EXPERT REVIEWS

Therapeutic implications of advances in our understanding of transitional B-cell development in humans

Expert Rev. Clin. Immunol. 6(5), 765–775 (2010)

Santi Suryani[1,2] and Stuart G Tangye[†1,2]

[1]*Immunology Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst 2010, NSW, Australia*
[2]*St Vincent's Clinical Clinical School, University of NSW, Kensington, NSW, 2033, Australia*
[†]*Author for correspondence:*
Tel.: +61 292 958 455
Fax: +61 292 958 404
s.tangye@garvan.org.au

B-cell development is characterized by the progressive maturation of hematopoietic stem cells through several stages to ultimately give rise to the mature B-cell pool that has been selected for reactivity against non-self antigens. Thus, the mature pool of naive B cells is capable of elicting high-affinity responses following natural infection with pathogens or vaccination and provides the host with protective long-lived humoral immunity. However, perturbations during the processes of B-cell development and differentiation can give rise to a diverse array of immunological diseases including autoimmunity, immunodeficiency and malignancy. While we have a very rich understanding of the processes underlying B-cell development in mice, our knowledge of the corresponding events occurring in human B cells is substantially less robust. Here, we overview the latest findings relating to human B cells in health and disease with a particular emphasis on the transitional stage of B-cell development.

Keywords: autoimmunity • B-cell development • human B cells • immunodeficiency • therapeutics • transitional B cells

During the past two decades, our understanding of human autoimmune diseases and primary immunodeficiencies has increased dramatically [1–5]. Many of these immunodeficient states are characterized by recurrent bacterial infection due to defects either in B-cell development or in the ability of naive B cells to differentiate into effector cells [1,4] such as plasma cells that produce protective levels of neutralizing immunoglobulin (Ig), or memory B cells, which differentiate into plasma cells following subsequent encounter with the intial immunizing antigen [6]. The identification and characterization of numerous human autoimmune diseases and primary B-cell immunodeficiencies provide *in vivo* glimpses of how B cells operate, how their dysfunction can manifest as disease and how these conditions could be treated. Thus, Ig replacement therapy in the form of intravenous Ig is often used to treat some of these immunodeficient conditions [1,4]. Conversely, rituximab, a chimeric anti-human CD20 monoclonal antibody (mAb), is used therapeutically for the treatment of some autoimmune conditions, owing to its ability to deplete B cells, even though not all of these cells are necessarily pathogenic [3,7–9]. Despite these advances, much of our current understanding of human B cells arises from animal studies. Furthermore, our understanding is potentially limited due to difficulties in accessing appropriate human tissue samples and the logistic and ethical issues surrounding performance of human *in vivo* research. Here, we will review recent findings regarding human B-cell development as well as the genetic and cellular defects underlying disease states that result from dysregulation of B-cell behavior.

B-cell development

B-cell development commences in the bone marrow (BM) from hematopoietic stem cell (HSC) progenitors that progress through the sequential stages that define the B-cell lineage pathway. HSCs initially develop into common lymphoid progenitors, which have the potential to give rise to T cells, B cells and natural killer cells (reviewed in [10]). Common lymphoid progenitors then mature into pro-B cells; it is at this stage that rearrangement of the Ig heavy chain genes commences. This involves the initial joining of

 10.1586/ECI.10.55 ISSN 1744-666X

one diversity (D_H) gene to a joining (J_H) gene at the early pro-B-cell stage and the subsequent alignment of a variable (V_H) gene to the DJ_H element at the late pro-B-cell stage. Successful VDJ_H rearrangement allows pro-B cells to progress to the pre-B-cell stage of development, which is defined by the expression of cytoplasmic Igμ heavy chains, which pair with a surrogate light chain to form the pre-B-cell receptor (BCR). Rearrangement of the Ig light chain (V–L) subsequently results in the expression of a functional surface IgM molecule that defines immature B cells (Figure 1) [11,12].

During B-cell development, positive and negative selection occurs to regulate the development and function of self-reactive B cells. Immature BM resident B cells that recognize self-antigen are either eliminated (clonal deletion) or rendered unresponsive (anergic), or their BCR is revised to avoid self-reactivity (receptor editing) (reviewed in [13]). High-affinity interactions between the BCR and multimeric self-antigen typically result in deletion, while low-affinity interactions, such as exposure to soluble self-antigen, render the B cells anergic or induce receptor editing [13]. Those cells that survive this selection process are exported to the periphery as transitional B cells.

Interestingly, a large proportion (~40%) of transitional B cells present in the peripheral blood of healthy human donors have reactivity to self-antigens [14,15]. The differentiation of transitional B cells into mature naive B cells is accompanied by a twofold reduction in the frequency of autoreactive cells (from 40% to 20%). This suggests the existence of another selection step at the transitional-to-mature naive stage of B-cell development. Therefore, only a small proportion of the cells generated in the

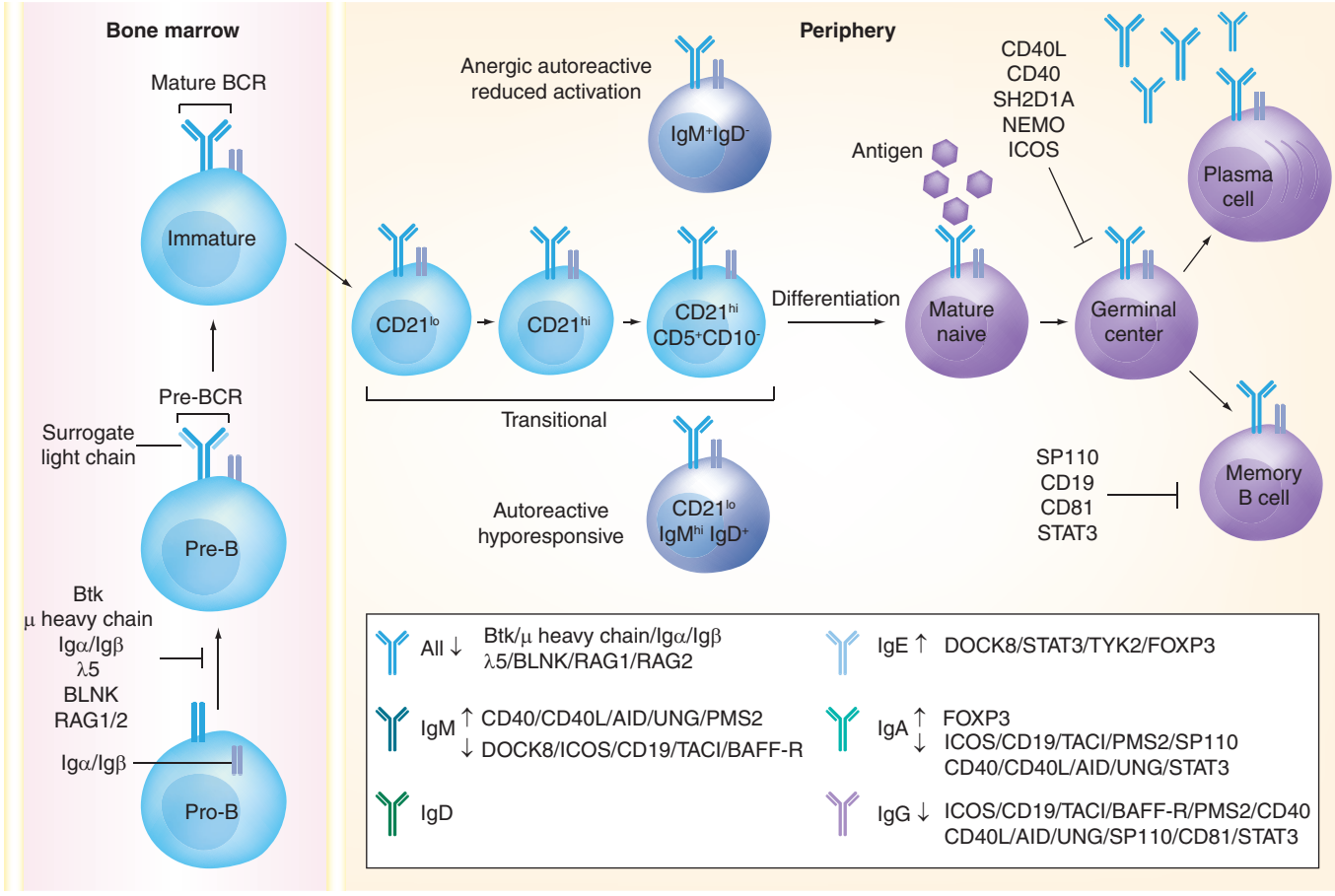

Figure 1. Human B-cell development/differentiation and specific mutations that cause diseases. B-cell development begins in the bone marrow from hematopoietic stem cells. Pro-B cells give rise to pre-B cells, which then become immature B cells. These cells then exit the bone marrow and enter the periphery as transitional B cells. To date, there are three known populations of human transitional B cells: $CD10^+CD21^{lo}$, $CD10^+CD21^{hi}$ and $CD10^-CD21^{hi}CD5^+$. Transitional B cells that survive selection then enter the mature B-cell repertoire as naive cells. Upon encountering T-cell-dependent antigen, naive B cells differentiate into germinal center cells that can follow one of two fates: plasma cells that produce antigen-specific immunoglobulin (Ig) and predominantly migrate to the bone marrow, or memory B cells that remain in the circulation or enter secondary lymphoid tissues and are capable of giving a rapid response following re-encounter with the immunizing antigen. Two populations of naive B cells (based on expression of unmutated Ig variable region genes) – IgM^+IgD^- and $CD21^{lo}IgM^+IgD^+$ B cells – have recently been described; however, their exact position in the development pathway of human B cells remains unclear. Specific mutations have been identified that affect distinct stages in the developmental and differentiation processes. Mutations affecting the ability of human B cells to produce total Ig, or specific isotypes, are indicated in the white box. Several of these are discussed in this review (e.g., *CD40*, *CD40L*, *ICOS*, *NEMO*, *STAT3*, *CD19*, *CD20*, *CD81* and *BAFF-R*); for the other genes indicated (e.g., *UNG*, *PMS2*, *AID*, *FOXP3*, *TYK2*, *DOCK8*, *SP110* and *TACI*, the reader is directed to [4,5] for an overview).

BM make it through the distinct stages of selection, thereby ensuring that only non-self-reactive cells are selected into the circulating mature B-cell pool. Transitional B cells have been a focus of intense research during the past decade as this cellular subset represents an important link between B-cell development in the BM and that in the periphery.

Human transitional B cells

Identification & initial phenotypic characterization

The term 'transitional B cell' was first coined by Carsetti *et al.* in 1995 [16]. Since then, it is now recognized that up to three subsets of murine transitional B cells can be identified (reviewed in [17]). In mice, two classifications have been described to distinguish subsets of transitional B cells: Loder *et al.* [18] defined transitional type 1 (T1) B cells as $CD24^{hi}CD21^{lo}CD23^{lo}IgM^{hi}IgD^{lo}$ and transitional type 2 (T2) as $CD24^{hi}CD21^{hi}CD23^{hi}IgM^{hi}IgD^{hi}$ while Allman *et al.* [19] defined T1 as $AA4^{+}CD23^{-}IgM^{hi}$, T2 as $AA4^{+}CD23^{+}IgM^{hi}$ and transitional type 3 (T3) as $AA4^{+}CD23^{+}IgM^{lo}$ (Table 1) [19]. By contrast, human transitional B cells were only first described in 2004 [20]. Consequently, our understanding of the human counterpart has greatly lagged behind that of mouse transitional B cells. Flow cytometric studies established that human transitional B cells have a distinct phenotype in that they are $CD19^{+}CD10^{+}CD24^{++}CD38^{++}$ while naive and memory B cells are $CD19^{+}CD27^{-}IgM^{lo}IgD^{hi}$ and $CD19^{+}CD27^{+}$, respectively (Table 1). Similar to naive B cells, transitional B cells also express IgM and IgD, while memory B cells can express IgM, IgG or IgA [21–23]. In contrast to naive and memory B cells, human transitional B cells express elevated levels of CD5 (Table 1) [21,22]. Human transitional B cells represent approximately 10% of adult peripheral blood B cells [21–23] but their frequencies in other tissues are variable. For example, 30% of B cells in umbilical cord blood and BM are transitional cells, while in secondary lymphoid tissues (i.e., the spleen, lymph node and tonsils), they comprise less than 0.5–5% of the B-cell compartment [21–23].

Delineation of subsets of human transitional B cells

Attempts have been made to further separate human transitional B cells into T1 and T2 subsets with the contention being that CD24 and CD38 expression is reduced as the earliest transitional B cells develop into subsequent stages [21,24]. A caveat to this is that the $CD24^{++}CD38^{++}$ B-cell subset is a rather homogeneous population and delineation of this population into distinct subset is subjective. We recently reported the identification of two human transitional B-cell subsets based on the differential expression of CD21: $CD10^{+}CD21^{lo}$ and $CD10^{+}CD21^{hi}$ transitional B cells [23].

Table 1. Phenotypic features of human and murine peripheral B-cell subsets.

B-cell subset	Phenotype	
	Murine B cells	*Human B cells*
Transitional 1	$B220^{+}$ $AA4.1^{+}$ $CD24^{hi}$ $CD21^{lo}$ $CD23^{lo/-}IgM^{hi}$ IgD^{lo}	$CD10^{+}$ $CD20^{+}$ IgM^{+} IgD^{lo} $CD27^{-}$ $CD40^{+}$ $CD5^{hi}$ $CD9^{hi}$ $CD24^{hi}$ $CD38^{hi}$ $CD21^{lo}$ $CD44^{lo}$ $CD23^{lo}$ $Bcl\text{-}2^{-/lo}$ MHC class II^{+} $BAFF\text{-}R^{lo}$ $TACI^{-}$
Transitional 2	$B220^{+}$ $AA4.1^{+}$ $CD24^{hi}$ $CD21^{hi}$ $CD23^{hi}$ IgM^{hi} IgD^{hi}	$CD10^{+}$ $CD20^{+}$ IgM^{+} IgD^{+} $CD27^{-}$ $CD40^{+}$ $CD5^{hi}$ $CD9^{hi}$ $CD24^{hi}$ $CD38^{hi}$ $CD21^{hi}$ $CD44^{+}$ $CD23^{+}$ $Bcl\text{-}2^{lo}$ MHC class II^{+} $BAFF\text{-}R^{hi}$ $TACI^{-}$
Transitional 3 (human 'pre-naive' $CD5^{+}$)	$B220^{+}$ $AA4.1^{+}$ $CD23^{+}$ IgM^{lo}	$CD5^{hi}$ $CD9^{lo}$ $CD10^{-}$ $CD23^{+}$ $CD24^{int}$ $CD38^{int}$ IgD^{+} $CD27^{-}$ $CD40^{+}$ MHC class II^{+} $BAFF\text{-}R^{hi}$ $TACI^{-}$
Naive	$B220^{+}$ IgD^{hi} IgM^{lo} $CD21^{lo}$ $CD23^{hi}$ MHC class II^{+}	$CD10^{-}$ $CD20^{+}$ IgM^{lo} IgD^{hi} $CD27^{-}$ $CD5^{lo}$ $CD9^{lo}$ $CD24^{+}$ $CD38^{+}$ $CD39^{+}$ $CD40^{+}$ $CD21^{hi}$ $CD44^{+}$ $CD23^{++}$ $CD77^{-}$ $CD80^{-}$ $CD86^{-}$ $CD95^{-}$ $Bcl\text{-}2^{+}$ $CXCR4^{+}$ $CXCR5^{+}$ $CCR7^{+}$ MHC class II^{+} $BAFF\text{-}R^{hi}$ $TACI^{-}$
Germinal center	$B220^{+}$ $CD95^{hi}$ $GL\text{-}7^{+}$ $CD38^{+/-}$ MHC class II^{+}	$CD10^{+}$ $CD20^{+}$ Ig^{lo} $CD23^{-}$ $CD27^{+}$ $CD24^{+}$ $CD38^{hi}$ $CD39^{-}$ $CD21^{hi}$ $CD44^{-/+}$ $CD23^{++}$ $CD40^{+}$ $CD77^{+}$ $CD80^{+}$ $CD86^{+}$ $CD95^{+}$ $Bcl\text{-}2^{-}$ $CXCR4^{+}$ $CXCR5^{+}$ $CCR7^{-}$ MHC class II^{+} $TACI^{-/+}$ $BCMA^{+}$
Memory	$B220^{+}$ $CD80^{+}$ $CD95^{+}$ $CD73^{+}$ $CD62L^{hi}$ MHC class II^{+}	$CD10$ $CD20^{+}$ IgM/G/A^{+} IgD^{lo} $CD27^{+}$ $CD24^{+}$ $CD38^{lo}$ $CD21^{hi}$ $CD44^{+}$ $CD23^{lo/-}CD77^{-}$ $CD39^{+}$ $CD40^{+}$ $CD80^{+}$ $CD86^{+}$ $CD95^{+}$ $Bcl\text{-}2^{hi}$ $CXCR4^{+}$ $CXCR5^{+}$ $CCR7^{+}$ MHC class II^{+} $BAFF\text{-}R^{lo}$ $TACI^{+}$ $BCMA^{-}$
Plasma cell	$B220^{lo/-}$ $CD138^{++}$ $CD19^{lo/-}$ $B220^{lo/-}$ MHC class II^{-} $CD38^{+/-}$ $CD43^{-}$ $CXCR5^{-}$ $CXCR4^{+}$ $BCMA^{+}$	$CD10^{-}$ $CD20^{lo}$ sIg^{lo} cIg^{hi} $CD9^{hi}$ $CD21^{-}$ $CD23^{-}$ $CD27^{hi}$ $CD38^{hi}$ $CD39^{hi}$ $CD40^{+}$ $CD21^{-/lo}$ $CD44^{+}$ $CD23^{-}$ $CD77^{-}$ $CD86^{+}$ $CD95^{+}$ $CD138^{-/+}$ $CXCR4^{+}$ $CXCR5^{-}$ $CCR7^{-}$ MHC class II^{+} $BAFF\text{-}R^{lo/-}$ $BCMA^{-/+}$

BAFF-R: B-cell-activating factor receptor; Bcl: B-cell lymphoma; BCMA: B-cell maturation antigen; CCR: C-C chemokine receptor; CXCR: C-X-C chemokine receptor; TACI: transmembrane activator and calcium modulator and cyclophilin ligand interactor.
Phenotype of human and murine B cells was derived from references [16,19,23,54,56,61–63,97–103].

Our data revealed that these two subsets have distinct phenotypes, with the CD21^{hi} subset expressing higher levels of Bcl-2, CD23, CD44, IgD and B-cell-activating factor receptor (BAFF-R) than the CD21^{lo} subset (Table 1). We also showed that the CD21^{hi} subset is the more mature subset based on the increased survival correlating with elevated Bcl-2 expression and greater proliferation and antibody production *in vitro* [23]. Lastly, another approach that has been used to distinguish B-cell subsets is the presence or absence of the ABCB1 transporter, the expression of which can be visualized by extrusion of the dye rhodamine 123 (R123). By using this method, Anolik and colleagues identified a third population of transitional B cells, T3 B cells, which are CD10^{-}CD24^{int}CD38^{int}IgD^{+}CD27^{-} and could be distinguished from naive B cells by their poor ability to extrude R123 [25]. We hypothesize that this population is similar, if not identical, to the recently described population of pre-naive B-cell populations that are CD5^{+} but lack expression of CD10 [26].

The sharp drop in the frequency of self-reactive cells at the transitional-to-naive stage of B-cell development indicates that a selection event occurs in the periphery. We also noted that the production of autoreactive antibodies by transitional B cells decreased as they developed from a CD21^{lo} to a CD21^{hi} phenotype [23]. This suggests that selection takes place at the CD21^{lo} to CD21^{hi} stage of transitional B-cell development and provided additional evidence that the CD21^{lo} transitional B cells were the precursors to the CD21^{hi} subset. We employed several approaches to establish a precursor/product relationship between these subsets. First, by examining patients with X-linked agammaglobulinemia due to mutations in *BTK* we found that, although these patients had a severe reduction in the number of peripheral B cells, the vast majority of these were of a CD21^{lo} transitional phenotype. This raised the likelihood that the first population of B cells exported from the BM to the periphery are CD10^{+}CD21^{lo} cells. Second, microarray analysis revealed differential gene expression between the CD21^{lo} and CD21^{hi} transitional B-cell subsets in that lymphoid-enhancing factor 1 (LEF-1) was highly expressed in the CD21^{lo} transitional B cells and expression decreased in the CD21^{hi} transitional subset [23]. Interestingly, a recent study by Hystad *et al.* that analyzed gene-expression profiles of early stages of human B-cell development in the BM showed that expression of LEF-1 was low or absent in CD34^{+}CD10^{+}CD38^{+}CD19^{-} early B cells, was induced in CD34^{+}CD10^{+}CD19^{+}IgM^{-} pro-B cells but then decreased as these cells matured into CD34^{-}CD10^{+}CD19^{+}IgM^{-} pre-B and CD34^{+}CD10^{+}CD19^{+}IgM^{+} immature B cells [27]. Such differential expression of LEF-1 would be consistent with a linear model of B-cell development whereby immature BM B cells give rise to CD21^{lo} B cells, which then give rise to CD21^{hi} transitional B cells (Figure 1).

Transitional B cells in human diseases

Immunodeficiency & stem cell transplant

Transitional B cells have a unique phenotype compared with other B-cell subsets in that they are CD10^{+}CD5^{+}CD9^{+} and IgM^{hi}CD38^{hi}CD24^{hi} [22,23]. This distinction has allowed detailed analysis of human transitional B cells to be performed. Transitional B cells have been found to be increased in the peripheral blood of several immunodeficient conditions such as X-linked lymphoproliferative disease (XLP) [22], STAT3 deficiency [28] and HIV infection [29], as well as following HSC transplant (HSCT) [22,23]. Analysis of lymphocyte reconstitution during the first 12 months post-HSCT indicated that the majority of B cells appearing in the initial stages are transitional cells [23]. This is consistent with early studies that established that B cells generated following HSCT are CD5^{+}, IgM^{hi}, IgD^{hi} and CD38^{hi}, which are all features of transitional B cells [30,31]. We extended these studies by demonstrating that the appearance of CD21^{lo} transitional B cells preceded that of CD21^{hi} transitional B cells in post-HSCT patients [23]. Post-HSCT, the frequency of transitional B cells decreased while the proportion of naive cells increased over time. Interestingly, B-cell reconstitution in patients with B-cell lymphoma or rheumatoid arthritis (RA) undergoing rituximab therapy follows a similar pattern to that observed for post-HSCT patients. Following reconstitution of the transitional B-cell compartment, mature cells began to increase in frequency, confirming that transitional B cells are the necessary intermediates for the development of mature B cells from the immature precursors present in the BM [22,23]. Meanwhile, the recovery of memory B cells is delayed and remains below that observed in normal healthy donors for up to 1 year post-therapy [24,32–34]. These studies showed that transitional B cells are the earliest cell to return when B cells are depleted either due to rituximab or chemotherapy/irradiation treatment.

Autoimmune diseases

In autoimmune diseases such as systemic lupus erythematosus (SLE) and RA, purging of self-reactive B cells from the mature B-cell repertoire at the transitional-to-mature naive stage is compromised. This is based on the observation that the frequency of autoreactive cells in the transitional and mature naive B-cell compartments of SLE and RA patients is the same (~40%), which contrasts with healthy donors where there is a 50% decrease (i.e., from 40% in the transitional subset to 20% in the naive subset) [14,15,35,36]. Perturbations to B-cell development at the transitional stage potentially cause autoimmune development since this B-cell subset carries autoreactive potential [14,23]. Indeed, the first detailed characterization of human transitional B cells noted that these cells are increased in frequency in the blood of patients with SLE [21], an observation recently confirmed in a study of a larger cohort of SLE patients [37]. This infers that an understanding of the biology of human transitional B cells, especially the CD21^{lo} transitional subset since these are enriched for autoreactive B cells [23], may aid the development of therapeutic interventions that target this population to improve the treatment of these autoimmune conditions.

Unexpectedly, a new study showed that transitional B cells potentially play an important role in regulating autoimmunity. The term 'regulatory' B cell (Breg) has been used to describe B cells with regulatory function in animal models of human diseases [38–40]. Murine Breg cells are contained with the CD1d^{hi} or CD1d^{hi}CD5^{+} subset of splenic B cells and are enriched for

IL-10-producing cells [41]. It is generally accepted that Bregs exert their regulatory effect through the production of IL-10. For instance, protection against the development of the multiple sclerosis model experimental autoimmune encephalomyelitis (EAE), the RA model collagen-induced arthritis and also diabetes in mice was compromised in the presence of B cells derived from IL-10-deficient mice [41]. These murine studies have provided convincing evidence that B cells are capable of not only positively regulating immune responses through the production of specific Ig and costimulating $CD4^+$ T cells, but also of suppressing the function of potentially autoreactive lymphocytes in the setting of inflammatory or autoimmune conditions. Until recently, no studies had characterized these cells in humans. Blair *et al.* found that in healthy individuals, $CD24^{hi}CD38^{hi}$ B cells (i.e., those with a transitional phenotype) have the ability to suppress production of IFN-γ and TNF-α by Th1 cells via an IL-10-dependent mechanism following stimulation through CD40 [37]. Interestingly, $CD24^{hi}CD38^{hi}$ (i.e., transitional) B cells isolated from SLE patients produced less IL-10 than those from healthy controls. It is possible that dysfunctional transitional B cells contribute to the development of autoimmunity on two levels. First, they are enriched for the production of autoreactive antibodies [14,23], and second, reduced production of IL-10 compromises the suppressive activity that ordinarily regulates the behavior of potentially pathogenic/proinflammatory Th1 cells [37]. While impaired IL-10 production by a subset of B cells in SLE patients contrasts with previous studies, which found that SLE B cells were responsible for the spontaneous secretion of IL-10 observed for mononuclear cells obtained from SLE patients, and that IL-10 is increased in the serum of patients with SLE [42], it provides further evidence that transitional B cells are an attractive cellular target for therapeutic intervention in SLE, and potentially other autoantibody-mediated diseases.

The requirements of B-cell development: differences between mice & humans

B-cell development depends not only on intrinsic factors such as Ig rearrangement and expression of a mature BCR, but also on extrinsic factors such as cytokines. Analysis of gene-targeted mice and human immunodeficiencies has revealed critical roles for key genes in B-cell development in both species. While the requirements for B-cell development in humans and mice are generally similar, there are notable differences. In this next section we contrast the roles of two cytokines – BAFF and IL-7 – in human and mouse B-cell development.

BAFF

B-cell-activating factor belonging to the TNF family (BAFF; also known as TALL-1, BLyS, THANK, zTNF4 and TNFSF13B) is important in murine B-cell development because it appears to be a potent survival factor for transitional B cells and is critical for the normal function of the immune system [43–47]. BAFF exerts its effects by interacting with three receptors belonging to the TNF receptor (TNF-R) superfamily: B-cell maturation antigen (BCMA), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and BAFF receptor (BAFF-R), also known as BLyS receptor 3 (BR3) [43–46]. The effects of BAFF on B-cell development are dependent on signaling through BAFF-R [43–47]. BAFF can also promote the survival of plasmablasts/plasma cells; however, this appears to involve BCMA rather than BAFF-R [48,49]. By contrast, TACI appears to be a negative regulator of B-cell function [43,44,46]. Much attention has been given to BAFF in the context of human disease for several reasons: there are phenotypic similarities between BAFF transgenic mice and humans with autoimmune diseases such as Sjögren's syndrome and SLE [43,44,47]; elevated levels of BAFF have been detected in the serum of patients with autoimmune diseases including SLE and Sjögren's syndrome [50,51]; excess BAFF can break peripheral B-cell tolerance in murine models of autoimmunity [52,53]; and disease severity in murine models of human SLE can be improved following BAFF blockade [43,44]. Collectively, these studies lead to the conclusion that abnormal levels of serum BAFF contribute to disease pathogenesis in autoimmune conditions by enhancing the survival of autoreactive B cells. For this reason, a great deal of enthusiasm has been generated over the prospect of BAFF inhibitors as therapies for the treatment of human autoantibody-mediated autoimmune conditions [3,9,47]. However, the results from clinical trials using the neutralizing anti-BAFF mAb belimumab have been underwhelming: Phase II trials failed to reach their primary end point, with no significant differences in disease score or autoantibody levels detected between the treatment and placebo groups [47,54,55]. Although two Phase III trials did reach their primary end point, the response rates were modest: 57.6 and 43.2% in the treatment groups versus 43.6 and 33.8% in the placebo groups for the two trials, respectively [56]. These studies indicated that therapeutic targeting of BAFF may not be a particularly efficacious strategy for treating B-cell-mediated autoimmune conditions such as SLE [47,55,56].

These findings are perhaps not that surprising, since there have been several indications in the literature that the function of BAFF on human (and nonhuman primate) B cells may be different to that on murine B cells. For instance, *in vivo* BAFF blockade in monkeys only reduced peripheral B-cell numbers by approximately twofold [57,58], while in mice the effect was more than tenfold [43,45,47]. Similarly, while BAFF is an efficient pro-survival factor for murine transitional B cells [59], it has no effect on human transitional B cells [21,23]. Lastly, the recent identification of humans with mutations in *TNFRSF13C*, which encodes BAFF-R, further highlighted differences in the function of BAFF during B-cell development and differentiation in mice and humans. Thus, while deficiency in BAFF-R in mice leads to a significant reduction in B cells, particularly at the transitional and mature B-cell stages of development [43,44], the percentage of human $CD10^+$ transitional B cells was increased in BAFF-R-deficient patients; however, the absolute number was still within the normal range [60]. There was also a detectable population of class-switched memory B cells, and strong T-cell-dependent immune responses in BAFF-R-deficient individuals [60]; by contrast, BAFF-R is required for T-cell-dependent responses in mice, as evidenced by a reduction in the formation of germinal centers [46], which are generally

considered to be required for the generation of high-affinity class-switched memory and plasma cell responses [6]. Perhaps the most striking difference between BAFF-R-deficient mice and humans is that the first BAFF-R-deficient patient did not exhibit any clinical symptoms of immunodeficiency until the age of 37 years, while the second individual remained healthy until 70 years of age [60]. This contrasts with *Baffr*-deficient mice, which develop B-cell deficiency at a relatively young age (6–8 weeks) [43,44]. Thus, it appears that, in contrast to mice, the immune systems of BAFF-R-deficient humans retain sufficient antibody-producing B cells to provide effective host defense following natural infection or immunization [60]. Taken together, the requirement of BAFF-R (and by inference BAFF) during B-cell development and differentiation is significantly different between mice and humans. The association of BAFF-R deficiency with adult-onset immunodeficiency implies that the predominant effect of BAFF on human B cells is not at the transitional stage of B-cell development but rather during the differentiation of memory B cells into plasmablasts/plasma cells [49]. Irrespective of these differences, it is clear that BAFF-R is an excellent marker of human mature B cells. Based on this, there is no reason why targeting BAFF-R with a specific mAb could not be used as an approach for B-cell depletion therapy akin to that achieved by rituximab. This may have an advantage over rituximab in that BAFF-R is expressed on some subsets of plasma cells – for example, tonsil plasma cells as well as plasmablasts [61] – whereas expression of CD20 is down-regulated from these cells [62]. Since pathogenic autoantibodies are produced by both short-lived plasmablasts and long-lived plasma cells [63], plasma cell depletion would be an important component of ameliorating the serum levels of autoantibodies. The elimination of all plasma cells would obviously also compromise humoral immunity provided by antigen-specific plasma cell-produced antibodies; this would need to be balanced with the benefit gained by reducing disease score in such cases of autoimmunity.

IL-7

Extensive focus has also been given to IL-7. IL-7 is produced by nonhematopoietic stromal cells and was initially identified and cloned by Namen *et al.* for to its ability to stimulate short-term proliferation of murine B cells in the absence of stromal cells [64]. IL-7 and its receptor subunits, the IL-7Rα chain and the common γ chain (γc) [65], have since been shown to be important in murine B-cell development. In the absence of IL-7/IL-7R signaling due to gene targeting or mAb-mediated neutralization, murine B-cell development *in vivo* and *in vitro* was essentially abolished [66–70]. In contrast to these studies of murine B cells, human B-cell development appears to be independent of IL-7. This is based on the following observations. First, the *in vitro* differentiation of human B cells from stem cell precursors can be achieved in the absence of endogenous or exogenous IL-7 [71]. Second, and more revealing, patients with mutations in IL-7R [72–74], γc [75] or JAK3 [76,77], which signals downstream of the IL-7R/γc complex, displayed normal or increased numbers of B cells in their peripheral blood. Thus, although IL-7 can have some effects on human B-cell precursors *in vitro* [78–80], it clearly does not have an obligatory role in B-cell development *in vivo*, which is in stark contrast to murine B cells. An important area for future investigation, therefore, is the elucidation of the critical cytokines required for human B-cell development *in vivo*.

Recent identification of novel autoreactive human B-cell subsets

Transitional B cells continue their maturation in the periphery where they are selected to become mature naive B cells that have reduced autoreactivity and produce non-self antigen-specific Ig in response to appropriate cognate stimulation. Despite the advances made in characterizing transitional B-cell subsets, several recent studies have identified additional subsets of B cells that share features with transitional and/or naive B cells but are in fact distinct from these populations.

Duty *et al.* identified a subset of $IgD^+IgM^-CD27^-$ (i.e., 'naive') B cells comprising less than 2% of all circulating B cells [81]. These cells express unmutated (i.e., germline) Ig variable region genes, and the Ig produced binds self-antigen. These cells also have impaired responsiveness to BCR engagement compared with IgD^+IgM^+ $CD27^-$ naive B cells. While the enrichment for autoreactivity and reduced responsiveness are features of human transitional B cells, these cells do not appear to be transitional B cells since their extended phenotype resembles that of naive cells (i.e., they are $CD10^-CD5^{lo}CD23^{hi}$). Rather, it was concluded that they are anergic B cells rendered unresponsive to antigen stimulation by peripheral tolerizing mechanisms. While this study showed that anergized self-reactive naive IgD^+IgM^- B cells are present in healthy individuals, it remains to be determined whether they are expanded and whether the tolerance mechanisms that ensure their hyporesponsiveness in normal individuals are compromised in cases of antibody-mediated autoimmune diseases. Furthermore, the exact precursor of this population – for example, a late transitional B cell or a naive B cell – is undefined at this stage (Figure 1).

Isnardi *et al.* and Rakhmanov *et al.* have both recently analyzed a unique B-cell subset, characterized by low expression of CD21 ($CD21^{lo}$), which is present at a low frequency (<1% of total B cells) in the peripheral blood of healthy individuals but is substantially expanded in a subset of patients with common variable immunodeficiency (CVID) (~20% of total B cells) or RA (>5% of total B cells) [82,83]. These $CD21^{lo}$ B cells bear a striking resemblance to human anergic B cells [81] in that they express unmutated Ig variable region genes but autoreactive BCRs and impaired signaling following BCR engagement. However, in contrast to anergic B cells, the $CD21^{lo}$ B cells appear to be activated, since they express CD86, CD95 and inflammatory chemokine receptors (CXCR3 and CXCR6) but have down-regulated cytokine (IL-4 receptor) and chemokine receptors (i.e., CXCR4, CXCR5 and CCR7) that are typically expressed by naive B cells. They have also undergone greater rounds of proliferation *in vivo* than naive B cells from healthy individuals [83] and express an array of inhibitory receptors not observed on B cells from healthy donors [82,83]. We would speculate that this population of $CD21^{lo}$ B cells is regulated in healthy individuals by specific tolerance mechanisms, while in diseases such as CVID and RA, such mechanisms are impaired, leading to their increased

frequency in these situations. It is likely that the production/expression of autoreactive Ig by the $CD21^{lo}$ B cells contributes to autoimmune pathology in autoimmune conditions; this is consistent with their over-representation not only in RA but also in the subset of CVID patients that have the greatest incidence of autoimmune cytopenias [84]. The precursor of these $CD21^{lo}$ B cells is unknown but it could be either $CD10^{+}CD21^{lo}$ transitional B cells, as indicated by their low expression of CD21 and high autoreactivity [23], or $CD10^{+}CD21^{hi}$ transitional or mature naive B cells that are not negatively selected against in the periphery (Figure 1). It is also unclear whether the anergic B cells reported by Duty *et al.* [81] correspond to the normal counterpart of the $CD21^{lo}$ B cells described by Rakhmanov *et al.* [83] and Isnardi *et al.* [82], since expression of CD21 on anergic B cells was not determined. Thus, it will be important to elucidate the origins of anergic B cells in healthy individuals and $CD21^{lo}$ B cells in cases of immune dyscrasias, and determine the relationship of these B-cell subsets with other well-defined stages of B-cell development. This will provide significant insights into the processes of B-cell positive and negative selection that under normal conditions ensures the generation of a repertoire of mature B cells purged of autoreactive clones, but can also yield a B-cell repertoire enriched with self-reactive specificities in cases of immune dysregulation.

Immunodeficiencies reveal requirements for specific molecules during B-cell differentiation

A prominent role of naive B cells is to differentiate into effector (memory and plasma) cells following interaction with, and subsequent activation by, foreign antigen. Several loss-of-function or loss-of-expression mutations in genes that cause specific immune-deficient conditions impair the ability of the naive B cells to undergo Ig class-switching to become memory B cells or Ig-producing plasma cells, thereby compromising long-lived humoral immunity in the affected patients [6]. Patients with mutations in *SH2D1A*/SAP (which causes XLP [85,86]), *CD40L*, *CD40* and *NEMO* (which cause hyper-IgM syndrome [87,88]), *ICOS* [89] or *CD19* (which cause CVID) [90,91] or *STAT3* (which causes autosomal dominant hyper-IgE syndrome [28,92]), all have reductions in memory B cells, particularly $CD27^{+}IgM^{-}IgD^{-}$ class-switched B cells (Figure 1). These conditions present with normal/increased levels of serum IgM but a deficiency in switched Ig isotypes and/or impaired antigen-specific antibody responses. Memory B cells fail to be generated in these patients, either because of an intrinsic requirement for expression and function of the mutant gene in the B cells (e.g., *CD40*, *NEMO*, *CD19* and *STAT3*), or the defect is extrinsic to the B cells reflecting impaired provision of $CD4^{+}$ T-cell help that is required for B-cell differentiation (e.g., CD40L, ICOS and SH2D1A/SAP). The deficit in memory B cells due to STAT3 mutations may also reflect a requirement for STAT3 in $CD4^{+}$ T cells (in addition to B cells), since studies in mice have demonstrated the importance of STAT3 in the differentiation of naive $CD4^{+}$ T cells in follicular T helper cells [93], the subset of $CD4^{+}$ T cells specialized to provide help to B cells for their maturation into memory and plasma cells.

Recently, homozygous mutations in CD81, which forms a multimolecular complex with CD19, CD21 and CD225 on the surface of mature B cells, were found to cause an antibody deficiency characterized by impaired formation of memory B cells and poor responses to both T-cell-dependent (i.e., tetanus) and polysaccharide (i.e., pneumococcal) antigens [94]. The phenotype of this individual was very similar to that of patients with mutations in CD19 [90,91] – this is consistent with the reduced levels of CD19 on their B cells (Figure 1). Apart from being required for the stable expression of CD19 on the B-cell surface, the exact function of CD81 in B-cell biology is unknown. Thus, it is unclear whether the antibody deficiency in this case of CD81 mutation resulted from a defect in CD81 function in B cells, or reflected impaired expression of the CD19 coreceptor. Irrespective of this uncertainty, what is clear is that the integrity of the multimeric complex comprising CD19, CD81, CD21 and CD225 is requisite for the normal functioning of human B cells in response to foreign antigens.

Interestingly, a patient with homozygous mutations in CD20 also developed an immune deficiency associated with a reduction in memory B cells. However, in contrast to the immune deficiencies detailed previously, the response of this patient to tetanus toxoid vaccination was normal, however, the recall response to pneumococcal polysaccharide vaccination was poor or absent, suggesting a defect in T-cell-independent B-cell responses [95].

These studies of monogenic immunodeficiencies have revealed key regulators of the differentiation of naive B cells into effector cells following encounter with T-dependent and/or T-independent antigens. This implies that pharmacological modulation of the function of molecules such as CD40/CD40L, ICOS, SAP, STAT3, CD19/CD81 or CD20 may allow the immune response of immunodeficient or autoimmune patients to be improved or attenuated, respectively, thereby alleviating some of the clinical features of these conditions. This is particularly relevant for cases of autoimmunity where defects in memory B-cell formation and function have been noted [2].

Expert commentary & five-year view

The past two decades have seen a great improvement in our understanding of the requirements for and regulators of human B-cell development and differentiation, as well as the identification and refined characterization of numerous stages of B-cell development. We now also have a real appreciation of the importance of studying human primary B cells to elucidate the mechanics of their behavior, rather than simply relying on murine models. Despite this, significant gaps remain in our understanding of the biology of human B cells and their development and function. There are substantial differences between murine and human B cells with respect to the factors necessary for their development. The fact that B-cell development is intact in humans with mutations in the IL-7R signaling pathway [72–77] and only mildly affected in those with mutations in *BAFF-R* [60], highlights the redundancy of these cytokines and the need to identify functional homologues of murine IL-7 and BAFF that play analogous roles during human B-cell development. Elucidation of such molecules may provide novel targets for the treatment of B-cell dyscrasias. It

will also be important to gain a greater understanding of human anergic B cells [81] and putative Bregs [37] and the relationship between these cells and other recently defined subsets, namely transitional/pre-naive B cells [23,25,26] and the $CD21^{lo/-}$ B cells identified in several cases of autoimmunity [82,96]. For example, the signals required for the maturation of $CD21^{hi}$ transitional B cells into the pre-naive $CD5^{+}CD10^{-}$ B-cell subset remain to be determined, as does the precursor/normal counterpart of the $CD21^{lo}$ B cells detected in diverse disease states. Further study of autoimmune and immunodeficient individuals with defined genetic mutations will no doubt shed substantial light on the requirements for the development, survival and function of these B-cell subsets. Since several of these subsets (anergic, transitional and $CD21^{lo}$) harbor autoreactive specificities [14,23,81,96], it will be important to elucidate regulatory mechanisms that prevent overt production of autoantibodies by these cells. Lastly, while murine Bregs and their contribution to controlling autoimmunity in models of human diseases have been the focus of intense research [41], the discovery of the human counterpart has only recently been reported [37]. The functionality of these cells in normal and autoimmune individuals requires validation, however, their initial characterization has clearly provided a platform for future studies. With recent discoveries relating to the molecular identification of genetic lesions underlying immunodeficiencies, together with improved tools for studying human B-cell subsets, function and dysfunction, there is no doubt that future investigation will continue to provide critical insights into the pathogenesis of B-cell-mediated diseases and the identification of molecules or signaling pathways that can be targeted for the improved treatment of conditions including autoimmunity, immunodeficiency and malignancy.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- The B-cell population present in human peripheral blood is heterogeneous, being comprised of transitional, naive and memory subsets.
- Transitional B cells correspond to recent bone-marrow emigrants, which enter the periphery and can then give rise to mature naive B cells.
- Three subsets of 'pre-naive' transitional B cells have been identified; these can be resolved from each other by the differential expression of CD21, CD10 and the ABCB1 transporter.
- The $CD21^{lo}$ subset of human transitional B cells appears to be the immediate product of immature B cells and gives rise to the more mature $CD21^{hi}$ subset of transitional B cells; these subsets differ with respect to gene expression, phenotype and autoreactive potential.
- Unlike murine B cells, the cytokine B-cell activating factor does not appear to exert a potent prosurvival effect on human transitional B cells; this suggests that alternative soluble factors regulate selection and survival of human B cells at this critical stage of development.
- $CD21^{-/lo}$ B cells represent a population of autoreactive naive B cells that are functionally unresponsive to signaling through the B-cell receptor. However, in contrast to *bona fide* naive B cells, these $CD21^{-/lo}$ cells exhibit a phenotype and *in vivo* proliferation history that is consistent with activated B cells. It is possible that these changes reflect chronic stimulation with self-antigen.
- Differentiation of naive B cells into memory B cells is compromised in many cases of monogenic immunodeficiencies. Thus, there is strong evidence for important roles of CD40/CD40 ligand, NEMO, ICOS, CD19, CD81, SH2D1A/SAP and STAT3 in T-cell-dependent B-cell activation and the subsequent generation of long-lived memory B cells/humoral immune responses.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Cunningham-Rundles C, Ponda PP. Molecular defects in T- and B-cell primary immunodeficiency diseases. *Nat. Rev. Immunol.* 5(11), 880–892 (2005).

2 Dorner T, Jacobi AM, Lipsky PE. B cells in autoimmunity. *Arthritis Res. Ther.* 11(5), 247 (2009).

3 Martin F, Chan AC. Pathogenic roles of B cells in human autoimmunity; insights from the clinic. *Immunity* 20(5), 517–527 (2004).

4 Notarangelo LD. Primary immunodeficiencies. *J. Allergy Clin. Immunol.* 125(2 Suppl. 2), S182–S194 (2010).

5 Notarangelo LD, Fischer A, Geha RS *et al.* Primary immunodeficiencies: 2009 update. *J. Allergy Clin. Immunol.* 124(6), 1161–1178 (2009).

6 Tangye SG, Tarlinton DM. Memory B cells: effectors of long-lived immune responses. *Eur. J. Immunol.* 39(8), 2065–2075 (2009).

7 Browning JL. B cells move to centre stage: novel opportunities for autoimmune disease treatment. *Nat. Rev. Drug Discov.* 5(7), 564–576 (2006).

8 Edwards JC, Cambridge G. B-cell targeting in rheumatoid arthritis and other autoimmune diseases. *Nat. Rev. Immunol.* 6(5), 394–403 (2006).

9 Martin F, Chan AC. B cell immunobiology in disease: evolving concepts from the clinic. *Annu. Rev. Immunol.* 24, 467–496 (2006).

10 Lai AY, Kondo M. T and B lymphocyte differentiation from hematopoietic stem cell. *Semin. Immunol.* 20(4), 207–212 (2008).

11 Hardy RR, Hayakawa K. B cell development pathways. *Annu. Rev. Immunol.* 19, 595–621 (2001).

12 Monroe JG, Dorshkind K. Fate decisions regulating bone marrow and peripheral B lymphocyte development. *Adv. Immunol.* 95, 1–50 (2007).

13 Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* 435(7042), 590–597 (2005).

14 Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC.

Predominant autoantibody production by early human B cell precursors. *Science* 301(5638), 1374–1377 (2003).

•• **The first description of increased frequencies of autoreactive B cells in the subset of human transitional B cells and that this stage of B-cell development represents a check point for the deletion of autoreactive cells.**

15 Wardemann H, Nussenzweig MC. B-cell self-tolerance in humans. *Adv. Immunol.* 95, 83–110 (2007).

16 Carsetti R, Kohler G, Lamers MC. Transitional B cells are the target of negative selection in the B cell compartment. *J. Exp. Med.* 181(6), 2129–2140 (1995).

17 Chung JB, Silverman M, Monroe JG. Transitional B cells: step by step towards immune competence. *Trends Immunol.* 24(6), 343–349 (2003).

18 Loder F, Mutschler B, Ray RJ *et al.* B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. *J. Exp. Med.* 190(1), 75–89 (1999).

19 Allman D, Lindsley RC, DeMuth W, Rudd K, Shinton SA, Hardy RR. Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation. *J. Immunol.* 167(12), 6834–6840 (2001).

• **Reported the refined resolution of murine transitional B cells into three phenotypic and functionally distinct subsets.**

20 Carsetti R, Rosado MM, Wardmann H. Peripheral development of B cells in mouse and man. *Immunol. Rev.* 197, 179–191 (2004).

21 Sims GP, Ettinger R, Shirota Y, Yarboro CH, Illei GG, Lipsky PE. Identification and characterization of circulating human transitional B cells. *Blood* 105(11), 4390–4398 (2005).

22 Cuss AK, Avery DT, Cannons JL *et al.* Expansion of functionally immature transitional B cells is associated with human-immunodeficient states characterized by impaired humoral immunity. *J. Immunol.* 176(3), 1506–1516 (2006).

•• **References 21 and 22 present the first detailed analyses of human transitional B cells with respect to phenotype and function.**

23 Suryani S, Fulcher DA, Santner-Nanan B *et al.* Differential expression of CD21 identifies developmentally and functionally distinct subsets of human transitional B cells. *Blood* 115(3), 519–529 (2010).

•• **Reported the delineation of human transitional B cells into distinct subsets according to CD21 expression and demonstrated that the CD21^{lo} subset was the progenitor of the CD21^{hi} subset. This study also established that the CD21^{lo} subset, rather than the CD21^{hi} subset, contains a high frequency of autoreactive cells, demonstrating that self-reactive B cells are purged from the repertoire as CD21^{lo} transitional B cells and mature into CD21^{hi} transitional B cells.**

24 Anolik JH, Friedberg JW, Zheng B *et al.* B cell reconstitution after rituximab treatment of lymphoma recapitulates B cell ontogeny. *Clin. Immunol.* 122(2), 139–145 (2007).

25 Palanichamy A, Barnard J, Zheng B *et al.* Novel human transitional B cell populations revealed by B cell depletion therapy. *J. Immunol.* 182(10), 5982–5993 (2009).

26 Lee J, Kuchen S, Fischer R, Chang S, Lipsky PE. Identification and characterization of a human CD5$^+$ pre-naive B cell population. *J. Immunol.* 182(7), 4116–4126 (2009).

• **References 25 and 26 describe a 'pre-naive' subset of human B cells, which probably represents a late stage of transitional B cells that arise from the CD21^{hi} subset of transitional cells.**

27 Hystad ME, Myklebust JH, Bo TH *et al.* Characterization of early stages of human B cell development by gene expression profiling. *J. Immunol.* 179(6), 3662–3671 (2007).

28 Avery DT, Deenick EK, Ma CS *et al.* B cell-intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *J. Exp. Med.* 207(1), 155–171 (2010).

29 Ho J, Moir S, Malaspina A *et al.* Two overrepresented B cell populations in HIV-infected individuals undergo apoptosis by different mechanisms. *Proc. Natl Acad. Sci. USA* 103(51), 19436–19441 (2006).

30 Storek J, Saxon A. Reconstitution of B cell immunity following bone marrow transplantation. *Bone Marrow Transplant.* 9(6), 395–408 (1992).

31 Antin JH, Ault KA, Rappeport JM, Smith BR. B lymphocyte reconstitution after human bone marrow transplantation. Leu-1 antigen defines a distinct population of B lymphocytes. *J. Clin. Invest.* 80(2), 325–332 (1987).

32 Rehnberg M, Amu S, Tarkowski A, Bokarewa MI, Brisslert M. Short- and long-term effects of anti-CD20 treatment on B cell ontogeny in bone marrow of patients with rheumatoid arthritis. *Arthritis Res. Ther.* 11(4), R123 (2009).

33 Leandro MJ, Cambridge G, Ehrenstein MR, Edwards JC. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum.* 54(2), 613–620 (2006).

34 Roll P, Palanichamy A, Kneitz C, Dorner T, Tony HP. Regeneration of B cell subsets after transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. *Arthritis Rheum.* 54(8), 2377–2386 (2006).

35 Yurasov S, Wardemann H, Hammersen J *et al.* Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J. Exp. Med.* 201(5), 703–711 (2005).

36 Yurasov S, Tiller T, Tsuiji M *et al.* Persistent expression of autoantibodies in SLE patients in remission. *J. Exp. Med.* 203(10), 2255–2261 (2006).

• **Describes defects in check points for the deletion of autoreactive B cells from the human B-cell repertoire in cases of autoimmune diseases.**

37 Blair PA, Norena LY, Flores-Borja F *et al.* CD19$^+$CD24^{hi}CD38^{hi} B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* 32(1), 129–140 (2010).

• **The first description of putative human regulatory B cells.**

38 Gray M, Miles K, Salter D, Gray D, Savill J. Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. *Proc. Natl Acad. Sci. USA* 104(35), 14080–14085 (2007).

39 Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF. A regulatory B cell subset with a unique CD1d^{hi}CD5$^+$ phenotype controls T cell-dependent inflammatory responses. *Immunity* 28(5), 639–650 (2008).

40 Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J. Clin. Invest.* 118(10), 3420–3430 (2008).

41 Bouaziz JD, Yanaba K, Tedder TF. Regulatory B cells as inhibitors of immune responses and inflammation. *Immunol. Rev.* 224, 201–214 (2008).

42 Llorente L, Richaud-Patin Y, Wijdenes J *et al.* Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. *Eur. Cytokine Netw.* 4(6), 421–427 (1993).

43 Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. *Nat. Rev. Immunol.* 2(7), 465–475 (2002).

44 Mackay F, Schneider P, Rennert P, Browning J. BAFF AND APRIL: a tutorial on B cell survival. *Annu. Rev. Immunol.* 21, 231–264 (2003).

45 Tangye SG, Bryant VL, Cuss AK, Good KL. BAFF, APRIL and human B cell disorders. *Semin. Immunol.* 18(5), 305–317 (2006).

46 Kalled SL. Impact of the BAFF/BR3 axis on B cell survival, germinal center maintenance and antibody production. *Semin. Immunol.* 18(5), 290–296 (2006).

47 Cancro MP, D'Cruz DP, Khamashta MA. The role of B lymphocyte stimulator (BLyS) in systemic lupus erythematosus. *J. Clin. Invest.* 119(5), 1066–1073 (2009).

48 O'Connor BP, Raman VS, Erickson LD *et al.* BCMA is essential for the survival of long-lived bone marrow plasma cells. *J. Exp. Med.* 199(1), 91–98 (2004).

49 Avery DT, Kalled SL, Ellyard JI *et al.* BAFF selectively enhances the survival of plasmablasts generated from human memory B cells. *J. Clin. Invest.* 112(2), 286–297 (2003).

50 Groom J, Kalled SL, Cutler AH *et al.* Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjogren's syndrome. *J. Clin. Invest.* 109(1), 59–68 (2002).

51 Cheema GS, Roschke V, Hilbert DM, Stohl W. Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. *Arthritis Rheum.* 44(6), 1313–1319 (2001).

52 Lesley R, Xu Y, Kalled SL *et al.* Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. *Immunity* 20(4), 441–453 (2004).

53 Thien M, Phan TG, Gardam S *et al.* Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity* 20(6), 785–798 (2004).

54 Wallace DJ, Stohl W, Furie RA *et al.* A Phase II, randomized, double-blind, placebo-controlled, dose-ranging study of belimumab in patients with active systemic lupus erythematosus. *Arthritis Rheum.* 61(9), 1168–1178 (2009).

55 Jacobi AM, Huang W, Wang T *et al.* Effect of long-term belimumab treatment on B cells in systemic lupus erythematosus: extension of a Phase II, double-blind, placebo-controlled, dose-ranging study. *Arthritis Rheum.* 62(1), 201–210 (2010).

56 Lateef A, Petri M. Biologics in the treatment of systemic lupus erythematosus. *Curr. Opin Rheumatol.* 22(5), 504–509 (2010).

57 Baker KP, Edwards BM, Main SH *et al.* Generation and characterization of LymphoStat-B, a human monoclonal antibody that antagonizes the bioactivities of B lymphocyte stimulator. *Arthritis Rheum.* 48(11), 3253–3265 (2003).

58 Vugmeyster Y, Seshasayee D, Chang W *et al.* A soluble BAFF antagonist, BR3-Fc, decreases peripheral blood B cells and lymphoid tissue marginal zone and follicular B cells in cynomolgus monkeys. *Am. J. Pathol.* 168(2), 476–489 (2006).

59 Batten M, Groom J, Cachero TG *et al.* BAFF mediates survival of peripheral immature B lymphocytes. *J. Exp. Med.* 192(10), 1453–1466 (2000).

60 Warnatz K, Salzer U, Rizzi M *et al.* B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. *Proc. Natl Acad. Sci. USA* 106(33), 13945–13950 (2009).

• **Reports the identification of the first individuals with loss-of function mutations in *TNFRSF13C* encoding BAFF-R; however, the phenotype of the affected individuals is relatively mild compared with *Baff* and *Baffr*-deficient mice.**

61 Darce JR, Arendt BK, Wu X, Jelinek DF. Regulated expression of BAFF-binding receptors during human B cell differentiation. *J. Immunol.* 179(11), 7276–7286 (2007).

62 Medina F, Segundo C, Campos-Caro A, Gonzalez-Garcia I, Brieva JA. The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. *Blood* 99(6), 2154–2161 (2002).

63 Hoyer BF, Moser K, Hauser AE *et al.* Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. *J. Exp. Med.* 199(11), 1577–1584 (2004).

64 Namen AE, Lupton S, Hjerrild K *et al.* Stimulation of B-cell progenitors by cloned murine interleukin-7. *Nature* 333(6173), 571–573 (1988).

65 Noguchi M, Nakamura Y, Russell SM *et al.* Interleukin-2 receptor γ chain: a functional component of the interleukin-7 receptor. *Science* 262(5141), 1877–1880 (1993).

66 Sudo T, Ito M, Ogawa Y *et al.* Interleukin 7 production and function in stromal cell-dependent B cell development. *J. Exp. Med.* 170(1), 333–338 (1989).

67 Sudo T, Nishikawa S, Ohno N *et al.* Expression and function of the interleukin 7 receptor in murine lymphocytes. *Proc. Natl Acad. Sci. USA* 90(19), 9125–9129 (1993).

68 Grabstein KH, Waldschmidt TJ, Finkelman FD *et al.* Inhibition of murine B and T lymphopoiesis *in vivo* by an anti-interleukin 7 monoclonal antibody. *J. Exp. Med.* 178(1), 257–264 (1993).

69 Peschon JJ, Morrissey PJ, Grabstein KH *et al.* Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J. Exp. Med.* 180(5), 1955–1960 (1994).

70 von Freeden-Jeffry U, Vieira P, Lucian LA, McNeil T, Burdach SE, Murray R. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J. Exp. Med.* 181(4), 1519–1526 (1995).

71 Prieyl JA, LeBien TW. Interleukin 7 independent development of human B cells. *Proc. Natl Acad. Sci. USA* 93(19), 10348–10353 (1996).

72 Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in $T^-B^+NK^+$ severe combined immunodeficiency. *Nat. Genet.* 20(4), 394–397 (1998).

73 Roifman CM, Zhang J, Chitayat D, Sharfe N. A partial deficiency of interleukin-7R α is sufficient to abrogate T-cell development and cause severe combined immunodeficiency. *Blood* 96(8), 2803–2807 (2000).

74 Giliani S, Mori L, de Saint Basile G *et al.* Interleukin-7 receptor α (IL-7Rα) deficiency: cellular and molecular bases. Analysis of clinical, immunological, and molecular features in 16 novel patients. *Immunol. Rev.* 203, 110–126 (2005).

75 Noguchi M, Yi H, Rosenblatt HM *et al.* Interleukin-2 receptor γ chain mutation results in X-linked severe combined immunodeficiency in humans. *Cell* 73(1), 147–157 (1993).

76 Russell SM, Tayebi N, Nakajima H *et al.* Mutation of *Jak3* in a patient with SCID: essential role of Jak3 in lymphoid development. *Science* 270(5237), 797–800 (1995).

77 Macchi P, Villa A, Giliani S *et al.* Mutations of *Jak-3* gene in patients with autosomal severe combined immune deficiency (SCID). *Nature* 377(6544), 65–68 (1995).

78 Parrish YK, Baez I, Milford TA *et al.* IL-7 dependence in human B lymphopoiesis increases during progression of ontogeny from cord blood to bone marrow. *J. Immunol.* 182(7), 4255–4266 (2009).

79 Johnson SE, Shah N, Panoskaltsis-Mortari A, LeBien TW. Murine and human IL-7 activate STAT5 and induce proliferation of normal human pro-B cells. *J. Immunol.* 175(11), 7325–7331 (2005).

80 Saeland S, Duvert V, Pandrau D *et al.* Interleukin-7 induces the proliferation of normal human B-cell precursors. *Blood* 78(9), 2229–2238 (1991).

81 Duty JA, Szodoray P, Zheng NY *et al.* Functional anergy in a subpopulation of naive B cells from healthy humans that express autoreactive immunoglobulin receptors. *J. Exp. Med.* 206(1), 139–151 (2009).

•• **Describes anergic human B cells in healthy indivduals.**

82 Isnardi I, Ng YS, Menard L *et al.* Complement receptor 2/CD21-negative human naive B cells mostly contain autoreactive unresponsive clones. *Blood* 115(24), 5026–5036 (2010).

83 Rakhmanov M, Keller B, Gutenberger S *et al.* Circulating CD21^{low} B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. *Proc. Natl Acad. Sci. USA* 106(32), 13451–13456 (2009).

• **References 82 and 83 describe an aberrant population of CD21^{lo} autoreactive B cells in patients with autoimmunity and common variable immunodeficiency.**

84 Warnatz K, Denz A, Drager R *et al.* Severe deficiency of switched memory B cells (CD27$^+$IgM$^-$IgD$^-$) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. *Blood* 99(5), 1544–1551 (2002).

85 Ma CS, Hare NJ, Nichols KE *et al.* Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4$^+$ T cells. *J. Clin. Invest.* 115(4), 1049–1059 (2005).

86 Ma CS, Pittaluga S, Avery DT *et al.* Selective generation of functional somatically mutated IgM$^+$CD27$^+$, but not Ig isotype-switched, memory B cells in X-linked lymphoproliferative disease. *J. Clin. Invest.* 116(2), 322–333 (2006).

87 Weller S, Faili A, Garcia C *et al.* CD40–CD40L independent Ig gene hypermutation suggests a second B cell diversification pathway in humans. *Proc. Natl Acad. Sci. USA* 98(3), 1166–1170 (2001).

88 Jain A, Ma CA, Lopez-Granados E *et al.* Specific NEMO mutations impair CD40-mediated c-Rel activation and B cell terminal differentiation. *J. Clin. Invest.* 114(11), 1593–1602 (2004).

89 Warnatz K, Bossaller L, Salzer U *et al.* Human ICOS deficiency abrogates the germinal center reaction and provides a monogenic model for common variable immunodeficiency. *Blood* 107(8), 3045–3052 (2006).

90 van Zelm MC, Reisli I, van der Burg M *et al.* An antibody-deficiency syndrome due to mutations in the *CD19* gene. *N. Engl. J. Med.* 354(18), 1901–1912 (2006).

91 Kanegane H, Agematsu K, Futatani T *et al.* Novel mutations in a Japanese patient with CD19 deficiency. *Genes Immun.* 8(8), 663–670 (2007).

92 Speckmann C, Enders A, Woellner C *et al.* Reduced memory B cells in patients with hyper IgE syndrome. *Clin. Immunol.* 129(3), 448–454 (2008).

93 Nurieva RI, Chung Y, Hwang D *et al.* Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* 29(1), 138–149 (2008).

94 van Zelm MC, Smet J, Adams B *et al.* *CD81* gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. *J. Clin. Invest.* 120(4), 1265–1274 (2010).

95 Kuijpers TW, Bende RJ, Baars PA *et al.* CD20 deficiency in humans results in impaired T cell-independent antibody responses. *J. Clin. Invest.* 120(1), 214–222 (2010).

96 Rakhmanov M, Keller B, Gutenberger S *et al.* Circulating CD21^{low} B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. *Proc. Natl Acad. Sci. USA* 106(32), 13451–13456 (2009).

97 Darce JR, Arendt BK, Chang SK, Jelinek DF. Divergent effects of BAFF on human memory B cell differentiation into Ig-secreting cells. *J. Immunol.* 178(9), 5612–5622 (2007).

98 Ellyard JI, Avery DT, Mackay CR, Tangye SG. Contribution of stromal cells to the migration, function and retention of plasma cells in human spleen: potential roles of CXCL12, IL-6 and CD54. *Eur. J. Immunol.* 35(3), 699–708 (2005).

99 Ellyard JI, Avery DT, Phan TG, Hare NJ, Hodgkin PD, Tangye SG. Antigen-selected, immunoglobulin-secreting cells persist in human spleen and bone marrow. *Blood* 103(10), 3805–3812 (2004).

100 Wolniak KL, Noelle RJ, Waldschmidt TJ. Characterization of (4-hydroxy-3-nitrophenyl)acetyl (NP)-specific germinal center B cells and antigen-binding B220- cells after primary NP challenge in mice. *J. Immunol.* 177(4), 2072–2079 (2006).

101 Kallies A, Hasbold J, Tarlinton DM *et al.* Plasma cell ontogeny defined by quantitative changes in blimp-1 expression. *J. Exp. Med.* 200(8), 967–977 (2004).

102 Tangye SG, Liu YJ, Aversa G, Phillips JH, de Vries JE. Identification of functional human splenic memory B cells by expression of CD148 and CD27. *J. Exp. Med.* 188(9), 1691–1703 (1998).

103 Liu YJ, Barthelemy C, Debouteiller O, Arpin C, Durand I, Banchereau J. Memory B-cells from human tonsils colonize mucosal epithelium and directly present antigen to T-cells by rapid up-regulation of B7–1 and B7–2. *Immunity* 2(3), 239–248 (1995).