Mechanisms of allergic diseases

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B-cell biology and development

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B cells develop from hematopoietic precursor cells in an ordered maturation and selection process. Extensive studies with many different mouse mutants provided fundamental insights into this process. However, the characterization of genetic defects causing primary immunodeficiencies was essential in understanding human B-cell biology. Defects in pre–B-cell receptor components or in downstream signaling proteins, such as Bruton tyrosine kinase and B-cell linker protein, arrest development at the pre–B-cell stage. Defects in survival-regulating proteins, such as B-cell activator of the TNF-α family receptor (BAFF-R) or caspase recruitment domain–containing protein 11 (CARD11), interrupt maturation and prevent differentiation of transitional B cells into marginal zone and follicular B cells. Mature B-cell subsets, immune responses, and memory B-cell and plasma cell development are disturbed by mutations affecting Toll-like receptor signaling, B-cell antigen receptor coreceptors (eg, CD19), or enzymes responsible for immunoglobulin class-switch recombination. Transgenic mouse models helped to identify key regulatory mechanisms, such as receptor editing and clonal anergy, preventing the activation of B cells expressing antibodies recognizing autoantigens. Nevertheless, the combination of susceptible genetic backgrounds with the rescue of self-reactive B cells by T cells allows the generation of autoreactive clones found in patients with many autoimmune diseases and even in those with primary immunodeficiencies. The rapid progress of functional genomic research is expected to foster the development of new tools that specifically target dysfunctional B lymphocytes to treat autoimmunity, B-cell malignancies, and immunodeficiency. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *B cell, B lymphocyte, immunodeficiency, development, humoral immunity, autoimmunity, tolerance*

Abbreviations used

AID:	Activation-induced cytidine deaminase
APRIL:	A proliferation-inducing ligand
BAFF:	B-cell activator of the TNF-α family (aka BLYS)
BAFF-R:	BAFF receptor
BCMA:	B-cell maturation factor
BCR:	B-cell antigen receptor
Blimp-1:	B lymphocyte–induced maturation protein 1
BLNK:	B-cell linker protein (aka Sark homology 2 domain–containing leukocyte protein of 65 kDa [SLP65])
BM:	Bone marrow
BTK:	Bruton tyrosine kinase
CARD11:	Caspase recruitment domain–containing protein 11 (aka CARMA1)
CNR2:	Cannabinoid receptor 2
CSR:	Class-switch recombination
CVID:	Common variable immunodeficiency
DOCK8:	Dedicator of cytokinesis 8
GC:	Germinal center
GFP:	Green fluorescent protein
H-chain:	Heavy chain
HEL:	Hen's egg lysozyme
L-chain:	Light chain
MyD88:	Myeloid differentiation primary response gene–88
MZ:	Marginal zone
N_{BH}:	B-cell helper neutrophil
NF-κB:	Nuclear factor κ light chain enhancer of activated B cells
nur77:	Nuclear receptor 77
S1P:	Sphingosine-1-phosphate
SHM:	Somatic hypermutation
TACI:	Transmembrane activator, calcium modulator, and cyclophilin ligand interactor
TLR:	Toll-like receptor
WAS:	Wiskott-Aldrich syndrome

B cells and their antibodies are the central elements of humoral immunity and protect, as part of the adaptive immune system, against an almost unlimited variety of pathogens. Defects in B-cell development, selection, and function lead to autoimmunity, malignancy, immunodeficiencies, and allergy. Combined with the enormous increase in knowledge about gene function and the genetic diversity of human subjects that has developed since the human genome was deciphered, primary immunodeficiencies are a still-growing source of mutations, providing unique opportunities to study the function of the human immune system. In addition, each newly discovered immunodeficiency represents a new challenge to develop the most optimal and personalized forms of treatment.

In this review we discuss human B-cell development (Fig 1) in light of genetic defects discovered by studying primary

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Terms in boldface and italics are defined in the glossary on page ■■■.

immunodeficiencies. By pointing out differences and common features with corresponding mouse models, we provide an overview on mechanisms regulating the maturation, survival, and selection of B lymphocytes in protective and autoimmune responses.

EARLY B-CELL DEVELOPMENT IN THE BONE MARROW

In adult human subjects, as in all mammals, B lymphocytes develop in the bone marrow (BM) from hematopoietic precursor cells. During embryonic life, the BM is seeded by hematopoietic stem cells developing in the fetal liver. Their precursor cells originate from the aorta-gonad-mesonephros,[1,2] which is formed by descendants of the ***mesoderm***. Early BM-dependent stages of B-cell development are structured along the functional rearrangement process of the immunoglobulin gene segments.[3] V_H, D_H, and J_H, rearrangements of the ***heavy chain (H-chain)*** together with the V_L-J_L rearrangements of the ***light chain (L-chain)*** gene segments generate a B-cell repertoire expressing antibodies capable of recognizing more than 5×10^{13} different antigens. According to the rearrangement of the H-chain and L-chain gene segments, 3 developmental stages are defined. In the first stage, pro–B cells rearrange the D and J segments of the H-chain, followed by a second rearrangement joining an upstream V region to the rearranged DJ segment.

The functional rearrangement of the μ–H-chain gene segments opens the entry into the next phase, the pre–B-cell stage. In human subjects pre–B cells undergo 1 or 2 cell divisions and rearrange the gene segments encoding the κ and λ chains.[4] Combined with the μ chain, an IgM molecule is formed and expressed on the cell surface. These cells are termed immature B cells. Immature B cells leave the BM and migrate to the spleen, where they finalize early development by differentiating into naive, follicular, or ***marginal zone*** (MZ) B cells.

Considering the enormous diversity of antibody specificities, the first challenge of the immune system is to find a balance between variable specificities against pathogens while avoiding autoreactivity. Therefore B cells are screened at several checkpoints during development for their degree of autoreactivity. The first screen takes place after differentiation of pro-B into pre-B cells.[5] Expression of the productively rearranged μ–H-chain, of the surrogate L-chains (in human subjects composed of λ-like and V-preB), and of the signal-transducing components Ig-α and Ig-β allows the formation of the so-called pre–B-cell antigen receptor (BCR) complex. The pre-BCR has 2 tasks. The first task is to shut down the activities and expression of the enzyme machinery catalyzing the rearrangements of the H-chain gene segments, a process termed allelic exclusion.[6] This prevents the expression of 2 H-chains with 2 different specificities by the same cell. The second task is to initiate the rearrangement of the L-chain genes.

Several genetic defects affecting components of the pre-BCR or downstream signaling proteins have brought to light human B-cell development and uncovered fundamental differences in B-cell lymphopoiesis between mice and human subjects. In human subjects, for example, mutations in genes encoding components of the ***IL-7*** signaling cascade, such as IL-2 receptor common γ,[7] the IL-7 receptor α chain,[8] or the associated kinase Janus kinase 3,[9] do not affect the B-cell compartment, but they do interrupt T-cell development, leading to severe combined immunodeficiency (B^+T^- severe combined immunodeficiency).

GLOSSARY

ACTIN: A protein found in microfilaments and active in muscular contraction, cellular movement, and maintenance of cell shape.

ACTIVATION-INDUCED CYTIDINE DEAMINASE (AID): An enzyme that catalyzes mutation of deoxycytidine to deoxyuracil in single-stranded DNA. AID is critical for somatic hypermutation. Mutations in AID result in autosomal recessive hyper-IgM syndrome.

AFFINITY MATURATION: As a consequence of somatic hypermutation, B cells increase their average affinity for antigen as the humoral immune response progresses. Such cells are preferentially activated and therefore have selective survival. Affinity maturation occurs in germinal centers.

5×10^{13}: For comparison purposes, there are roughly 10^{14} cells in the human body, of which only 10^{13} are human. 5×10^{13} is roughly 40 billion more than the number of stars in the Milky Way Galaxy. There are 5×10^{12} known digits in π. One million years is approximately 3.2×10^{13} seconds.

GERMINAL CENTER: A central proliferative area of a lymphoid follicle. It forms during T cell–dependent humoral immune responses.

HEAVY CHAIN (H-CHAIN): Part of the core structure of an antibody molecule. Antibodies contain 2 identical H-chains that consist of variable and constant regions. The constant regions of the H-chain mediate effector functions of the antibody.

IL-7: A hematopoietic cytokine binding to cytokine receptors of the common γ chain family. In addition to being vital to the survival and expansion of both precursor T lymphocytes, IL-7 stimulates the proliferation and differentiation of cytotoxic T and natural killer cells and stimulates the antitumor properties of monocytes and macrophages.

LIGHT CHAIN (L-CHAIN): An antibody molecule also contains 2 identical L-chains that contain 1 variable domain and 1 constant domain. The variable region of 1 H-chain is juxtaposed with the variable region of 1 L-chain to form the antigen-binding site of the antibody molecule.

MARGINAL ZONE: A splenic zone located next to the marginal sinuses, the site of entry into the spleen for lymphocytes, macrophages, and dendritic cells in human subjects. The marginal sinus separates the white pulp from the red pulp.

MESODERM: The 3 primary germ layers of an embryo are the mesoderm, ectoderm, and endoderm. The mesoderm develops from the ectoderm on the 15th day of life. The mesoderm is the source of bone, muscle, connective tissue, and dermis.

NUCLEAR FACTOR κB (NF-κB): A family of transcription factors that promote the expression of a variety of survival and differentiation factors, as well as inflammatory mediators. NF-κB is present in an unstimulated state in the cytoplasm, where it is bound by IκB, an inhibitory protein.

PEYER PATCHES: Aggregates of lymphoid follicles that are a component of gut-associated lymphoid tissue. Antigen-driven priming and maturation of naive T and B lymphocytes occurs here. They are located primarily in the ileum.

SOMATIC HYPERMUTATION: High-frequency point mutations that occur in a mature B cell at the hypervariable sites of both the V_H and V_L genes. The amino acid products of these sites, particularly at V, D, and J junctions, are the specific points of contact with antigen within the binding groove.

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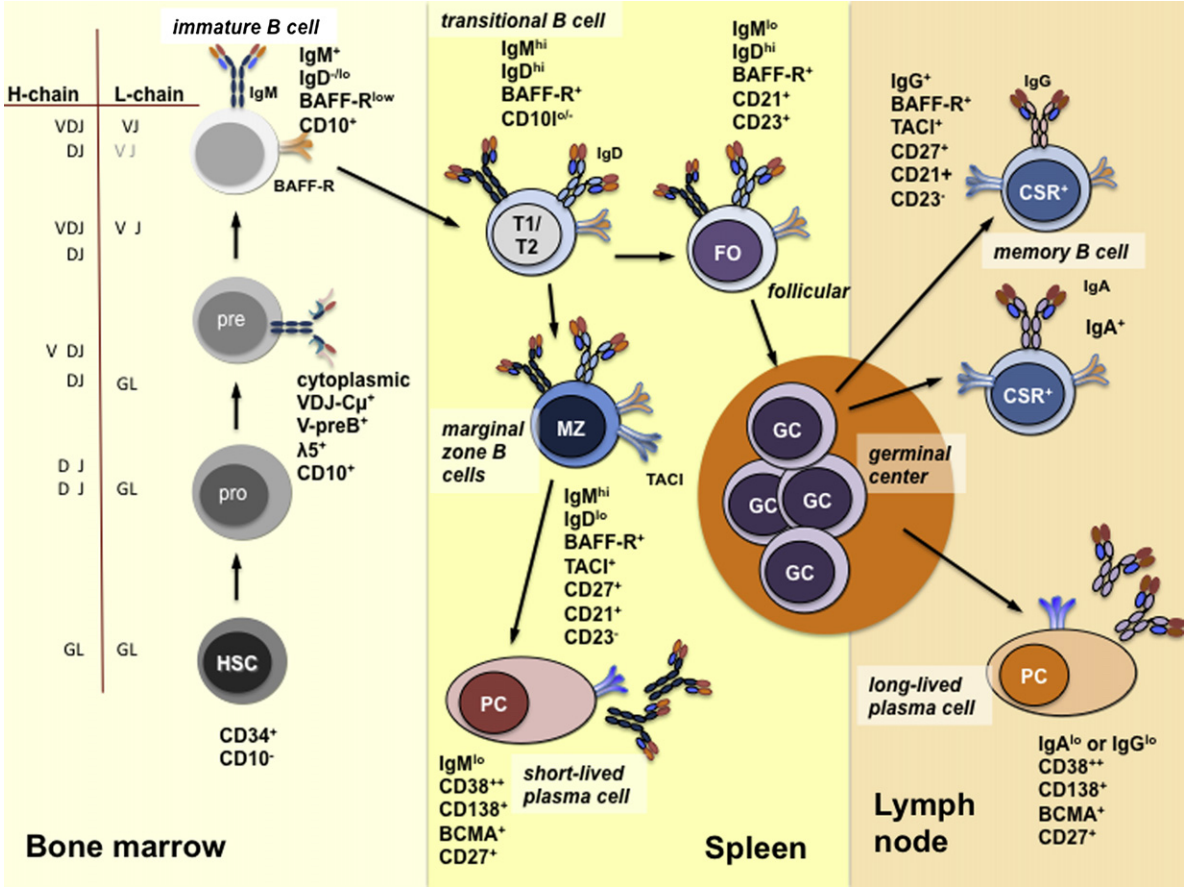

FIG 1. B-cell development and B-cell subsets. B cells develop in the BM from hematopoietic precursor cells *(HSC)*. Recombination-activating gene (RAG) 1/2–dependent rearrangement of the H-chain, D-gene, and J-gene segments from germline *(GL)* starts at the pro–B-cell stage. V-gene segment rearrangement follows in the early pre-B cell stage. In CD10$^+$ CD19$^+$ pre-B cells, functional H-chains (VDJ-Cμ) pair with V-preB and λ-like, forming the pre-BCR, which is expressed within a cell and not detected on the surface. Pre-BCR–induced signals shut down RAG expression, preventing the rearrangement of the second H-chain allele and inducing proliferation. Next, RAG genes are re-expressed to initiate V-J rearrangement of L-chains. Successfully rearranged κ or λ L-chains replace V-preB/λ5 of the pre-BCR pair with the H-chain and form IgM. IgM expressed by immature B cells changes the expression pattern of many genes and initiates egress into the circulation. Immature B cells enter the spleen as transitional B cells, where they receive survival signals through BAFF-R and complete the first stage of development as MZ B cells or follicular B cells, depending on the specificity of their BCR. On contact with antigen and supported by N_{BH} cells, MZ B cells develop into short-lived plasma cells. Follicular B cells are activated by antigen binding and develop in GCs supported by T_H cells into memory B cells (CSR$^+$) or plasma cells *(PC)*. Activation of B cells induces AID and other components of the SHM/class-switch machinery, thus changing the affinity of the BCR and the isotype (IgM to IgG, IgA, or IgE).

Bruton tyrosine kinase (BTK), a central signaling component downstream of the pre-BCR and the BCR, links the activation of the pre-BCR and the BCR to Ca^{2+} influx,[10] the activation of the mitogen-activated protein kinase cascade, and changes in the activity of transcription factors, including ***nuclear factor κB (NF-κB)***.[11] In contrast to mice,[12] the development of human B cells in the BM is strongly dependent on BTK activity.[13,14] Up to 90% of patients who receive diagnoses of early-onset hypogammaglobulinemia carry mutations in the *BTK* gene located on the X chromosome (X-linked agammaglobulinemia).[15-17] In infants and adults the lack of peripheral blood B cells leads to severely reduced antibody titers of all isotypes. Analysis of BM samples from patients with X-linked agammaglobulinemia revealed normal levels of the earliest B-cell progenitors, whereas cytoplasmic Ig-μ$^+$ pre-B cells showed abnormalities in cell numbers and proliferation.[18] In contrast, mice lacking *Btk* have a milder reduction in peripheral B-cell numbers and close to normal IgG_1, IgG_{2a}, IgG_{2b}, and IgA antibody titers; only a significant reduction in IgM and IgG_3 levels in serum was demonstrated.[19,20] Thus mice on a *Btk*-deficient background are still able to mount humoral immune responses.

Similar to BTK, defects in B-cell linker protein (*BLNK*; SLP65) have different outcomes in human subjects and mice. Deletion of *Blnk* in mice inhibits B-lymphocyte development at the stage of small pre-B cells,[21] but because the block is permissive, *Blnk*-deficient B cells populate peripheral lymphoid tissues in adult mice and mount T cell–dependent immune responses.[22] In human subjects *BLNK*-deficient B cells stop development at the stage of pro-B cells, resulting in a phenotype very similar to BTK deficiency.[23] Circulating B cells are almost absent, and

serum concentrations of IgM and IgA are less than detection levels. Moreover, the lack of BLNK/SLP65 function in mice[24] but not in human subjects[21,25] promotes the proliferation of pro-B/pre-BI cells and the spontaneous development of pre–B-cell acute lymphoblastic leukemia. Because BLNK/SLP65 and BTK are both critical elements of pre-BCR function, signals induced by the pre-BCR in human subjects induce pre–B-cell development and inhibit rearrangement of the second H-chain allele at the same time. In mice the pre-BCR–derived signals also inhibit IL-7–driven pre-B-cell proliferation.

BCR signaling has most recently received the utmost clinical attention not only because most of the monogenetic defects in patients with agammaglobulinemia and different hypogammaglobulinemias have been identified in this pathway but also because the specific inhibitor of BCR signaling ibrutinib (PCI-32765), a BTK inhibitor, is about to revolutionize not only the treatment of B-cell lymphomas[26,27] but also might turn out to be beneficial in B cell–driven autoimmune conditions.[28,29] In general terms, the causes of all antibody deficiencies arising from conditions with B-cell dyscrasia will teach us about B-cell biology, which in turn might become premier targets for hematologists treating B-cell malignancies.

However, mutations affecting proteins of the BCR complex, such as the μ-chain, Ig-α, and Ig-β, lead to similar phenotypes in mice and human subjects. Patients with a μ-chain deletion present as having severe hypogammaglobulinemia caused by a block in early B-cell development.[30,31] Although pro-B cells are present at normal levels, patients lack pre-B cells in the BM and, consequently, all mature B-cell subsets in the periphery, which is similar to what is observed in mice with a deletion of the μ-chain.[32,33]

Similarly, B-cell development is arrested at the pro–B-cell stage in patients with null mutations in genes encoding Ig-α (CD79a)[34,35] and Ig-β (CD79b).[36] A missense mutation in the *CD79B* gene results in an amino acid substitution near a cysteine residue, thus preventing the formation of the disulfide bond between Ig-β and Ig-α and the assembly of a functional pre-BCR.[37] Phenotypically, the defect results in severe B-cell lymphopenia with less than 0.1% peripheral B cells.

Recently, a homozygous deletion of the downstream BCR signaling component caspase recruitment domain–containing protein 11 (CARD11) was shown to result in an antibody deficiency by blocking B-cell differentiation.[38,39] CARD11 interacts with B-cell lymphoma 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) and functions in the signaling pathway by activating canonical NF-κB after BCR engagement.[40] Interestingly, work conducted in mice has demonstrated that CARD11 acts also as a modulator of B-cell tolerance[41] because point mutations in *Card11* equivalent to those found in B-cell lymphomas induce the proliferation of self-reactive B cells instead of apoptosis.

Although mutations affecting major components of the BCR signaling machinery, such as the μ-chain, Ig-α, Ig-β, and BTK, lead to a developmental block of early B cells in the BM, mutations in genes encoding components of the B-cell coreceptor complex have been shown to be less severe. Human subjects lacking CD19,[42,43] CD21,[44] or CD81[45] showed normal numbers of B cells in the periphery. However, ***affinity maturation*** and antibody responses were impaired, thus leading to hypogammaglobulinemia and increased susceptibility to infections.

Synopsis: Striking differences exist between murine and human B-cell development. In contrast to murine pre-B cells, human B-cell precursors do not depend on IL-7 as a pre–B-cell growth factor, whereas mutations in genes encoding components downstream of pre-BCR signaling completely block development in the BM. Mutations in BCR coreceptor genes impair humoral immune responses and increase susceptibility to bacterial infections. Inhibitors of BCR signaling are promising new therapeutics to treat B-cell malignancies and B cell–dependent autoimmune diseases.

BM EGRESS AND GUIDANCE TO THE SECONDARY LYMPHOID ORGANS

Circulating through the body through the bloodstream and the lymph, B cells regularly visit secondary lymphoid organs, such as the spleen, lymph nodes, tonsils, ***Peyer patches***, and mucosal tissues. Homing to B-cell follicles, formation of ***germinal centers*** (GCs), and egress from tissues back to the circulation are regulated by adhesion molecules and by the interplay between different G protein–coupled receptors. Although chemokine receptors respond to a gradient of chemokines produced by tissue stromal cells, sphingosine-1-phosphate (S1P) receptors react against S1P. S1P is a multipotent lipid mediator synthesized by platelets,[46] erythrocytes,[47] and vascular endothelial cells.[48] High S1P concentrations in blood and lymph attract B cells and induce the egress from the lymphoid tissues, where S1P concentrations are low.[49]

Mouse models with deleted S1P receptor genes have shown that the egress of immature B cells from the BM is in part regulated by S1P receptor 1.[50] S1P receptor 1 knockout mice have less circulating transitional B cells, whereas the number of immature B cells in the BM is increased, where they stay longer.[51]

Cannabinoid receptor 2 (CNR2) was shown to play a role in the sinusoidal retention of immature B cells in the BM.[52] CNR2 antagonists displaced immature B cells from the sinusoids, and moreover, CNR2 deficiency in mice resulted in a reduction in λ^+ B-cell numbers in the periphery, mainly because immature B cells in the BM had less time for secondary rearrangements of their L-chain genes.[52]

After leaving the BM, B lymphocytes home to the spleen. Here B cells populate niches to form the MZ and the follicular B-cell compartment. The positioning of cells into the MZ of mice was shown to be guided by the expression of CNR2. Mice deficient for CNR2 have fewer numbers of MZ B cells.[53,54] Antagonists of endocannabinoids were able to deplete the B cells from the MZ, and *vice versa*, overexpression of CNR2 guided B cells to the MZ.[55]

Synopsis: In mice G protein–coupled receptors, such as S1P receptors and CNR2, regulate the egress of B cells from the BM, as well as positioning in the B-cell follicles and the splenic MZ.

MZ B CELLS, TOLL-LIKE RECEPTORS, AND CYTOSKELETAL REMODELING

T cell–independent humoral responses are mounted by B cells of the MZ and the mucosa. In the spleen MZ B cells are the first line of defense against blood-borne pathogens.[56] During the first 3 days of an infection, they rapidly develop into extrafollicular plasma cells secreting IgM,[57] which forms immune complexes with the pathogen.

Human MZ B cells express IgM carrying ***somatic hypermutations*** (SHMs) within the variable regions and recirculate, whereas their murine counterparts have unmutated variable regions and

remain in the spleen.[58,59] Several findings indicate that the SHM in IgM variable regions of human MZ B cells originate independently from antigen-driven clonal expansion in GCs. First, young children show a higher clonal diversity in the MZ subset than in switched B cells.[59] Second, the MZ compartment found in children less than 2 years of age shows expression of low levels of ***activation-induced cytidine deaminase (AID)***, a critical enzyme for SHM,[60] whereas AID expression was not detected in MZ B cells from adult spleens.[61,62] Third, although their numbers are reduced, MZ B cells are found in patients with genetic defects in the *CD40LG* or *CD40* genes encoding 2 molecules essential for T_H cell–dependent GC reactions.[63,64] Therefore it was proposed that MZ B cells develop and mutate during the first years of age independently from immune responses.

Recently, it was shown that human MZ B cells strongly depend on myeloid differentiation primary response gene–88 (MyD88), IL-1 receptor–associated kinase 4 (IRAK4), and Toll–IL-1 receptor domain–containing adaptor protein (TIRAP), which are downstream signaling components of many Toll-like receptors (TLRs).[65] However, abnormalities in MZ B-cell numbers were not found in patients with mutations in Toll-like IL-1 receptor domain–containing adapter inducing IFN-β *(TRIF)* and uncoordinated-93B (UNC93B). Both factors are essential for signaling through TLRs located in endosomes, such as TLR3, TLR7, TLR8, and TLR9. Thus the authors postulated that the development, homeostasis, or both of human MZ B cells might depend on TIRAP-transmitted signals induced by TLRs expressed on the cell surface, such as TLR10.

Two studies of patients with severe immunodeficiencies have uncovered dedicator of cytokinesis 8 (DOCK8) as an important factor for the generation of humoral immune responses.[66,67] DOCK8 is a guanine exchange factor interacting directly with the Rho GTPase cell division control protein 42 (cdc42),[68] a critical component of signaling pathways regulating cell morphology[69] and division.[70] The clinical manifestations linked to DOCK8 deficiency are characterized by a highly increased susceptibility to bacterial infections and to increased IgE levels, resulting in an hyper-IgE syndrome.[66] DOCK8-deficient patients lack MZ B cells, do not produce protective antibodies after vaccination, and have extremely low numbers of switched memory B cells.[66,67,71] In addition to the role of DOCK8 as a guanine nucleotide exchange factor regulating cytoskeletal remodeling and immunologic synapse formation, it was recently shown that DOCK8 associates with MyD88 and serves as an adaptor in the TLR9 signaling pathway in B cells.[72] Because B-cell proliferation was affected in DOCK8-deficient B cells after TLR9 but not after CD40 costimulation, DOCK8 might be more important for T-independent than for T-dependent B-cell responses.

Apart from DOCK8, another regulator of the cytoskeletal reorganization, termed L-plastin, was shown to be essential for the development of murine MZ B cells.[73] In addition to a greater than 80% reduction in MZ B-cell numbers, $Lpl^{-/-}$ mice have only 60% of follicular B cells. As an ***actin***-binding protein, L-plastin plays a role in BCR signaling, as well as in S1P-directed positioning of B cells into the MZ zone.

Another defect disturbing cytoskeletal organization and B-cell responses is caused by mutations in the *WAS* gene, resulting in Wiskott-Aldrich syndrome (WAS).[74] Because it encodes a critical regulator of actin polymerization, mutations in *WAS* result in defective B-cell migration and breaking of B-cell tolerance.[75] Therefore in addition to immunodeficiency, patients with WAS have autoimmunity as well.[76] Because MZ B cells shuttle in response to S1P and chemokine signals between follicles and the MZ,[50] *WAS* mutations inhibit the development and maintenance of MZ B cells.[77]

Synopsis: MZ B cells form a first line of defense against blood-borne pathogens and elicit a T-independent immune response. In human subjects mutations in TLR signaling components impair MZ B-cell responses.

SURVIVING IN THE PERIPHERY: THE B-CELL ACTIVATOR OF THE TNF-α FAMILY AND A PROLIFERATION-INDUCING LIGAND SYSTEM

The survival of B cells in the periphery depends on the expression of a functional BCR and on signals generated by B-cell activator of the TNF-α family (BAFF) binding to BAFF receptor (BAFF-R).[78] During B-cell maturation, BAFF-R is expressed first by immature B cells in the BM.[79] In addition to BAFF-R, BAFF binds to 2 receptors termed transmembrane activator, calcium modulator, and cyclophilin ligand interactor (TACI) and B-cell maturation factor (BCMA).[80] These 3 receptors form a ligand-receptor system that includes the homologous ligand a proliferation-inducing ligand (APRIL). APRIL, in contrast to BAFF, binds only TACI and BCMA but does not interact with BAFF-R (Fig 2).

BAFF-R expression increases when transitional B cells differentiate into MZ and follicular B cells. However, BAFF-R is not found on long-lived plasma cells in the BM, which express BCMA,[81] whereas TACI is expressed by B cells of the MZ and switched memory B cells.[82]

The role of BAFF-R in B-cell survival was first demonstrated in mice.[78,83] The main function of BAFF-R is to provide survival signals for immature and mature B cells.[84,85] Mice with a deletion in the *Baff-r* or *Baff* genes showed a marked reduction in B-cell numbers and a developmental block at the transitional T2 stage. Specific antibody production was low in BAFF-R–deficient mice but increased after immunization with T cell–independent and T cell–dependent antigens.[83] The phenotype of BAFF-R–deficient mice is less severe than in $Taci^{-/-}$ mice because they show a robust T cell–independent antibody response that can be maintained by TACI. Reflecting the main role of BAFF-R in survival, the overexpression of transgenic Bcl2 in $Baff\text{-}r^{-/-}$ mice allowed mature B cells to develop.[83]

In human subjects BAFF-R deficiency was discovered by screening patients with common variable immunodeficiency (CVID) with low numbers of B cells.[86] In addition to a strong B-cell lymphopenia, B-cell phenotyping revealed a relative increase in numbers of transitional B cells. The strong reduction in MZ and switched memory B-cell numbers was accompanied by reduced serum IgM and IgG levels and impaired T cell–independent immune responses against pneumococcal polysaccharides. Unlike in other patients with CVID, IgA serum levels were normal, and IgA^+ plasma cells were found in the gut.[86] Likewise, $Baff^{-/-}$ mice have normal IgA levels.[87] In contrast to BAFF-R deficiency, deletion of Taci[88] or April[89] in mice and mutations in the TACI-encoding *TNFRSF13B* gene in human subjects have significantly reduced IgA levels.[90,91] Therefore it seems that serum IgA concentrations depend on functional TACI-APRIL interactions.

TACI deficiency is also associated with humoral immunodeficiency, as documented by severely decreased T cell–independent

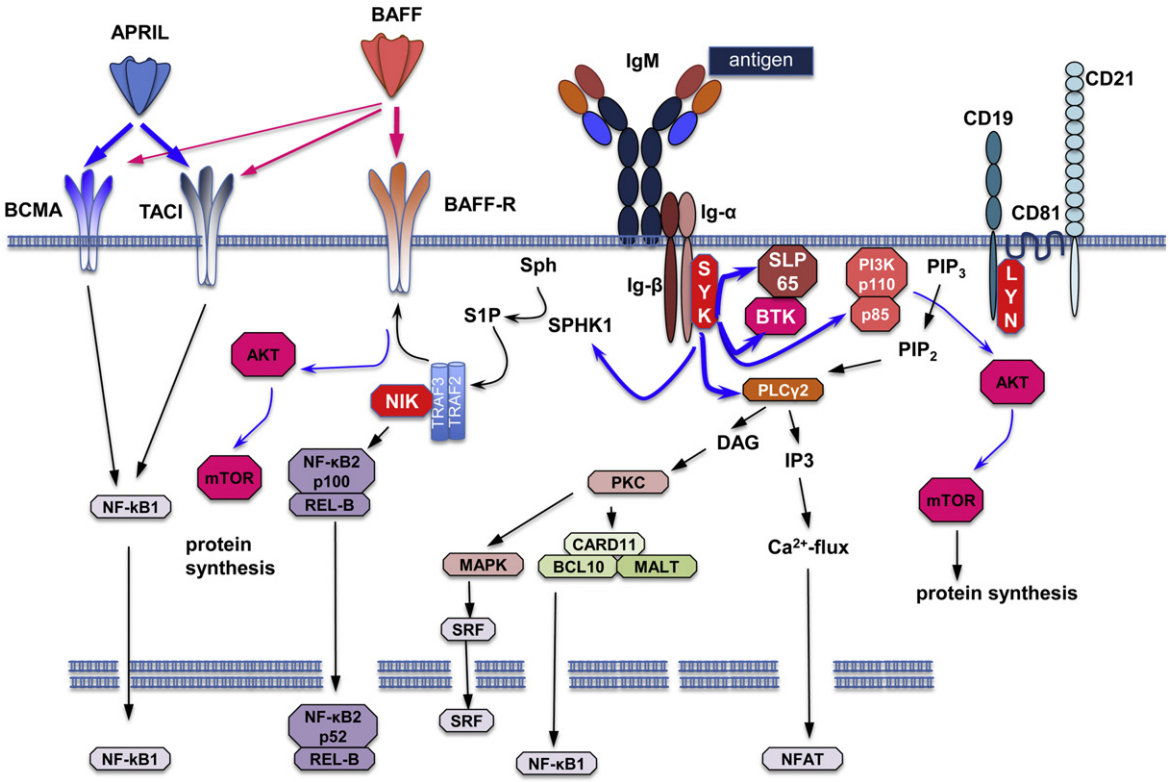

FIG 2. BCR and BAFF-R signaling cascades in B cells. Antigen binding to surface IgM induces conformational changes in the BCR, including the signaling components Ig-α (CD79A) and Ig-β (CD79B). The conformational changes allow binding of the tyrosine kinases, such as spleen tyrosine kinase *(SYK)*, and initiates several key signaling cascades composed of protein phosphorylation and processing reactions. Spleen tyrosine kinase phosphorylates Ig-α/Ig-β and the adapter protein SLP65 (BLNK), serving as scaffold for other substrates, including BTK. Phosphorylation of downstream substrates, including the phosphatidylinositol-4.5 bisphosphate 3-kinase *(PI3K)* and phospholipase C *(PLCγ2)*, activates downstream transcription factors, such as NF-κB1, nuclear factor of activated T cells *(NFAT)*, and serum response factor *(SRF)*. AKT (PKB) induces protein synthesis and cellular fitness, prolonging cell survival. BCR signals activate sphingosine-kinase 1 *(SPHK1)*. The enzyme phosphorylates sphingosine *(Sph)*, a metabolite of the membrane lipid sphingomyelin, to generate S1P, which is required by TNF receptor–associated factor 2 *(TRAF2)* as a cofactor. BAFF binds to BAFF-R and with lower affinity to TACI and BCMA, whereas APRIL only binds to TACI and BCMA. BAFF-R is expressed by all B cells, TACI by MZ and memory B cells, and BCMA by activated B and plasma cells. The alternative NF-κB pathway is activated by BAFF binding to BAFF-R. Conformational changes of BAFF-R promote TRAF binding and allow release of the NF-κB–inducing kinase *(NIK)*. NIK activates inhibitor of NF-κB kinase α *(IKKα)*, which phosphorylates NF-κB2 p100. Phosphorylated p100 is processed on ubiquitination into the active form p52, which assembles with relB into a transcriptional activator upregulating prosurvival genes. BAFF-R signaling also induces AKT and protein synthesis, thus increasing cellular fitness. *DAG*, Diacylglycerol; *IP3*, inositol-1,4,5-trisphosphate; *MALT*, mucosa-associated lymphoid tissue lymphoma translocation protein; *MAPK*, mitogen-activated protein kinase; *mTOR*, mammalian target of rapamycin; *PKC*, protein kinase C.

immune responses to polysaccharide antigens. Correspondingly, TACI is expressed on MZ B-cell subsets, strong responders to T cell–independent type II antigens. Because TACI-deficient mice have high numbers of B cells, it was proposed that TACI acts as a negative regulator of B-cell survival. Consistent with this, TACI-deficient mice tend to have lymphoproliferative disorders and autoimmune diseases with high titers of autoantibodies.[92,93] Patients with CVID share immunologic features of *Taci*$^{-/-}$ mice, including immunodeficiency, lymphoproliferation, and autoimmunity. In fact, 5% to 10% of patients with CVID carry at least 1 germline mutation in *TACI*.[90,91] B cells from TACI-deficient patients do not initiate class-switch recombination (CSR) when cocultured with either APRIL or BAFF.[91] Low IgA and IgG antibody titers in TACI-deficient patients underline the role of this receptor in CSR in human subjects. The absence of pneumococcal cell wall polysaccharide-specific IgG antibodies in some TACI-deficient patients also supports the role of TACI in immune responses to blood-borne pathogens. It has also been suggested that decreased numbers of BAFF-binding receptors caused by the absence of TACI give rise to more circulating BAFF binding to BAFF-R, thus supporting B-cell survival.[94] In human subjects it was recently shown that the numbers of BAFF binding sites can regulate steady-state BAFF concentrations.[95] High levels of BAFF, as demonstrated by BAFF transgenic mice, increase numbers of mature B cells and lead to a progressive autoimmune disease similar to systemic lupus erythematosus.[96]

In the human spleen the areas surrounding the MZ are colonized by a special subtype of neutrophils termed B-cell helper neutrophils (N_{BH} cells), which are distinct from their circulating counterparts, N_C cells.[97] The MZ colonization with N_{BH} cells occurs through a noninflammatory pathway in the absence of an infection. N_{BH} cells provide an MZ B cell–interacting

structure facilitating antibody production and SHM by activating MZ B cells with APRIL and BAFF. In contrast to T_H cells, which support follicular B cells in the GC reaction, N_{BH} cells induce a potent extrafollicular B-cell response against T cell–independent antigens. Correspondingly, patients with congenital neutropenia had fewer MZ B cells with less SHMs.

The role of BCMA as a survival factor for plasma cells is still unclear. Originally, it was described that mice deficient for BCMA had a normal B-cell compartment. However, the survival of long-lived plasma cells was impaired.[98] On a Fas/CD95-deficient background, *Bcma*$^{-/-}$ mice have a fatal lymphoproliferative syndrome caused by an enormous increase in both short- and long-lived plasma cells.[99] The pathologic features include high titers of autoantibodies against nuclear antigen, rheumatoid factor, and deposition of immune complexes. Similar to *Bcma*$^{-/-}$ mice, *Fas*$^{-/-}$*Bcma*$^{-/-}$ mice had lower numbers of BM plasma cells. Therefore BCMA might have dual function. Because BCMA is rapidly upregulated together with Fas on activated B cells, both receptors seem to control synergistically B-cell proliferation and selection in the GC reaction. However, in the BM BMCA might act as a survival factor for long-lived plasma cells.

Synopsis: B-lymphocyte survival depends on BAFF-R signaling because BAFF-R deficiency blocks B-cell development at the stage of transitional B cells. Mutations in the related receptor TACI impair the development of IgA- and IgG-secreting plasma cells and promote lymphoproliferation. N_{BH} cells surrounding the splenic MZ are critical for the production of BAFF and APRIL, 2 TNF-like ligands binding to BAFF-R and TACI, respectively.

DEVELOPMENT AND MAINTENANCE OF MEMORY B CELLS AND PLASMA CELLS

Studies with survivors of the 1918 influenza pandemic have shown that antigen-specific memory B cells can persist for decades in the absence of antigen.[100] Antigen-induced T cell–dependent B-cell activation takes place in GCs in the spleen and lymph nodes and leads to development of plasma and switched memory B cells.[101,102] B-cell activation in GCs upregulates AID. The enzyme deaminates cytidine residues in the DNA, thus generating uracil residues, which are marked by the uracil-N-glycosylase *(UNG)* to be recognized by repair enzymes. This process is part of the enzymatic machinery generating SHMs in the variable region of the expressed immunoglobulin gene.[103] Hypermutations in the variable regions increase or decrease the affinity of antibodies to antigen. Therefore they represent, in addition to the immunoglobulin gene rearrangement in the BM, a second diversification step. This has evolved to transform antibodies with intermediate or low affinities into highly specific tools against pathogenic antigens. In parallel, immunoglobulins change their effector functions by replacing the IgM constant region against those of IgG, IgA, or IgE. This process, termed immunoglobulin CSR, is catalyzed by components of the SHM machinery, including AID and enzymes of the nonhomologous end-joining complex. Ultimately, GC B cells become long-lived memory B cells or plasma cells. They can reside either in secondary lymphoid organs or migrate to the BM. Also shown by humoral immunodeficiencies, the development of long-lived memory and plasma cells is interrupted by mutations in genes encoding components of the SHM/CSR machinery, such as AID,[60] UNG,[104] and postmeiotic segregation increased 2 (PMS2).[105] As shown by humoral immunodeficiencies, other components and receptor-ligand pairs are required for successful B/T-cell interactions, such as CD40,[106] CD40 ligand,[107] or inducible costimulator,[108] which is expressed by follicular T_H cells. Similar to these defined genetic defects, patients with CVID with impaired plasma cell development have almost no switched memory B cells. This suggests that the development of switched memory cells is intimately connected to plasma cell development. However, it is still unknown where exactly switched memory B cells branch off from the path to plasma cell development.

The human memory B-cell compartment is far less uniform than originally thought. A recent publication has defined the existence of several memory B-cell subsets differentiating from 3 different origins.[64] The origin of $CD27^-IgG^+$ memory B cells is the spleen, whereas $CD27^+IgM^+IgD^-$ and class-switched $CD27^+IgG/IgA^+$ memory B cells develop in the GCs. Sharing common features with IgA^+ B cells in the lamina propria, $CD27^-IgA^+$ B cells can come from the gut, where they are generated outside of the GCs, even in CD40 ligand–deficient patients. This memory B-cell subset is characterized by a limited replication history and dominant IgA_2 H-chain and Igλ L-chain use.

Switched memory B cells also differ from their precursors by their requirements for survival signals. Treatment of patients with systemic lupus erythematosus with the BAFF-neutralizing mAb belimumab revealed that persistence of memory B cells does not require BAFF/BAFF-R interactions, as is the case for transitional, follicular, and MZ B cells.[109]

Treatment of patients with autoimmune diseases with the B cell–depleting anti-CD20 mAb rituximab revealed that plasma cells in human subjects can be both short and long lived.[110,111] Long-lived plasma cells survive in specialized niches of the BM[112,113] or through the continuous, antigen-driven differentiation of memory B cells.[114] Analyses of serum immunoglobulin titers against viral antigens or vaccines in rituximab-treated patients, whose B cells were entirely eliminated, showed that $CD20^-$ long-lived plasma cells can persist for years. Part of the long-lived plasma cell repertoire of the BM is replaced by newly incoming plasma cells generated during acute immune responses.[115] It has been widely accepted that long-lived plasma cells originate from B cells activated in concert with T-follicular helper cells in the GC reaction. However, recent findings revealed T cell–independent methods of long-lived plasma cell formation. For example, long-lived plasma cells can be generated in T cell–deficient mice that have been treated with haptenated LPS.[116] Although IgM antibodies were thought to be secreted by short-lived plasma cells, it has now been reported that chronic infections with *Ehrlichia muris* induce $CD138^{high}IgM^{high}$ BM plasma cells, contributing to a long-lasting IgM titer.[117]

Development of both short-lived and long-lived plasma cells depends on the expression of the transcription factor B lymphocyte–induced maturation protein 1 (Blimp-1),[118] a master gene of plasma cell development. Blimp-1 induces X-box binding protein 1 (XBP1),[119] a key transcription factor, to initiate the unfolded protein response. Blimp-1 activity is also required to downregulate the transcription factor paired-box protein 5 (PAX5),[120] the master gene for B-cell development induced in common lymphocyte progenitor cells to initiate B-lineage commitment.

The guidance of plasma cells to their niches is achieved by changing the responsiveness to tissue-specific chemokines. Downregulation of GC-related chemokine receptors, such as CXCR5, as well as upregulation of CXCR4, promotes plasma cell homing to sites with high expression of the CXCR4 ligand

CXCL12, which is produced, for example, by BM stromal cells.[121-123]

Long-lived plasma cells are terminally differentiated and seem not to divide. Therefore they need survival signals to ensure long-term existence. Potent *in vitro* plasma cell survival factors are IL-6 and ligands for CD44, such as hyaluronic acid.[124]

Recently, it has been shown that human and murine plasma cells express CD28 together with its ligands, B7.1 and B7.2. CD28 is expressed on T cells, providing costimulatory signals on binding to B7 molecules on antigen-presenting cells. Mice deficient for CD28 have been used to uncover a role of this receptor in both short-lived and long-lived plasma cells. This is because plasma cells secrete higher levels of antibodies in the absence of CD28. Because the deficiency in the B7 molecules causes the same phenotype,[125] CD28-B7 interactions seem to negatively regulate the rate of antibody secretion by plasma cells.[126] However, in malignant plasma cells CD28 signaling was shown to induce cell survival, thus protecting them against apoptosis induced by cytostatics.[127]

Despite these extensive studies on the role of ligands, receptors, signaling components, and transcription factors, the mechanisms for how long long-lived plasma cells persist in the BM are not yet completely understood.

Synopsis: In T-dependent immune responses, class-switched memory B cells and plasma cells develop in GCs. Mutations affecting components of the class-switch and SHM machinery prevent the formation of memory B and plasma cells expressing IgA, IgG, and IgE. The human memory B-cell compartment is more complex than originally thought, including extrafollicular memory B lymphocytes and cells originating from the gut and spleen. Nondividing, long-lived plasma cells reside in the BM. These cells are not eliminated by rituximab treatment. Fig 3 summarizes genetic defects interrupting B-cell development and activation.

B-CELL TOLERANCE

It was already recognized by Paul Ehrlich, after he postulated the "side-chain-theory" in 1897 to describe the function of the yet to be discovered antibodies, that reactivity against self-antigens represents a thread to the organism.[128] Bearing this in mind, he coined the term "horror autotoxicus." Decades later, Frank Mcfarlane Burnet developed the concept of clonal deletion or selection of autoreactive or nonautoreactive B cells, respectively. This concept explains how reactivity against foreign antigens and pathogens can be achieved without generating a large spectrum of potentially harmful autoreactivities.[129] With the onset of transgenic and knockout/knock-in mouse technology, it was then possible to prove these concepts directly for defined antibody specificities *in vivo*. Chris C. Goodnow developed a very elegant mouse model to demonstrate clonal B-cell anergy and clonal deletion as mechanisms evolved to silence autoreactive B cells. The model is based on transgenic mice expressing hen's egg lysozyme (HEL)–specific IgM/IgD immunoglobulins from μ-δ/κ transgenes that were combined with another mouse strain expressing transgenic HEL either in a soluble or membrane-bound form.[130-132] It has been widely used to investigate the selection mechanisms acting on B cells and the molecules involved in this process. Clonal deletion of autoreactive B cells at the stage of immature B cells was also shown by several other groups using different transgenic mouse models.[133-135] In addition to clonal anergy and deletion, secondary rearrangements of L-chain genes expressed by autoreactive, IgM^+ immature B cells in the BM were found to be one of the key regulatory mechanisms. This prevents the exit of highly autoreactive B cells from the BM into the circulation.[136,137] Which of these mechanisms plays a role in the selection of human B cells and in shaping the B-cell repertoire in human subjects? The comparison of antibody specificities between newly formed immature B cells in the BM, circulating transitional B cells, and mature B cells has clearly shown that the repertoire of immature B cells contains a large proportion of specificities recognizing self-antigens, such as insulin or DNA.[138] In contrast, these specificities represent only a small part within the repertoire of mature B cells. Repertoire analysis with B cells from patients with defects in the BCR,[139] TLRs,[140] and accessory receptor signaling[141,142] revealed that in human subjects BCR and TLR signals are critical in guiding the selection of B cells.[143] These analyses show that the human immune system is equipped with an effective counterselection system precluding the exit of autoreactive B cells into the pool of mature, circulating B cells. However, autoimmune diseases and the many specificities of autoantibodies clearly prove that this selection process leaves many holes, allowing autoreactive B cells to sneak through. Where are these holes, and which mechanisms might allow autoreactive B cells to develop into short- or even long-lived plasma cells?

The anti-HEL IgM/D X HEL transgenic mouse model clearly showed that B cells expressing surface IgM/IgD with high affinity to soluble HEL as autoantigen are "silenced" because their BCR does not respond to antigen triggering. The unresponsiveness is mainly due to the different rates of receptor internalization between surface IgM and surface IgD because surface IgD is more stable[144] and less rapidly downregulated than IgM on binding to HEL as a self-antigen.[145]

Regulation of B-cell responsiveness by different recycling rates of autoantigen-binding surface IgM and surface IgD has very recently been shown by Zikherman et al[146] in a transgenic mouse model. It is based on the observation that transcription of nuclear receptor 77 (nur77), a steroid-thyroid hormone retinoid receptor family member, is rapidly induced after antigen binding to the BCR. Expressing a green fluorescent protein (GFP) transgene under the transcriptional control of nur77 regulatory elements, B cells of these mice emit green fluorescence when the BCR binds antigen. There the system is an ideal reporter to monitor BCR engagement *in vivo*. Surprisingly, and in contrast to current concepts, all mature B cells expressed nur77-driven GFP, although at different intensities, suggesting that all B cells are autoreactive. Autoantigen binding was detected first in transitional B cells on entering the spleen, whereas immature and pre-B cells in the BM did not activate nur77-GFP expression. This might be explained by assuming that the signals sensed by autoantigen-binding immature B cells in the BM might not be strong enough to upregulate nur77 transcription. However, in the spleen nur77-GFP fluorescence intensity correlated directly with the pattern of IgM and IgD surface expression. The highest GFP levels were found in $IgD^{hi}IgM^{lo}$ follicular B cells. Because $IgD^{hi}IgM^{lo}$ B cells had a stronger response by Ca^{2+} flux to anti-IgD than to IgM cross-linking, these follicular B cells have all the attributes of silenced anergic B cells, as described in the HEL model. In human subjects most of the follicular B cells have an $IgD^{hi}IgM^{lo}$ phenotype. Therefore a major part of our B-cell repertoire seems to be formed by self-reactive B cells that are kept alive by BAFF/BAFF-R signaling waiting to be activated by T_H cells on antigen

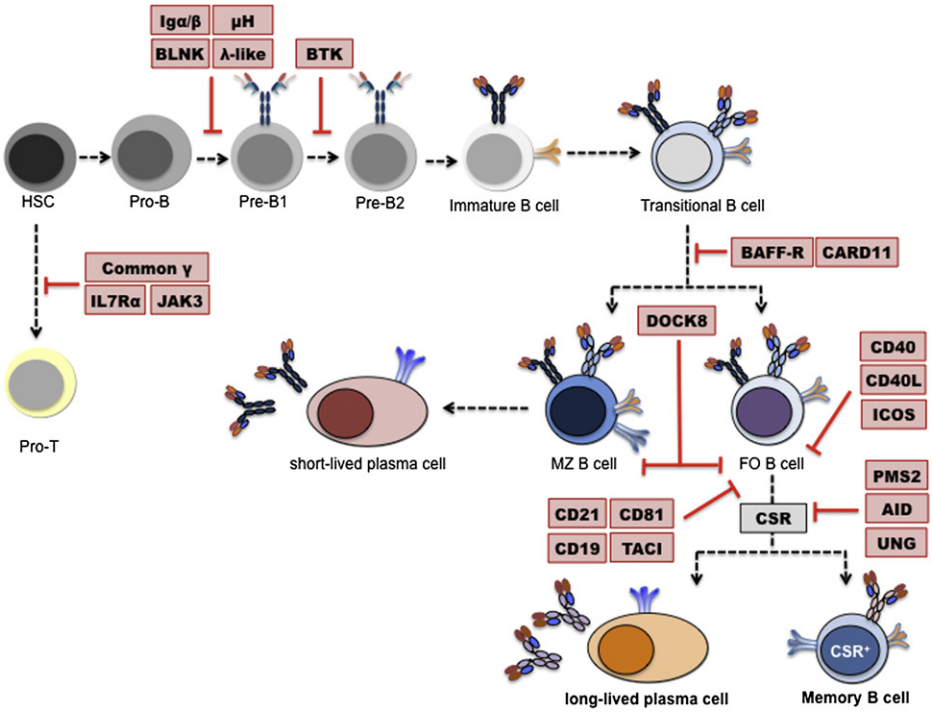

FIG 3. Genetic defects interrupting B-cell development. Genetic defects interrupting B-cell development at different stages are *boxed in red*.

binding. Thus everyone has a large repertoire of autoreactive B cells forming a reservoir of clones that have the potential to differentiate in the GC reaction into plasma cells secreting highly autoreactive pathogenic autoantibodies.[147,148]

In another anti-HEL/HEL transgenic mouse model, it was shown that the development of such clones is regulated by their affinity for the self-antigen and by the distance of the autoantigen from the GC, in which autoreactive B cells are activated by cross-reactive foreign antigens.[149] Sufficiently high levels of self-antigens within the GC effectively prevent the differentiation of autoreactive GC B cells into plasma cells, even if their BCRs are highly cross-reactive to foreign antigens. However, if self-antigens are expressed in other tissues and organs outside of the GC, self-reactive B cells escape deletion and can be positively selected to initiate a high-affinity autoimmune response.[149] This mechanism might account, for instance, for the presence of cross-reactive autoantibodies in antiviral immune responses[150] and in hepatitis C–related autoimmune thrombocytopenia. Therefore the balance between the relative affinity of BCRs to self-antigens and foreign antigens and the distance of self-antigens from activated B-cell clones in the GC are key parameters in determining the fate of self-reactive GC B cells and in discriminating between protective immune responses and autoimmunity. Fig 4 summarizes checkpoints for autoreactive B cells.

Synopsis: In the BM autoreactive B cells are rescued by secondary rearrangement of their L-chains, resulting in new antibody specificities. In the spleen most if not all B cells seem to bind self-antigens to some extent. Cells with higher affinity are rendered unresponsive to IgM-dependent signaling. They can be rescued by antigen binding to their BCR in cooperation with T-cell help. Self-antigens expressed in GCs block the formation of memory and plasma cells expressing high-affinity autoantibodies. Because B cells do not discriminate between foreign antigens and self-antigens found only outside the GC, the latter might serve as targets for high-affinity autoantibodies.

SUMMARY

Many different lines of genetically engineered mice carrying germline mutations in immunologically important genes were and still are indispensable to study the molecular mechanisms regulating B-cell development, selection, and response. However, the comparison of mouse mutants with corresponding genetic defects in human subjects revealed significant differences between human and murine B cells with respect to early phases of development, subset composition, and responses to antigens. Human B-cell development, for instance, does not depend on IL-7, whereas mutations in genes like BTK and BLNK block maturation of human but not murine pre-B cells. Human subjects, but not mice, have circulating MZ B cells carrying somatic mutations right after birth, and human subjects have a large compartment of circulating class-switched memory B cells that is much smaller in adult mice. Because primates and rodents diverged from a common ancestor at least 65 million years ago, such differences reflect the adaptation of the respective immune systems to the different physiologies, the different life spans, the environment, pathogens, commensal agents, and so on.

Despite the increase in knowledge about human B-cell biology, many basic questions still remain. For example, how do GC B cells decide whether they prefer to develop into switched memory B cells or plasma cells. Do different switched memory B-cell subsets have different functions in immune responses? Do they develop into short- or long-lived plasma cells? Which survival signals keep memory B cells and plasma cells alive over decades, or how can we exploit the characteristics of human B cells to improve vaccination? With the current

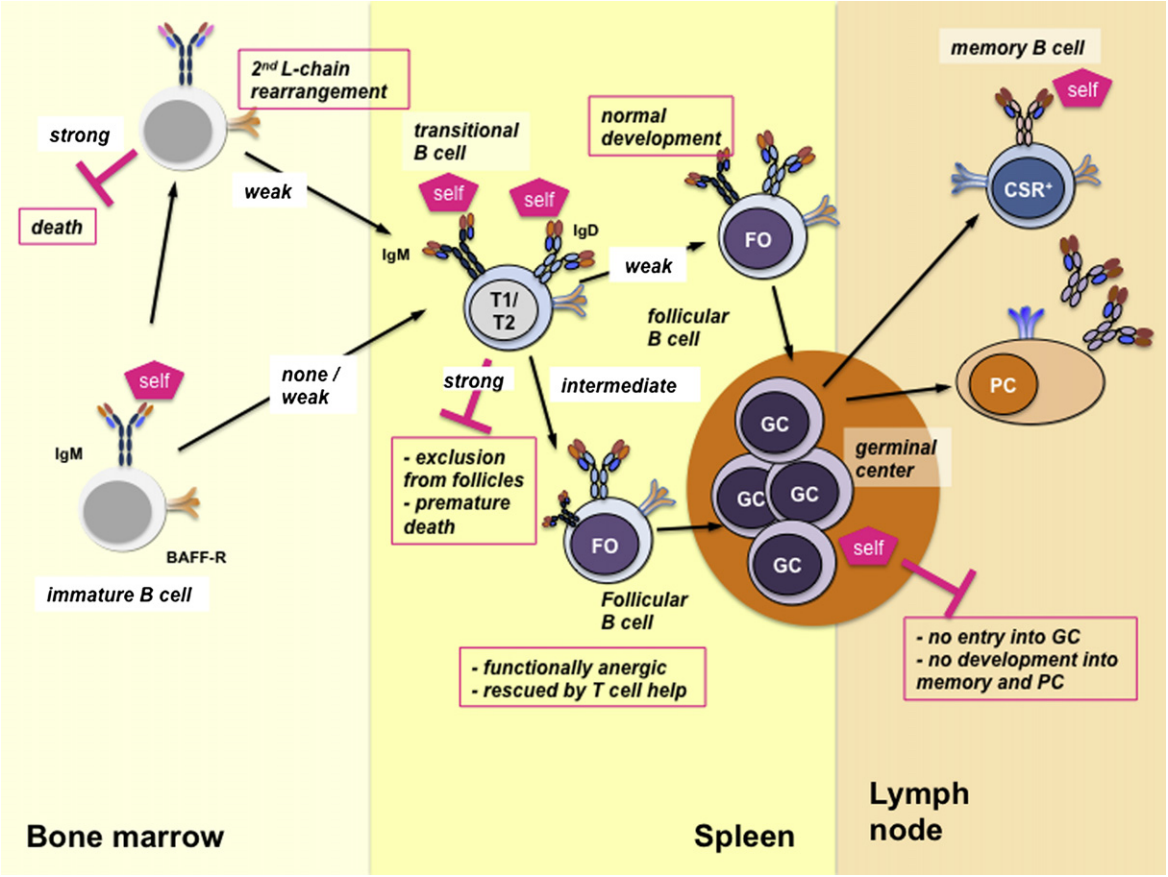

FIG 4. B-cell tolerance models. If immature B cells bind to self-antigens, they can undergo secondary rearrangement of L-chain loci to generate new specificities with lower affinity to self-antigens. Strongly binding cells will die in the BM; all other cells emigrate to the spleen, where BCRs bind to self-antigens with various affinities. Strong binding can lead to exclusion from B-cell follicles, and intermediate binding can lead to enhanced IgM internalization and functional anergy. Binding of (foreign) antigens to IgD combined with T-cell help rescues anergic B cells and allows activation and entry into the GC reaction. Cells exposed to self-antigens in the GCs are not selected into the pool of long-lived switched memory B cells and plasma cells. B cells expressing BCRs reactive against self-antigens located outside of the GCs are selected into the memory B lymphocyte and plasma cell pool. This mechanism might account for the generation of autoreactive B cells in patients with autoimmune diseases.

pace of discovering new genetic defects and deciphering the functions of their gene products, we can expect to be able to understand human B-cell biology in the near future to foster the development of new tools that specifically target dysfunctional B lymphocytes to treat autoimmunity, B-cell malignancies, and immunodeficiency.

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