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New insights in the regulation of human B cell differentiation

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Abstract

B lymphocytes provide the cellular basis of the humoral immune response. All stages of this process, from B cell activation to formation of germinal centers and differentiation into memory B cells or plasma cells, are influenced by extrinsic signals and controlled by transcriptional regulation. Compared to naïve B cells, memory B cells display a distinct expression profile, which allows for their rapid secondary responses. Indisputably, many B cell malignancies result from aberrations in the circuitry controlling B cell function, particularly during the GC reaction. Here we review new insights into memory B cell subtypes, recent literature on transcription factors regulating human B cell differentiation, and further evidence for B cell lymphomagenesis emanating from errors during the GC cell reactions.

Introduction

B cell differentiation into antibody (Ab)-secreting cells (ASCs) forms the basis of the humoral adaptive immune system. This process requires careful control to ensure sufficient specific humoral immunity, whilst simultaneously avoiding the production of autoantibodies.

Several supopulations of mature B cells with distinct functions and predispositions to differentiate into ASC can be distinguished. Upon encounter with foreign antigen (Ag), B cells are activated in either a T-cell independent (TI) or T-cell dependent (TD) manner. TD-Ag responses often involve the establishment of a germinal center (GC) leading to the production of ASC, but also memory B cells, that drive a superior immune response upon re-exposure to the same antigen. B cell differentiation in the GC reaction is regulated by an elaborate transcription factor network whose details are being progressively elucidated. Importantly, B cell malignancies are often associated with aberrant expression of GC transcription factors.

Despite the obvious limitations, it is becoming increasingly important to investigate the signaling pathways and regulation mechanisms in human cells since findings from animal studies cannot always be easily extrapolated to human lymphocyte biology [1] or the clinical setting (see the recent discussion by Davis [2]). For example, the Toll-like receptor (TLR)-4 agonist LPS alone efficiently activates murine naïve B cells but not human naïve B cells [3] due to absence of TLR4 expression [4]. Unlike in mice, B cell development is independent of IL-7 signaling in humans; XSCID patients with mutations in the common γ chain have normal B cell numbers (reviewed in [1]). Also, deficiencies in intracellular signaling molecules, such as BLNK or Btk, or in T cell-mediated co-stimulatory molecules as ICOS have different effects on B cell development or function, respectively, in the mouse compared to the human. Several

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genetic deficiencies that specifically affect B cell function have been described in humans, and these provide valuable insights into the molecular regulation of B cell function. Molecular research of human B cells was boosted lately with the availability of new tools such as stable viral transduction to genetically manipulate primary lymphocytes. Furthermore, insights into the extrinsic factors required for B cell activation allow the mimicking of many B cell differentiation pathways *in vitro* to further increase our knowledge of the molecular processes.

This review shows that studies of transcriptional regulation in the murine GC can largely be extrapolated to humans. In line with this, the differential expression of the factors involved within the various GC stages is similar between both the human and murine systems [5]. Not all pathways described in murine B cells have been confirmed in humans, and *vice versa*, some findings are only described in human systems. It is of note that no mechanism established thus far in human B cells contradicts the regulatory pathways described in the murine GC.

Differential gene expression in naïve and memory B cell subsets

In both humans and mice, several functionally distinct subpopulations of mature B cells can be distinguished (Box 1). Most B cells ultimately serve to yield ASCs, but the subsets differ in location, ability to migrate, activation by TI- or TD-Ag, in the rate of ASC differentiation and the stimulation requirements for ASC differentiation. Secondary humoral immune responses are mediated by memory B cells and are characterized by more rapid B cell activation, proliferation, and differentiation and the secretion of higher affinity Ab. It has become clear that the qualitative and quantitative superiority of the secondary antibody response is not only due to the increased frequency and affinity of Ag-specific B cells, but also to intrinsic differences between memory versus naïve B cells [6,7] (Figure 1).

Initial microarray studies of human B cells identified few differences in the gene expression patterns between naïve and memory B cells; only some known surface proteins like tumor necrosis factor receptor superfamily (TNFRSF) members CD27 and CD95, as well as the co-stimulatory molecule CD80 were differentially expressed [8]. Later it was found that expression of the ABCB1 transporter by human resting naïve B cells discriminates them from cycling transitional and memory B cells [9] (Figure 1). More recent studies have shown that naïve B cells, as compared to memory B cells, express higher levels of the promyelocytic leukemia zinc finger (PLZF) factor and the Krüppel-like factors (KLF)4 and KLF9, which are transcription factors important in maintaining cellular quiescence [10]. In addition memory B cells express increased levels of TNFRSF member TACI, signaling lymphocytic activation molecule (SLAM) family members CD84 and CD229, TLR-related molecule CD180 as well as CD86 [11]. Collectively, these differences in expression levels might account for the accelerated response to Ag and enhanced proliferation and eventual Ab secretion of memory B cells compared to naïve B cells. In line with the longevity of the memory B cell pool, which can persist for the lifetime of the host [3], memory B cells also express higher levels of the anti-apoptotic factors Bcl-2, A1 and Mcl-1 as compared to naïve B cells [11].

TLR stimulation is an important mechanism by which humoral memory is maintained [12, 13]. Contrasting results have been reported on the expression of TLRs on naïve and memory B cells. Earlier studies suggested that TLRs were only expressed by memory B cell subsets [13], and that responsiveness of naïve B cells to CpG-ODN could only be induced by upregulation of TLR9 by B-cell receptor (BCR) or CD40 stimulation [14]. More recently it was reported that naïve human B cells also express detectable levels of TLR9 and TLR10 [11,15]. This expression also appears to be functional because the TLR9 agonist CpG-oligodeoxynucleotide (ODN) can stimulate both naïve and memory B cells in the absence of BCR cross-linking. This supports the notion that naïve B cells are not intrinsically unresponsive to TLR triggers [16,17]. TLR9-mediated activation of human naïve B cells not only triggers

their differentiation into $CD138^+$ PCs, but in addition a positive feedback loop is created by increasing TLR9 expression [18]. Transitional B cells, which represent the most immature peripheral B cells, differentiate into IgM memory-like B cells and IgM-secreting PCs upon TLR9 signaling [19]. In addition to the role of TLR9, engagement of TLR7 in naïve human B cells induces their proliferation and differentiation into IgG producing cells in the absence of BCR cross-linking and CD40-CD40L interaction [20].

The memory B cells are themselves not homogenous and differences in gene expression can also be observed in subsets within this population ([21] and Figure 1). The so-called IgM^+ memory B cells (Box 1) might represent the human counterpart of murine marginal zone B cells, as they are positive for the marginal zone B cell marker CD1c [22] and express Notch2 [23], a factor indispensable for the development of murine marginal zone B cells [24] (Figure 1). A distinct human B-cell memory subset expressing the inhibitory receptor Fc-receptor-like 4 (FCRL4) has been described [25], which is largely restricted to mucosal tissues and mesenteric lymph nodes. The $FCRL4^+$ and $FCRL4^-$ memory B cell subsets differ in the expression of transcription factors, cell cycle regulators, signaling molecules and surface proteins [26] (Figure 1), suggesting that $FCRL4^+$ and $FCRL4^-$ memory B cells might have different functions in immune responses. While $FCRL4^+$ B cells from healthy donors exhibit a high degree of ASC differentiation potential [26], in the peripheral blood of HIV^+ viraemic patients these cells express low levels of the co-stimulatory molecules CD80 and CD86 and are impaired in their capacity to proliferate or differentiate into ASCs in response to polyclonal stimuli [27]. This exhausted phenotype might contribute to poor antibody responses in HIV–infected individuals. The developmental relationship between $FCRL4^+$ and $FCRL4^-$ memory B cells is elusive and remains to be established [28].

Regulation of B cell activation and differentiation in the human germinal center

In response to TD-Ag, activated B cells enter the lymph node follicle and following extensive proliferation form GC structures, where PCs and memory B cells are generated (Box 2). The ensuing differentiation of B cells during the GC reaction is regulated by a complex network of transcription factors [29,30] (Table 1 and Figure 2). The function of these factors has largely been studied in (conditional) deletion and transgenic mouse models, although the exact hierarchy and relationship among these factors remains incompletely understood. Extensive data is also available on the regulation of the GC reaction in human B cells.

IL-21 is a cytokine that has attracted a lot of attention and is produced by the follicular helper T cells (Tfh) in the GC (Box 3). Engagement of the IL-21 receptor activates STAT1, STAT3, STAT5a and STAT5b [31], and in human B cells clearly phosphorylates STAT3 the most sustained, while STAT5 is only weakly and transiently activated [32,33]. Some effects of IL-21 are exclusively mediated by STAT3 activation, including the induction of IgE, as was demonstrated using B cells from patients with loss-of-function mutations in STAT3 [32]. Other effects of IL-21 on B cells, including induction of apoptosis or proliferation, might be mediated through STAT1 or STAT5 [34]. While somewhat paradoxical, these findings can be interpreted by assuming that IL-21 favors growth arrest and apoptosis of aberrantly activated B cells, while promoting B cell maturation during a productive T cell-dependent B cell response; but how this dichotomous activity is regulated remains elusive. In both mouse and human B cells, IL-21 induces the expression of both B cell lymphoma (BCL)6 and B lymphocyte induced maturation protein (BLIMP)1 [33,35,36] (Figure 2). As BCL6 is known to inhibit PC differentiation, while BLIMP1 promotes PC differentiation [29], IL-21 might transiently induce an intermediate stage in this process. Due to differential kinetics of STAT activation [33], the duration of the IL-21 stimulus might determine the outcome of this balance. Alternatively, BLIMP1 or BCL6 expression might be induced by IL-21 in distinct cells and thereby determine the cell's fate. In

line with this, in human tonsil mutually exclusive expression of BCL6 and BLIMP1 is described [37], but this was not studied for IL-21 stimulated cells *in vitro*. It has been proposed that BCL6 is directly induced by STAT3 in mouse B cells [38], although studies in primary human B cells do not to confirm this [33]. Rather, in line with our earlier findings that BCL6 is a direct target of STAT5 [39], BCL6 induction by IL-21 might be STAT5-dependent. STAT3 plays a profound role in terminal B cell differentiation, as demonstrated by STAT3-deficient mouse B cells which do not differentiate into IgG-secreting PCs upon TD immunization [40]. Similarly in human B cells, STAT3 activation induces expression of PC genes, phenotypic PC formation and Ab secretion [33] (Figure 2). Importantly, activation of STAT3 also initiates early PC differentiation and BLIMP1 expression in human B cells ectopically expressing high levels of BCL6 [33]; just like *ex vivo* $BCL6^{+}$ tonsil B cells rapidly differentiate into PCs in the presence of IL-21. Thus, premature PC differentiation in response to cytokine triggering can be regulated by controlling STAT3 levels. Interestingly, STAT3 is transcriptionally repressed by BCL6 in human B cells [41], providing an additional mechanism of action for BCL6 in regulating PC differentiation. These findings collectively suggests that STAT signaling through cytokine triggering plays a role in influencing GC B cell differentiation in both humans and mice.

Several members of the v-ets erythroblastosis virus E26 oncogene homolog 1 (Ets) family have been implicated in B cell differentiation. We recently described a novel role for the Ets factor Spi-B in regulating B cell differentiation [42] (Figure 2). Spi-B protein levels were high in naïve, memory and activated human tonsil B cells, but undetectable in human PCs. Enforced expression of Spi-B in human B cells directly represses *PRDM1* (encoding BLIMP1) and *XBP1* (encoding X-box binding protein-1) gene expression, which are required for PC formation and Ab production. Spi-B-deficient mice do not show increased numbers of PCs or elevated serum Ab titers, which is probably explained by the impaired GC formation also seen in these mice, therefore suggesting additional contributions of Spi-B during B cell differentiation [43]. A role for another Ets factor, Ets-1, was recently described in murine B cell differentiation [44]. Upon TLR9 triggering, enhanced differentiation of Ets-$1^{-/-}$ B cells into IgM-secreting PCs was observed, which was attributed to the fact that Ets-1 was able to impair BLIMP1 activity [44]. In line with the observation that Ets-1 and Spi-B are differentially expressed in naïve and GC B cells, it is tempting to speculate that Spi-B prevents premature differentiation of activated GC B cells, whereas Ets-1 contributes to the prevention of spontaneous differentiation and Ab secretion by naïve B cells [42,45].

Prerequisite for PC formation is BLIMP1 activity, which is regulated by the deactivation of its repressors (including Spi-B [42], BCL6 [46] and PAX-5 [47], Box 2 and Figure 2) and by positive induction of gene expression. The minimal promoter to confer expression of the human *PRDM1* gene includes a phylogenetically conserved GC-box, which is targeted by multiple transcription factors such as the SP1 and SP3 isoforms as well as early growth response 1 (EGR-1) [48]. Several repressor binding sites in the human *PRDM1* and mouse *Prdm1* loci have also been described. Spi-B bound most effectively at a region 1.5kb upstream of the transcription start of *PRDM1* [42], PAX-5 targets to the first exon of the human *PRDM1* gene [49], whereas BCL6 bound to a conserved element in intron 5 of murine *Prdm1* (corresponding to intron 4 of human *PRDM1*) [50]. However, ChIP-on-chip analysis did not show BCL6 binding to this site in the human *PRDM1* promotor but rather to a novel site in intron 3 [51]. Thus, while the sites may differ, it is clear that in both human and mouse, BCL6 can directly repress the expression of BLIMP1. Reciprocally, expression of *BCL6*, *SPI-B* and *PAX-5* were directly repressed by BLIMP1 in mice [47] (Figure 2), thus creating autoregulatory loops between these factors. An issue still under debate is which factor is the first to be downregulated to relieve suppression of BLIMP1 expression. Downregulation of PAX-5 alone is sufficient to allow BLIMP1 expression and PC differentiation in the mouse [52,53]. Evidence that PAX-5, in addition to its role in BLIMP1 repression, may also maintain BCL6 was obtained in studies

using a chicken B-cell line [54]. Collectively, it appears that PAX-5 is an important regulator of B cell differentiation, though determining the exact sequence of events occurring at the transition into PCs presents a difficult challenge. Whereas several pathways to downregulate BCL6 in GC B cells have been proposed [55], it is unclear how PAX-5 activity is initially shut down in order to initiate the differentiation process [53]. Similarly not much information is available on the regulation of Spi-B expression. Spi-B is a direct transcriptional target of OBF-1 [56], a factor that regulates B cell differentiation at two levels; OBF-1 represses early stages of TD-differentiation, and is critical for the completion of ASC development in mice [57]. OBF-1 itself is a direct target of the PC factor XBP-1 [58]. Based on the premise that PAX-5 directly regulates Spi-B expression in murine pro-B cells [59] one could hypothesize that PAX-5 also regulates Spi-B in mature B cells, but this has not been validated. Another scenario that could be proposed is that downregulation of PAX-5 and BCL6 results in increased BLIMP1 levels, which consequently downregulates Spi-B, since Spi-B is a direct target of BLIMP1 [47]. While we cannot exclude that this contributes to the downregulation of Spi-B, we observed that Spi-B levels decreased prior to the increase of BLIMP1 levels in differentiating human memory B cells [42]. This suggests that Spi-B is downregulated initially by a factor other than BLIMP1.

Similar to mice, little is known about the regulation of human memory B cell formation. The balance of activated STAT5 and STAT3 might be an important determinant in the differentiation of human GC B cells into memory B cells or PCs, respectively [33] (Figure 2). We previously proposed BCL6, induced by STAT5, to be a candidate for *in vivo* memory B cell development due to its high expression in a subset of GC B cells that were biased against PC differentiation [39]. Expression profiling of human memory B cells obtained *in vitro* from centroblasts revealed a unique pattern of costimulatory molecules, cytokine receptors, anti-apoptotic proteins, T cell chemokines, and transcription factors [60]. BCL6 was not expressed in these *in vitro* generated memory B cells [60]. This, together with the observation that ectopic expression of BCL6 in tonsil B cells reduced memory B cell formation [60] suggests that BCL6 might not be required for post-GC memory B cell formation. Further research should definitively establish the role of BCL6 in memory B cell development and whether additional factors determine the memory B cell fate.

Germinal center factors and B cell malignancies

The majority of B cell lymphomas originate from GC B cells, as indicated by the presence of somatically mutated IgV_H genes [61]. Chromosomal translocations causing the dysregulated expression of genes associated with B cell lymphomas often involve the Ig locus. These translocations are either associated with SHM or CSR, or they occur during V(D)J recombination of immature B cells. Well described examples are the t(14;18) and t(8;14) translocations, involving the Ig and *BCL2* or *MYC* loci in follicular or Burkitt lymphoma, respectively. Aberrant expression or activity of transcription factors controlling B cell development and differentiation is typically associated with B-cell lymphomagenesis [55] (Table 1). Recent findings have extended the list of regulators of the GC reaction that are associated with the formation of B cell tumors.

Several subsets of diffuse large B cell lymphoma (DLBCL), the most common form of non-Hodgkin lymphoma, are distinguished by gene-expression profiling [62,63]. Chromosomal translocations involving BCL6 are characteristic for the germinal center B-cell (GCB) DLBCL subtype. Dysregulated BCL6 expression prevents the silencing and termination of the GC response and furthermore maintains the specific pro-proliferative, DNA-damage tolerant centroblastic phenotype [55]. Constitutive activity of the NF-kB signaling pathway is implicated in the pathogenesis of the least curable activated B-cell (ABC)-type of DLBCL.

ABC-DLBCL is also associated with inactivation of *PRDM1* (encoding BLIMP1), probably resulting in malignant transformation by blocking terminal differentiation.

A role for Ets factors in oncogenesis has been appreciated for some time [64]. Recently it was observed that the Spi-B locus was translocated and inserted in the proximity of the Ig 3′α–enhancer in an ABC-DLBCL cell line, resulting in relatively high Spi-B levels [65]. In general, Spi-B levels were higher in ABC-DLBCL than in the GCB-type of DLBCL [66]. Introduction of shRNA against Spi-B in an ABC-DLBCL cell line identified the requirement of Spi-B for proliferation and cell survival, reinforcing the notion that Spi-B has potential as an oncogene [66].

Inappropriate activation of JAK/STAT signaling occurs with high frequency in human cancers and correlations exist with cancer cell survival and proliferation, also for B cell lymphomas (Table 1). Besides deviant NF-κB activation in ABC-DLBCL, additional oncogenic pathways, including the constitutive activation of STAT3, have been reported [41,67]. In agreement with the observation that BCL6 under normal conditions repressed STAT3 gene expression [41], ABC-DLBCL express low levels of BCL6 resulting in higher levels of STAT3 expression. As expected, inactivation of STAT3 by RNA interference inhibited cell proliferation and triggered apoptosis, accompanied by decreased expression of several STAT3 targets. Constitutive STAT3 activation might result from autocrine or paracrine IL-6 and/or IL-10 induced signaling, since these cytokines were highly expressed in some ABC-DLCBL as a consequence of increased levels of NF-κB activity [67]. Considering the prominent role of IL-21 in the GC and being a potent inducer of STAT3 activation [31–33] it will be interesting to establish the role of IL-21 in the oncogenesis of this DLBCL subtype. In general IL-21 has ambivalent effects on B cell malignancies, as it has the capacity to induce cell death in certain B cell malignancies (B-CLL, follicular lymphoma (FL), DLBCL) while inducing proliferation in others (multiple myeloma) [68].

Classical Hodgkin lymphoma (cHL) is characterized by the presence of B cell-derived Hodgkin and Reed-Sternberg (HRS) cells [61]. In the malignant HRS cells of cHL the expression of B cell-specific genes is lost, and B lineage-inappropriate genes are up-regulated. Functional disruption of the B lineage-specific transcription factor program contributes to this process [69]. Recently we and others have reported that IL-21 is expressed by cHL cells [70,71]. Consistent with expression of the IL-21R on cHL cells, autocrine IL-21 activates both STAT3 and STAT5 [70,71]. Moreover, constitutive activation of the STAT5 and NF-kB pathways leads to rapid transformation of peripheral blood B cells closely resembling cHL cells [70]. A picture is emerging that, in analogy to the STAT3-NF-κB cooperation in DLBCL, a collaboration between the STAT5 and NF-κB pathways is operational in cHL.

Interferon regulatory factors (IRFs) play a role in normal and malignant B cell function. IRF-8, which is highly expressed in the GC and in lymphomas of GC origin [72], induced the expression of BCL6 [73]. Further research is warranted to establish the role of IRF-8 in B cell malignancies. IRF-4 plays a critical role in multiple myeloma by positively regulating c-myc [74]. Interestingly, IRF-4 was not genetically altered in myeloma cells, but rather the cells appeared “addicted” to an aberrant IRF-4 regulatory network that fused the gene expression programs of normal PCs and activated B cells. It was proposed that IRF-4 might function as an important regulator of the GC reaction protecting against lymphomagenesis.

Collectively, many of the transcription factors involved in the regulation of the GC reaction are associated with lymphomagenesis, including STATs, NF-kB, BCL6, Pax-5 and BLIMP1 [55] and more recently described factors such as Spi-B [66] and IRF-4 [74] (Table 1). Several other factors, which are described to regulate B cell differentiation, include MTA3, IRF-8,

XBP-1 and MiTF. It would not be unexpected if future research reveals aberrant expression of these factors in B cell lymphomas as well.

Future prospects

An emerging line of enquiry leading to insights in the regulation of lymphocyte development and maturation is by means of microRNAs (miRNAs) [75]. These post-transcriptional regulators of gene expression show a highly unique profile in GC lymphocytes [76–78]. It is now well-accepted that the malignant transformation process is associated with altered expression of multiple miRNA [79]. Also for B cell lymphomas, miRNA signatures have been reported [76–78,80], and it is expected that differential expression of miRNAs between B-cell lymphomas and their normal counterparts (GC lymphocytes) contributes to the process of B-lymphomagenesis. Some of the important candidates include miR-155, which regulates AID activity and PU.1 expression [81,82] and was increased in cHL, non-HL and CLL [83], miR-21, which was found in association with elevated BCL-2 levels, was increased in DLBCL and CLL, and the miR-17~92 cluster, which targets the pro-apoptotic protein Bim is associated with B-cell lymphomagenesis when overexpressed [84]. As such, miRNAs appear to be potential targets for therapeutic intervention. Efficient miRNA silencing using high-affinity targeting by chemically modified antisense oligonucleotides will highlight the utility of such compounds in the development of miRNA-based cancer therapeutics. In addition, these reagents will be valuable tools to gain further insight in the transcriptome that controls the differentiation of B cells into PCs.

In conclusion, modulation of gene expression patterns in B cells by transcriptional and miRNA-based mechanisms is intricately involved in the correct regulation of GC function. Since these mechanisms may go awry during B cell lymphomagenesis and in autoimmunity it is critical to understand these regulatory networks, particulary in human, to fully explore the potential for design of novel therapies or diagnostics in the future.

BOX 1

Human B cell subsets and IgM memory B cells

Classically, mature human peripheral CD19+ B cells are subdivided into separate pools based on IgD and CD38 expression [85,86] (reviewed in [21,87]). $IgD^{+}CD38^{-}$ B cells are naïve and have unmutated Ig V region sequences, whereas $IgD^{-}CD38^{-}$, $IgD^{-}CD38^{+}$, and $IgD^{+}CD38^{+}$ B cell pools are defined as memory, germinal center (GC), and pre-GC B cells. A putative GC founder cell (pro-GC) was recently identified in the human tonsil, which exhibited a unique phenotype ($IgD^{+}CD38^{-}CD23^{-}FSC^{hi}CD71^{+}$) and morphology and might represent an intermediate population between naïve B cells and GC B cells [88]. CD27 has been identified as a marker of human memory B cells [89] and in peripheral blood $IgM^{+}CD27^{-}$, $IgM^{-}CD27^{+}$ and $IgM^{+}CD27^{+}$ B cells can be distinguished. Isotype switched memory B cells lacking CD27 have also been found in systemic lupus erythematosus patients as well as healthy subjects [21], though their exact function is currently not fully understood.

In mice, naïve B cells are generally divided into three subsets, B1 B cells, follicular B cells, and marginal zone (MZ) B cells [90]. It is still debated whether, in analogy to mice, the human secondary B-cell repertoire also comprises a pool of B1 cells, a subset that produces high amounts of natural antibodies [91]. In humans $IgM^{+}CD27^{+}$ B cells are found in the region of the human spleen that is analogous to the murine MZ [21]. This circulating population has somatically mutated Ig genes, but continues to express IgM. It is not clear whether $IgM^{+}CD27^{+}$ B cells are the human counterpart of murine MZ cells, which have been described as largely naïve, long-lived, non-circulating cells that respond rapidly to T-

cell independent (TI) antigen. Much research has investigated the nature, development and function of these so-called IgM memory B cells (see for instance [92]). It has been proposed that IgM^+CD27^+ B cells participate in T cell independent immune responses specifically against encapsulated bacteria. As IgM^+CD27^+ B cells were generated in humans unable to form GCs, it was suggested that they are formed independently of GCs and thus, despite mutated Ig loci and CD27 expression, are not *bona fide* memory B cells. SHM in these cells might be induced during pre-diversification of the B cell repertoire. More recent findings studying $IgM^+IgD^+CD27^+$ B cells in children of <2 yrs of age [93], in the human fetus, and in T cell-deficient $Rag2^{-/-}\gamma_c^{-/-}$ mice repopulated with human hematopoietic stem cells indicate that the IgM^+ $CD27^+$ B cells with mutated Ig loci can develop independently of TI or TD immune responses [23].

BOX 2

The germinal center reaction

Memory B cells that exhibit rapid antibody (Ab) production when re-exposed to the same antigen (Ag) and plasma cells (PCs) that produce high-affinity Abs are generated in a structure called the germinal center (GC). Upon engagement of cognate Ag, naïve B cells enter the primary follicles of secondary lymphoid organs including spleen, lymph nodes, Peyer's patches, or tonsils where they undergo rapid cell proliferation and form GCs. In the GC, Ab diversification processes (i.e. somatic hypermutation, SHM, and class switch recombination, CSR) take place leading to the generation of high affinity Ag-specific Abs. SHM introduces point mutations into the Ig V gene and CSR alters the effector function of the subsequent Ab by switching the constant region to a secondary isotype. SHM and CSR depend on the activity of the enzyme activation-induced cytidine deaminase (AID). Subsequently in the so-called affinity maturation process, GC centrocytes compete for binding to antigen associated with follicular dendritic cells (FDC) and for T-cell help by follicular helper T cells (Tfh). Selected B cells differentiate and leave the germinal center as either memory B cells or PCs.

The GC reaction and the differentiation of B cells into PCs and memory B cells is regulated by a network of transcription factors [29,30] (Figure 2). PC differentiation is most likely initiated by the downregulation of PAX-5, the 'identity' gene of B cells. B cell lymphoma (BCL)-6, which is strongly upregulated in GC B cells, is the master regulator of the GC reaction by suppressing apoptosis and promoting proliferation. Expression of B lymphocyte induced maturation protein (BLIMP)-1, a zinc finger-containing transcriptional repressor, is induced in subsets of GC B cells and in PCs. BLIMP1 promotes PC development by suppressing genes associated with the GC program (BCL6, PAX-5, Spi-B) and induces the expression of those essential for PC development. The X-box binding protein 1 (XBP-1), which is upregulated as part of the endoplasmic reticulum (ER) stress response, is expressed at a high level in PCs and is essential for inducing the secretory phenotype of the PCs. More recently interferon regulatory factor (IRF)-4 was identified as additional master gene in PC differentiation.

BOX 3

The role of IL-21 in human B cell differentiation

The most potent cytokine in activation and differentiation of human B cells is undeniably IL-21, as became clear from several recent studies (reviewed in [31,68]). Human tonsil $CD4^+CXCR5^+CCR7^-$ follicular helper T cells (Tfh) are a rich source of IL-21, which induces the differentiation of all mature human B cells subsets into ASCs, including naïve

and memory B cells [94]. IL-21 is required for B-cell activation, proliferation, PC differentiation, and Ab production induced by interaction of B cells with activated T cells [95]. Although IL-21 can also be pro-apoptotic for murine B cells depending on the context such as BCR engagement in the absence of CD40 mediated T cell help [96,97], this effect has not been observed in human B cells. In fact, IL-21 may promote autoantibody production in autoimmune diseases where the stimulation context may be altered, such as lack of T cell help [68].

Stimulation of naïve B cells with IL-21 induced the transcription of AID [36], and while IL-21 induced CSR in naïve B cells, it is notable that IL-21 does not induce SHM, not even when concomitantly triggered by CD40 and BCR. This might suggest that IL-21 is unable to induce relevant co-factors to mediate SHM [68]. IL-21 preferentially induces switching into IgG1 and IgG3 and, in synergy with IL-4, IL-21 increases the generation of $IgG1^+$ B cells, but prevents switching to IgA. So the production of IgG subclasses and IgA is regulated by the interaction between IL-4 and IL-21, suggesting that the cytokines may act sequentially in humoral immune responses to specific pathogens [98].

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References

1. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol 2004;172:2731–2738. [PubMed: 14978070]
2. Davis MM. A prescription for human immunology. Immunity 2008;29:835–838. [PubMed: 19100694]
3. Lanzavecchia A, et al. Understanding and making use of human memory B cells. Immunol Rev 2006;211:303–309. [PubMed: 16824137]
4. Muzio M, et al. Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. J Immunol 2000;164:5998–6004. [PubMed: 10820283]
5. Cattoretti G, et al. Stages of germinal center transit are defined by B cell transcription factor coexpression and relative abundance. J Immunol 2006;177:6930–6939. [PubMed: 17082608]
6. Tangye SG, et al. Intrinsic differences in the proliferation of naive and memory human B cells as a mechanism for enhanced secondary immune responses. J Immunol 2003;170:686–694. [PubMed: 12517929]
7. Anderson SM, et al. Intrinsic properties of human and murine memory B cells. Immunol Rev 2006;211:280–294. [PubMed: 16824135]
8. Klein U, et al. Transcriptional analysis of the B cell germinal center reaction. Proc Natl Acad Sci USA 2003;100:2639–2644. [PubMed: 12604779]
9. Wirths S, Lanzavecchia A. ABCB1 transporter discriminates human resting naive B cells from cycling transitional and memory B cells. Eur JImmunol 2005;35:3433–3441. [PubMed: 16259010]
10. Good KL, Tangye SG. Decreased expression of Kruppel-like factors in memory B cells induces the rapid response typical of secondary antibody responses. Proc Natl Acad Sci USA 2007;104:13420–13425. [PubMed: 17673551]
11. Good KL, et al. Resting human memory B cells are intrinsically programmed for enhanced survival and responsiveness to diverse stimuli compared to naive B cells. J Immunol 2009;182:890–901. [PubMed: 19124732]
12. Bernasconi NL, et al. Maintenance of serological memory by polyclonal activation of human memory B cells. Science 2002;298:2199–2202. [PubMed: 12481138]

13. Bernasconi NL, et al. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. Blood 2003;101:4500–4504. [PubMed: 12560217]
14. Ruprecht CR, Lanzavecchia A. Toll-like receptor stimulation as a third signal required for activation of human naive B cells. Eur JImmunol 2006;36:810–816. [PubMed: 16541472]
15. Cognasse F, et al. Identification of two subpopulations of purified human blood B cells, CD27(−) CD23(+) and CD27(high) CD80(+), that strongly express cell surface Toll-like receptor 9 and secrete high levels of interleukin-6. Immunology. 2008
16. Jiang W, et al. TLR9 stimulation drives naive B cells to proliferate and to attain enhanced antigen presenting function. Eur J Immunol 2007;37:2205–2213. [PubMed: 17621369]
17. Bekeredjian-Ding I, et al. TLR9-activating DNA up-regulates ZAP70 via sustained PKB induction in IgM+ B cells. J Immunol 2008;181:8267–8277. [PubMed: 19050243]
18. Huggins J, et al. CpG DNA activation and plasma-cell differentiation of. Blood 2007;109:1611–1619. [PubMed: 17032927]
19. Capolunghi F, et al. CpG drives human transitional B cells to terminal differentiation and production of natural antibodies. J Immunol 2008;180:800–808. [PubMed: 18178818]
20. Glaum MC, et al. Toll-like receptor 7-induced naive human B-cell differentiation and immunoglobulin production. J Allergy Clin Immunol 2009;123:224–230. [PubMed: 18995892]
21. Sanz I, et al. Phenotypic and functional heterogeneity of human memory B cells. Semin Immunol 2008;20:67–82. [PubMed: 18258454]
22. Weller S, et al. Human blood IgM “memory” B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. Blood 2004;104:3647–3654. [PubMed: 15191950]
23. Scheeren FA, et al. T cell-independent development and induction of somatic hypermutation in human IgM+IgD+CD27+ B cells. J Exp Med. 2008
24. Saito T, et al. Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. Immunity 2003;18:675–685. [PubMed: 12753744]
25. Ehrhardt GR, et al. Expression of the immunoregulatory molecule FcRH4 defines a distinctive tissue-based population of memory B cells. J Exp Med 2005;202:783–791. [PubMed: 16157685]
26. Ehrhardt GR, et al. Discriminating gene expression profiles of memory B cell subpopulations. J Exp Med 2008;205:1807–1817. [PubMed: 18625746]
27. Moir S, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med 2008;205:1797–1805. [PubMed: 18625747]
28. Kuppers R. Human memory B cells: Memory B cells of a special kind. Immunol Cell Biol. 2008
29. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. Nat Rev Immunol 2005;5:230–242. [PubMed: 15738953]
30. Tarlinton D, et al. Plasma cell differentiation and survival. Curr Opin Immunol 2008;20:162–169. [PubMed: 18456483]
31. Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. Annu Rev Immunol 2008;26:57–79. [PubMed: 17953510]
32. Avery DT, et al. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. Blood 2008;112:1784–1793. [PubMed: 18579794]
33. Diehl SA, et al. STAT3-Mediated Up-Regulation of BLIMP1 Is Coordinated with BCL6 Down-Regulation to Control Human Plasma Cell Differentiation. J Immunol 2008;180:4805–4815. [PubMed: 18354204]
34. Gowda A, et al. IL-21 mediates apoptosis through up-regulation of the BH3 family member BIM and enhances both direct and antibody-dependent cellular cytotoxicity in primary chronic lymphocytic leukemia cells in vitro. Blood 2008;111:4723–4730. [PubMed: 18182577]
35. Ozaki K, et al. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. J Immunol 2004;173:5361–5371. [PubMed: 15494482]
36. Ettinger R, et al. IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. J Immunol 2005;175:7867–7879. [PubMed: 16339522]

37. Cattoretti G, et al. PRDM1/Blimp-1 is expressed in human B-lymphocytes committed to the plasma cell lineage. J Pathol 2005;206:76–86. [PubMed: 15772984]
38. Arguni E, et al. JunD/AP-1 and STAT3 are the major enhancer molecules for high Bcl6 expression in germinal center B cells. Int Immunol 2006;18:1079–1089. [PubMed: 16702165]
39. Scheeren FA, et al. STAT5 regulates the self-renewal capacity and differentiation of human memory B cells and controls Bcl-6 expression. Nat Immunol 2005;6:303–313. [PubMed: 15711548]
40. Fornek JL, et al. Critical role for Stat3 in T-dependent terminal differentiation of IgG B cells. Blood 2006;107:1085–1091. [PubMed: 16223771]
41. Ding BB, et al. Constitutively activated STAT3 promotes cell proliferation and survival in the activated B-cell subtype of diffuse large B-cell lymphomas. Blood 2008;111:1515–1523. [PubMed: 17951530]
42. Schmidlin H, et al. Spi-B inhibits human plasma cell differentiation by repressing BLIMP1 and XBP-1 expression. Blood 2008;112:1804–1812. [PubMed: 18552212]
43. Su GH, et al. Defective B cell receptor-mediated responses in mice lacking the Ets protein, Spi-B. EMBO J 1997;16:7118–7129. [PubMed: 9384589]
44. John SA, et al. Ets-1 regulates plasma cell differentiation by interfering with the activity of the transcription factor Blimp-1. J Biol Chem. 2007
45. Bhattacharya D, et al. Transcriptional profiling of antigen-dependent murine B cell differentiation and memory formation. J Immunol 2007;179:6808–6819. [PubMed: 17982071]
46. Shaffer AL, et al. BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. Immunity 2000;13:199–212. [PubMed: 10981963]
47. Shaffer AL, et al. Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. Immunity 2002;17:51–62. [PubMed: 12150891]
48. Mora-Lopez F, et al. Transcription of PRDM1, the master regulator for plasma cell differentiation, depends on an SP1/SP3/EGR-1 GC-box. Eur J Immunol 2008;38:2316–2324. [PubMed: 18604866]
49. Mora-Lopez F, et al. Human BSAP and BLIMP1 conform an autoregulatory feedback loop. Blood 2007;110:3150–3157. [PubMed: 17682124]
50. Tunyaplin C, et al. Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. J Immunol 2004;173:1158–1165. [PubMed: 15240705]
51. Parekh S, et al. BCL6 programs lymphoma cells for survival and differentiation through distinct biochemical mechanisms. Blood. 2007
52. Delogu A, et al. Gene repression by Pax5 in B cells is essential for blood cell homeostasis and is reversed in plasma cells. Immunity 2006;24:269–281. [PubMed: 16546096]
53. Kallies A, et al. Initiation of Plasma-Cell Differentiation Is Independent of the Transcription Factor Blimp-1. Immunity 2007;26:555–566. [PubMed: 17509907]
54. Nera KP, et al. Loss of Pax5 promotes plasma cell differentiation. Immunity 2006;24:283–293. [PubMed: 16546097]
55. Klein U, Dalla-Favera R. Germinal centres: role in B-cell physiology and malignancy. Nat Rev Immunol 2008;8:22–33. [PubMed: 18097447]
56. Bartholdy B, et al. The Ets factor Spi-B is a direct critical target of the coactivator OBF-1. Proc Natl Acad Sci USA 2006;103:11665–11670. [PubMed: 16861304]
57. Corcoran LM, et al. Differential requirement for OBF-1 during antibody-secreting cell differentiation. J Exp Med 2005;201:1385–1396. [PubMed: 15867091]
58. Shen Y, Hendershot LM. Identification of ERdj3 and OBF-1/BOB-1/OCA-B as direct targets of XBP-1 during plasma cell differentiation. J Immunol 2007;179:2969–2978. [PubMed: 17709512]
59. Pridans C, et al. Identification of Pax5 target genes in early B cell differentiation. J Immunol 2008;180:1719–1728. [PubMed: 18209069]
60. Kuo TC, et al. Repression of BCL-6 is required for the formation of human memory B cells in vitro. J Exp Med. 2007
61. Kuppers R. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer 2005;5:251–262. [PubMed: 15803153]
62. Alizadeh AA, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503–511. [PubMed: 10676951]

63. Wright G, et al. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci USA 2003;100:9991–9996. [PubMed: 12900505]
64. Seth A, Watson DK. ETS transcription factors and their emerging roles in human cancer. Eur J Cancer 2005;41:2462–2478. [PubMed: 16213704]
65. Lenz G, et al. Aberrant immunoglobulin class switch recombination and switch translocations in activated B cell-like diffuse large B cell lymphoma. J Exp Med 2007;204:633–643. [PubMed: 17353367]
66. Lenz G, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proc Natl Acad Sci USA 2008;105:13520–13525. [PubMed: 18765795]
67. Lam LT, et al. Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor-{kappa}B pathways in subtypes of diffuse large B-cell lymphoma. Blood 2008;111:3701–3713. [PubMed: 18160665]
68. Ettinger R, et al. The role of IL-21 in regulating B-cell function in health and disease. Immunol Rev 2008;223:60–86. [PubMed: 18613830]
69. Kuppers R. The biology of Hodgkin's lymphoma. Nat Rev Cancer 2009;9:15–27. [PubMed: 19078975]
70. Scheeren FA, et al. IL-21 is expressed in Hodgkin lymphoma and activates STAT5: evidence that activated STAT5 is required for Hodgkin lymphomagenesis. Blood 2008;111:4706–4715. [PubMed: 18296629]
71. Lamprecht B, et al. Aberrant expression of the Th2 cytokine IL-21 in Hodgkin lymphoma cells regulates STAT3 signaling and attracts Treg cells via regulation of MIP-3alpha. Blood 2008;112:3339–3347. [PubMed: 18684866]
72. Martinez A, et al. Expression of the interferon regulatory factor 8/ICSBP-1 in human reactive lymphoid tissues and B-cell lymphomas: a novel germinal center marker. Am J Surg Pathol 2008;32:1190–1200. [PubMed: 18580679]
73. Lee CH, et al. Regulation of the germinal center gene program by interferon (IFN) regulatory factor 8/IFN consensus sequence-binding protein. J Exp Med 2006;203:63–72. [PubMed: 16380510]
74. Shaffer AL, et al. IRF4 addiction in multiple myeloma. Nature 2008;454:226–231. [PubMed: 18568025]
75. Baltimore D, et al. MicroRNAs: new regulators of immune cell development and function. Nat Immunol 2008;9:839–845. [PubMed: 18645592]
76. Lawrie CH, et al. MicroRNA expression distinguishes between germinal center B cell-like and activated B cell-like subtypes of diffuse large B cell lymphoma. Int J Cancer 2007;121:1156–1161. [PubMed: 17487835]
77. Malumbres R, et al. Differentiation-stage-specific expression of microRNAs in B-lymphocytes and diffuse large B-cell lymphomas. Blood. 2008
78. Zhang J, et al. Patterns of microRNA expression characterize stages of human B cell differentiation. Blood. 2009
79. Lu J, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–838. [PubMed: 15944708]
80. Pichiorri F, et al. MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis. Proc Natl Acad Sci USA 2008;105:12885–12890. [PubMed: 18728182]
81. Teng G, et al. MicroRNA-155 Is a Negative Regulator of Activation-Induced Cytidine Deaminase. Immunity. 2008
82. Vigorito E, et al. microRNA-155 Regulates the Generation of Immunoglobulin Class-Switched Plasma Cells. Immunity 2007;27:847–859. [PubMed: 18055230]
83. Lindsay MA. microRNAs and the immune response. Trends Immunol 2008;29:343–351. [PubMed: 18515182]
84. Xiao C, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol 2008;9:405–414. [PubMed: 18327259]
85. Liu YJ, et al. Five human mature B cell subsets. Adv Exp Med Biol 1994;355:289–296. [PubMed: 7709838]

86. Pascual V, et al. Analysis of somatic mutation in five B cell subsets of human tonsil. J Exp Med 1994;180:329–339. [PubMed: 8006591]
87. Jackson SM, et al. Chapter 5 human B cell subsets. Adv Immunol 2008;98:151–224. [PubMed: 18772006]
88. Kolar GR, et al. A novel human B cell subpopulation representing the initial germinal center population to express AID. Blood 2007;109:2545–2552. [PubMed: 17132718]
89. Klein U, et al. Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. J Exp Med 1998;188:1679–1689. [PubMed: 9802980]
90. Allman D, Pillai S. Peripheral B cell subsets. Curr Opin Immunol 2008;20:149–157. [PubMed: 18434123]
91. Sagaert X, et al. The dynamics of the B follicle: understanding the normal counterpart of B-cell-derived malignancies. Leukemia 2007;21:1378–1386. [PubMed: 17495967]
92. Tangye SG, Good KL. Human IgM+CD27+ B cells: memory B cells or “memory” B cells? J Immunol 2007;179:13–19. [PubMed: 17579014]
93. Weller S, et al. Somatic diversification in the absence of antigen-driven responses is the hallmark of the IgM+ IgD+ CD27+ B cell repertoire in infants. J Exp Med 2008;205:1331–1342. [PubMed: 18519648]
94. Bryant VL, et al. Cytokine-mediated regulation of human B cell differentiation into Ig-secreting cells: predominant role of IL-21 produced by CXCR5+ T follicular helper cells. J Immunol 2007;179:8180–8190. [PubMed: 18056361]
95. Kuchen S, et al. Essential role of IL-21 in B cell activation, expansion, and plasma cell generation during CD4+ T cell-B cell collaboration. J Immunol 2007;179:5886–5896. [PubMed: 17947662]
96. Jin H, et al. Distinct activation signals determine whether IL-21 induces B cell costimulation, growth arrest, or Bim-dependent apoptosis. J Immunol 2004;173:657–665. [PubMed: 15210829]
97. Mehta DS, et al. IL-21 induces the apoptosis of resting and activated primary B cells. J Immunol 2003;170:4111–4118. [PubMed: 12682241]
98. Avery DT, et al. IL-21-induced isotype switching to IgG and IgA by human naive B cells is differentially regulated by IL-4. J Immunol 2008;181:1767–1779. [PubMed: 18641314]
99. Cattoretti G, et al. Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. Cancer Cell 2005;7:445–455. [PubMed: 15894265]
100. Iida S, et al. The t(9;14)(p13;q32) chromosomal translocation associated with lymphoplasmacytoid lymphoma involves the PAX-5 gene. Blood 1996;88:4110–4117. [PubMed: 8943844]
101. Pasqualucci L, et al. Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. J Exp Med 2006;203:311–317. [PubMed: 16492805]
102. Tam W, et al. Mutational analysis of PRDM1 indicates a tumor-suppressor role in diffuse large B-cell lymphomas. Blood 2006;107:4090–4100. [PubMed: 16424392]
103. Catlett-Falcone R, et al. Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity 1999;10:105–115. [PubMed: 10023775]
104. Holtick U, et al. STAT3 is essential for Hodgkin lymphoma cell proliferation and is a target of tyrphostin AG17 which confers sensitization for apoptosis. Leukemia 2005;19:936–944. [PubMed: 15912144]
105. Lai R, et al. Expression of STAT3 and its phosphorylated forms in mantle cell lymphoma cell lines and tumours. J Pathol 2003;199:84–89. [PubMed: 12474230]
106. Melzner I, et al. Biallelic mutation of SOCS-1 impairs JAK2 degradation and sustains phospho-JAK2 action in the MedB-1 mediastinal lymphoma line. Blood 2005;105:2535–2542. [PubMed: 15572583]
107. Niu H, et al. Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. Genes Dev 1998;12:1953–1961. [PubMed: 9649500]
108. Saito M, et al. A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma. Cancer Cell 2007;12:280–292. [PubMed: 17785208]

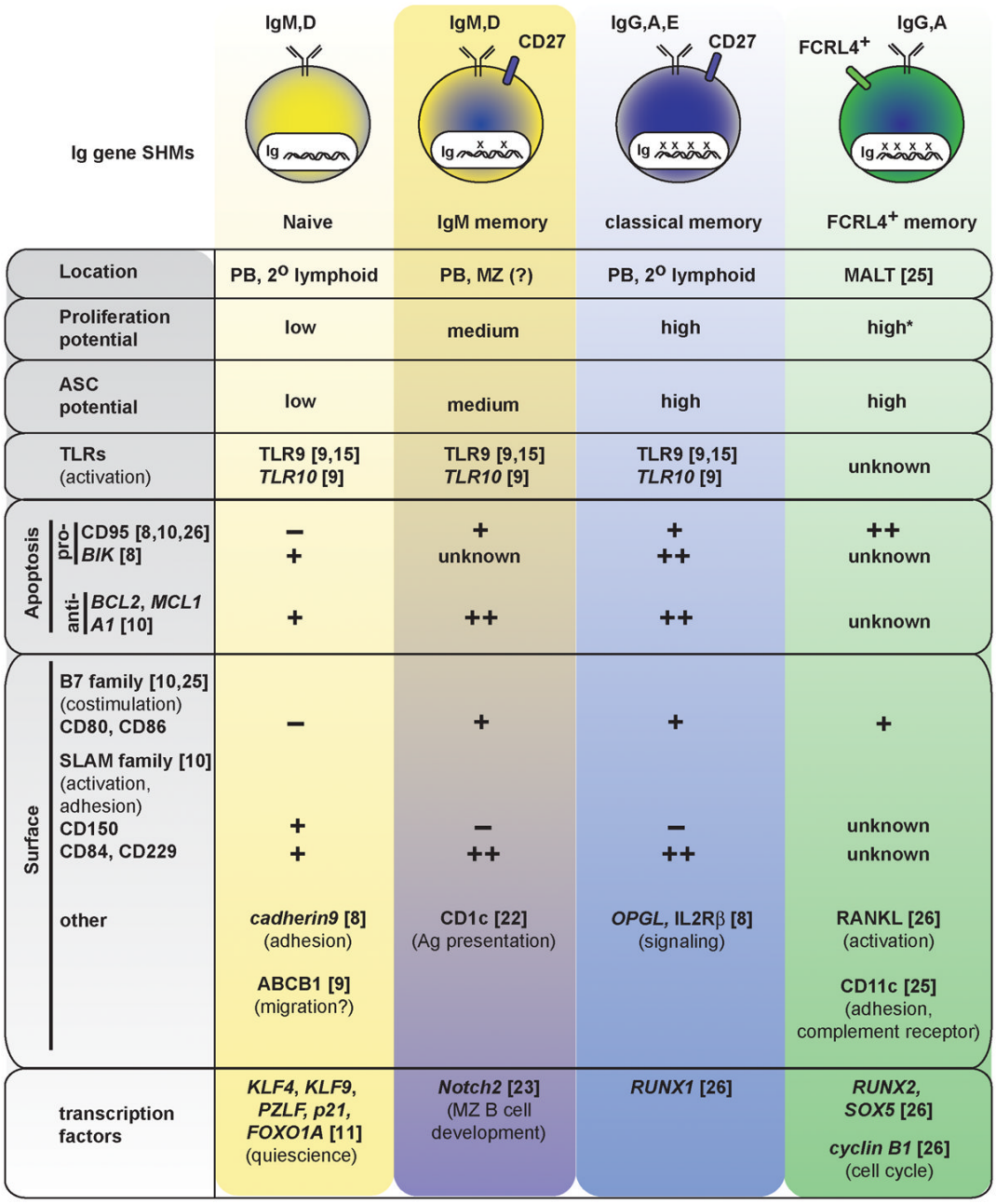

Figure 1. Characteristics and factors among mature human naïve and memory B cell subsets

Naïve B cells are identified as IgM^+IgD^+cells, which possess no somatic hypermutations (SHMs) and little capacity to proliferate or differentiate into antibody secreting cells (ASCs), likely due to intrinsic differences in expression of factors involved in quiescence including Kruppel-like factors (KLF)4, KLF9 and promyelocytic leukemia zinc finger (PZLF) [10]. $IgM^+IgD^+CD27^+$ memory B cells probably form the human counterparts of murine splenic marginal zone B cells, exhibit SHMs and require Notch2 for their development [23]. Classical switched $CD27^+$ memory B cells readily proliferate and differentiate into ASCs upon stimulation and are maintained through expression of anti-apoptotic Bcl-2 family members [11]. Fc-receptor-like 4 (FCRL4) is expressed on a subset of primarily $CD27^-$ memory B cells

[25,26] that resides in epithelial tissue-based niches and shows a distinct expression profile. *The proliferation of $FCRL4^+$ B cells is only high in response to CD40L and cytokines, they fail to proliferate in response either to BCR ligation or *Staphylococcusaureus* stimulation. 2° lymphoid, secondary lymphoid organs; PB, peripheral blood; MALT, mucosa-associated lymphoid tissue. Factors for which protein levels have been assessed are indicated in regular font, whereas italics indicates that only transcription has been detected. Functions of factors are indicated in parentheses.

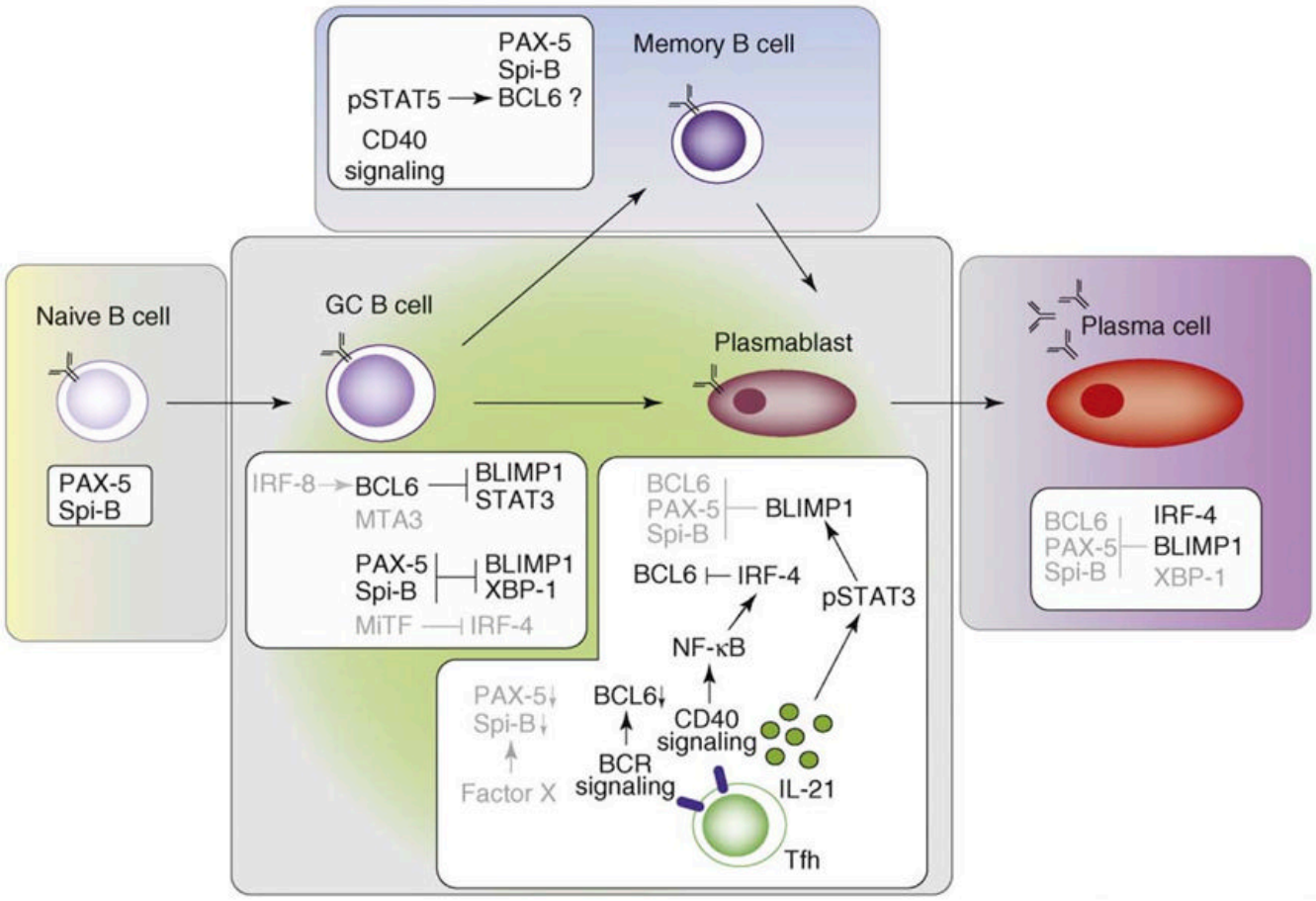

Figure 2. The 'checks and balances' system of human PC differentiation

T-cell dependent (TD) activation of Ag-specific B cells leads to formation of germinal centers (GCs) in lymphoid organs. Several transcription factors are maintained or upregulated in GC B cells to allow affinity maturation to take place, i.e. inhibiting the differentiation of GC B cells into PCs by direct repression of the transcription of factors that are required for PC formation [29,30]. Consequently, expression levels of these repressors must decrease to allow the differentiation of GC B cells once B cell selection is successfully achieved. Once expressed, PC factors repress the factors required for B cell identity and GC phenotype. Also, PC factors can induced their own and each others expression, in order to fully establish the PC phenotype. Research with human B cell systems has confirmed and extended the factors involved in the GC repression network established in murine studies. In the figure, protein expression and repression mechanism that are confirmed or discovered in human systems are displayed in black, results from mouse studies only are displayed in grey. For human B cells it was shown that BCL6 is highly expressed in GC center B cells [5] and represses BLIMP1 [39] as well as STAT3 [41]. PAX-5 and Spi-B are expressed in naïve, memory and activated human B cells [5,42] and repress BLIMP1 and XBP-1 in activated GC B cells [42,49]. BLIMP1 and IRF-4 levels are high in human plasmablasts and PCs [5,37]. IL-21 secreted by Tfh induces BLIMP1 in a STAT3-dependent manner [33]. BCR Ag stimulation induces phosphorylation and degradation of BCL6 [107], while CD40 triggering results in downregulation *of BCL6* expression [108], suggesting a role for Ag and T cell help in GC differentiation by modulating BCL6 levels. $CD25^{+}$ GC B cells express high levels of pSTAT5, a direct inducer of BCL6, resulting in memory differentiation [39]. BCL6 may or may not be required for the differentiation of memory B cells in the GC [60].

Table 1

Transcription factors that regulate B cell differentiation are associated with B cell malignacies.

Transcription factor	Expression in mature B cells subsets*	Role in terminal B cell differentiation*	Associated B cell malignancies
BCL6	Specifically expressed in GC B cells	GC formation and maintenance / GC B cell proliferation during genotoxic stress / Regulation of PC differentiation / Role in memory B cell formation unresolved [39,60]	GCB-DLBCL [99]
MTA3	Specifically expressed in GC B cells	Regulation of PC differentiation	Unknown
PAX-5	All, except PCs	Regulation of PC differentiation	LPL [100]
MiTF	Naive B cells	Regulation of PC differentiation	Unknown
Spi-B	All, except PCs	Regulation of PC differentiation [42]	ABC-DLBCL [65,66]
Ets-1	All, except PCs	Regulation of PC differentiation. Blocks BLIMP1 activity [44]	Unknown
IRF-8	Specifically expressed in GC B cells, absent in PCs	Regulates AID and BCL6 [73]	Unknown
OBF-1	All	Represses early differentiation but critical for the completion of PC development [57]	Unknown
BLIMP1	High in PCs	Crucial for PC formation and Ab secretion	ABC-DLBCL [101,102]
XBP-1	High in PCs	Required for Ab secretion	Unknown
IRF-4	Expressed in some centrocytes, high in PCs	Required for PC formation	MM [74]
Active STAT3	High in GC B cells	Activated by the Tfh cytokine IL-21. Induces BLIMP1 expression and PC formation [33,40]	ABC-DLBCL [41,67], MM [103], cHL [71,104], MCL [105]
Active STAT5	High in CD25+ centrocytes	Differentiation into memory B cells [39]	PMBL [106], cHL [70]

* For more details and additional primary references see other reviews [29,30,55]. Abbreviations: cHL: classical Hodgkin lymphoma, DLBCL: Diffuse large B cell lymphoma (GCB: Germinal center B-cell-like, ABC: Activated B-cell-like), LPL: lymphoplasmacytoid lymphoma, MM: Multiple myeloma, MCL: Mantel-cell lymphoma, PMBL: Primary mediastinal B-cell lymphoma.