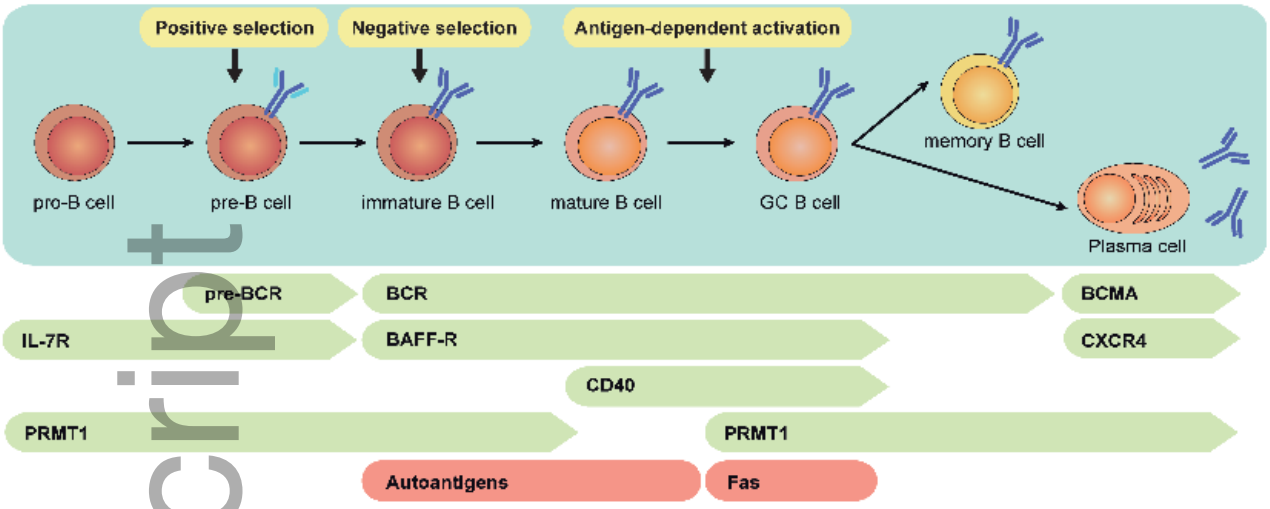
693 **Table 2. Overview of B-cell cancers and their corresponding B-cell subtypes.**

B-cell malignancy	**Closely related normal B-cell**
Acute Lymphoblastic Leukaemia (B-ALL)	pre-B-cell
Hodgkin Lymphoma (HL)	GC-B-cell
Non-Hodgkin lymphoma (NHL)	
Chronic Lymphocytic Leukaemia (B-CLL)	
IGHV-unmutated	Mature pre-GC B-cell
IGHV-mutated	Mature post-GC B-cell

Diffuse Large B-cell lymphomas (DLBCL)	
Germinal Centre B-cell-like phenotype (GCB-DLBCL)	GC-B-cell
Activated B-cell like (ABC-DLBCL)	Plasmablast
Primary Mediastinal B-cell Lymphoma (PMBL-DLBCL)	medullary thymic B-cell
Follicular Lymphoma (FL)	GC-B-cell
Mantle Cell Lymphoma (MCL)	Mature pre-GC B-cell
Burkitt's Lymphoma (BL)	GC-B-cell
Hairy Cell Leukaemia (HCL)	post-GC memory B-cell
Marginal Zone Lymphomas (MZL)	
Splenic marginal zone lymphoma (SMZL)	MZ B-cell
Nodal Marginal Zone Lymphoma (NMZL))	MZ B-cell
Mucosa-Associated Lymphoid Tissue (MALT) lymphoma	MZ B-cell
Multiple Myeloma (MM)	Plasma cell

FIGURE LEGEND

Figure 1: Key homeostatic control mechanisms. In the bone marrow, progenitor B (pro-B) and precursor B (pre-B) cells require signalling through the IL-7R. Constitutive pre-BCR and BCR signalling support survival and development. In the periphery, BAFF-R and tonic BCR signalling are essential for survival. Autoreactive B cells are removed or rendered anergic, at multiple checkpoints throughout development in the bone marrow and in the periphery. Antigen activation through the BCR and CD40 co-stimulation drive Germinal Centre (GC) formation, where GC B cells undergo somatic hypermutation to select for B cells with optimal antigen affinity. GC B cells with suboptimal affinity are deleted via Fas-mediated apoptosis. PRMT1 activity is essential for B-cell development and it is required for the generation of immune responses. Plasma cell survival is supported by APRIL signalling through BCMA and CXCL12 through CXCR4.



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