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Antigen-Specific B-Lymphocyte Activation

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ABSTRACT: B lymphocytes comprise a major component of the adaptive mammalian immune system, having the exclusive ability to produce and secrete immunoglobulins (Ig) of various forms (isotypes). This property alone renders B-cell activation critical to immunity, but the fully activated B cell also functions in antigen presentation and the production of a variety of cytokines and chemokines. There are many signals that must be coordinated to achieve and properly regulate antigen-specific B-lymphocyte activation and the development of humoral memory. This review seeks to summarize these components, and to highlight recent advances in each area that contribute to a greater understanding of the complex processes involved in B-cell activation.

KEY WORDS: receptors, signal transduction, immunoglobulin, TNF-R family, memory

I. INTRODUCTION

The study of mammalian immunity over many decades has clearly shown that an effective immune system requires the normal functioning of all types of hematopoietic cells. Protection from pathogens requires that both the innate and adaptive arms of immunity function well and in concert. One of the major components of adaptive immunity is the humoral, or antibody-mediated immune response—the exclusive purview of the B lymphocyte. A variety of immune system cells produce lymphokines and chemokines, but only B lymphocytes make immunoglobulins (Ig), and a lack of antibodies, even of certain isotypes, leads to profound compromises in immunity.[1–4] Although Ig production is a unique B-cell function, effective activation of B lymphocytes is important to normal immune function in additional ways. Dendritic cells are the most effective antigen-presenting cells (APC) for most activation events of naïve T lymphocytes,[5] but B lymphocytes can serve as important APC in certain situations and play roles in the stimulation of normal immunity, autoimmunity, and tolerance.[6–23] It is thus not surprising that a complete lack of B cells or a failure in B-cell activation pathways also leads to defects in T-cell activation,[24–27] so while it has long been recognized that adaptive humoral immunity requires normal cell-mediated immune function, it is now becoming appreciated that the converse is also true.

There are two major avenues for B-lymphocyte activation: activation that occurs in the context of cognate interaction with an activated T lymphocyte, whose receptor recognizes antigen presented by the B cell, or activation by "T-independent" (TI) antigens. The latter can bind either all B-cell antigen receptors, regardless of specificity (TI type 1), or bind to other antigen nonspecific activation receptors expressed by all B cells (TI type 2). Because each type of B-cell activation is itself a major topic, in this review we will confine ourselves to T-dependent, antigen- specific B-cell activation. The reader is referred to other excellent articles

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to begin to explore the topic of T-independent B-cell activation.[28,29]

T-dependent B-cell activation is initiated by binding of antigen to the combining site of the B-cell antigen receptor (BCR), the membrane form of Ig. This binding has two important consequences. First, it initiates internalization of the antigen into specialized intracytoplasmic compartments, where antigen is processed and its peptide fragments loaded onto MHC class II molecules for membrane presentation to T cells.[30] Second, engagement of the BCR by antigen stimulates formation of a membrane signaling complex, consisting of both the BCR and a variety of co-receptors, which regulates B-cell activation in both positive and negative ways. Upon successful antigen presentation to an activated T lymphocyte, B cells receive many additional regulatory signals. These include signals delivered via contact-mediated receptor–ligand interactions, as well as receipt of signals delivered by soluble chemokines and lymphokines. The culmination and interaction of all these various signaling pathways determines the ultimate outcome of the encounter with antigen—clonal expansion and differentiation, plasma cell formation, or long-term survival as a memory B cell in a germinal center. These signals also regulate the development of B-cell tolerance. Each of these signals and steps in activation are discussed in turn in the following review. It is clear that much is now known about B-cell activation, including the identity of many of the receptors involved, their signaling mechanisms, and the functional consequences of signal delivery. But it is equally clear that much remains to be understood. Previously unidentified receptors involved in B-cell activation are being discovered at a rapid pace. How each plays its roles in the process, and, especially, how the B cell integrates all this information from its environment, are questions that await further elucidation.

II. THE BCR COMPLEX

The B-cell antigen receptor (BCR) is absolutely required during B-cell development and differentiation. The BCR plays an essential role early in B-cell development in many processes, including allelic exclusion and gene recombination at the light-chain loci, negative and positive selection, and anergy and receptor editing.[31,32] Targeted disruption of the BCR complex has revealed its necessary role in selection into the peripheral B-cell population, maintenance of the B-cell repertoire, response to antigen, and selection into the memory B-cell pool.[31,32] Because of the important physiological role of B lymphocytes in the immune system, extensive research is devoted to understanding the molecular mechanisms and signaling pathways that regulate BCR-mediated B-cell activation. The individual events and molecules involved are described below and summarized in schematic form in Figure 1.

The BCR is a multimeric protein complex in which the antigen binding and the signal transduction subunits are distinct. The antigen recognition/binding module is the membrane Ig (mIg), which is a tetrameric complex of immunoglobulin heavy (IgH) and light (IgL) chains. The signal-transmitting component consists of Ig-α (CD79a) and Ig-β (CD79b), which form a disulfide-bonded heterodimer.[33]

The critical role of the membrane form of the μ chain is evident in mice lacking this gene, which results in blockade at the pro-B-cell stage and loss of H chain allelic exclusion.[34,35] Elegant studies by Lam et al. using Cre-*loxP*-mediated inducible gene targeting demonstrated that BCR expression is required for the persistence of mature B cells in the peripheral immune system, and its deletion leads to elimination via apoptosis of receptorless B cells.[36] Association of μ chain with its signaling modules, Ig-α and Ig-β, is essential for allelic exclusion and developmental progress to the pre-B-cell stage, evident in mice transgenic for a mutant μ chain that is not able to associate with endogenous Ig-α and Ig-β.[37]

The contribution of Ig-α and Ig-β in B-cell development and BCR signaling has been assessed in a systematic manner by generations of mice either deficient in or containing mutant versions of each of the components. Recent studies have revealed distinct and complementary roles for Ig-α and Ig-β. Reichlin et al. compared B-cell development and BCR signaling among mice carrying a deletion in the cytoplasmic domain of Ig-α (Ig-αΔC) or Ig-β (Ig-βΔC).[38] Whereas both mouse mutants show a dramatic decrease in the

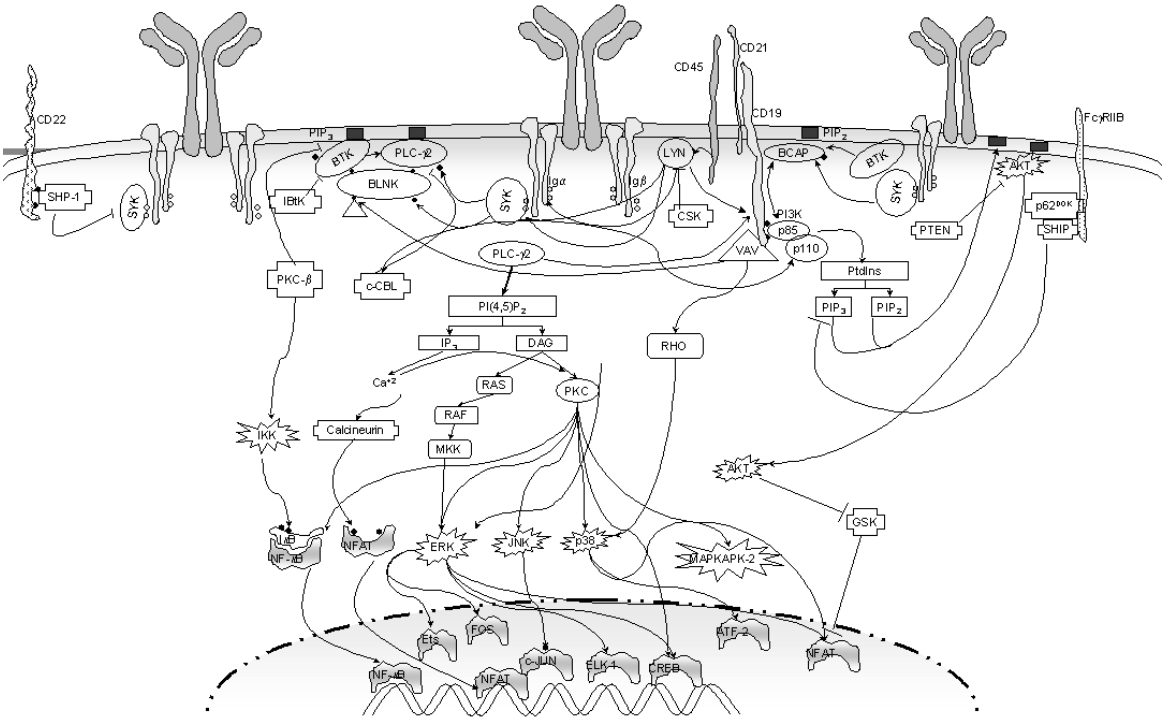

FIGURE 1. Antigen recognition by the BCR leads to activation of several protein tyrosine kinases, including Lyn, Syk, and Btk. Tyrosine phosphorylation of adaptor proteins, such as BLNK, links BCR engagement to downstream signaling pathways regulated by PI-3K, PLC-γ2, PKC, and Ras. Cell surface co-receptors activate additional signal cascades that influence BCR-initiated signals, both positively (e.g., CD19) and negatively (e.g., CD22 and CD32). The integration of multiple signaling pathways culminates in activation of transcription factors and gene regulation, as detailed in the text.

number of splenic B cells, B-cell development is abruptly terminated at the immature stage in Ig-βΔC mice, versus Ig-αΔC mice in which B cells are lost throughout development. Additionally, B cells from Ig-βΔC mice display normal calcium (Ca^{2+}) mobilization upon BCR engagement,[39] whereas this response is enhanced and prolonged in Ig-αΔC mice and associated with strong protein tyrosine phosphorylation, suggesting a negative role for Ig-α.[40] Further analysis of Ig-αΔC mice showed that immature B cells are unexpectedly activated, thereby mimicking self-reactive B cells, which might explain why they are eliminated.[39] Therefore, Ig-α and Ig-β play differential roles, depending on the differentiation stage of B cells.

The signaling functions of Ig-α and Ig-β depend primarily on the immunoreceptor tyrosine-based activation motif (ITAM) found in the cytoplasmic (CY) domain.[41,42] Each ITAM has the amino acid sequence $D/Ex_7D/ExxYxxI/Lx_7YxxI/L$. The two tyrosine residues serve as protein tyrosine kinase (PTK) substrates and, once phosphorylated, function as docking sites for downstream signaling molecules.[41,42] BCR engagement leads to Ig-α ITAM phosphorylation by Lyn, a Src kinase family member. The crucial role of ITAM tyrosine phosphorylation is apparent in mice expressing Igα, in which the two tyrosines in the ITAM motif were replaced by phenylalanines ($Ig\text{-}\alpha^{FF/FF}$).[43] These mice show defects in development of B1 and marginal zone B cells, and in response to T-cell-dependent (TD) antigens. Confirming a negative role of Ig-α,[39,40] these mutants displayed exaggerated Ca^{2+} flux. Interestingly, the phosphorylation of Lyn and Syk is not completely abolished, but is delayed and reduced, suggesting a role for the non-ITAM tyrosines in BCR-signaling. Recent studies have demonstrated a role for the non-ITAM tyrosines, Y176 and

Y204, in the Ig-α CY tail, in linking Syk activation to B-cell linker protein (BLNK)-dependent pathways.[44,45] Despite similarities between the ITAM motifs in Ig-α and Ig-β, there seem to be differences in magnitude of phosphorylation, which could account for the differences in PTKs associated with each of the receptors.[46,47] Upon BCR engagement, Syk has been shown to bind primarily to Ig-β, rather than Ig-α,[48] whereas BLNK interacts primarily with Ig-α.[45] For a more extensive coverage of the role of Ig-α and Ig-β in B-cell activation refer to recent excellent reviews.[33,49]

A. Protein Tyrosine Kinases

The transmission of signals from the BCR to the nucleus relies on the activation of protein tyrosine kinases (PTKs), which occurs immediately after BCR engagement. Extensive work in this area has shown that the BCR activates three distinct types of PTKs: the Src, Syk, and Tec family kinases. This section will focus on representatives from each family.

Lyn is a member of the Src family of tyrosine kinases, which is expressed in various cell types.[50] It was initially identified as a homologous gene to other *src* family members,[51] and it exists in two isoforms, $p56^{lyn}$ and $p53^{lyn}$.[52] Structurally, Lyn consists of SH2 and SH3 domains, enabling it to interact with other proteins by recognizing phosphotyrosine-containing or proline-rich regions, respectively.[53] In addition, Lyn has a catalytic/kinase domain necessary for regulation of other proteins via tyrosine phosphorylation. Src kinases contain two primary regulatory tyrosine residues, whose phosphorylation can lead to enhancement (Tyr-397 within the kinase domain) or inhibition (Tyr-508 within the C-terminal tail) of activity.[53,54] BCR engagement leads to rapid tyrosine phosphorylation and activation of Lyn,[55,56] which leads to further phosphorylation of the ITAM of Ig-α, thereby creating docking sites for SH2 domain-containing downstream signaling molecules.[57] Activation of Src family kinases, such as Lyn, is counterbalanced by **Csk**, a cytoplasmic PTK, that exerts its regulatory effect via phosphorylation of the C-terminal inhibitory tyrosine residue.[58,59] Mice deficient for Csk die early in development and display enhanced Lyn kinase activity.[60,61] This is also evident in Csk-deficient B cells,[62] supporting a critical negative role for Csk in Lyn activation.

Mice deficient for Lyn have decreased numbers of peripheral B cells and are defective in their responses to TD and TI antigens.[63] In addition to the positive roles that Lyn plays in BCR-signaling initiation, the knockout mice suggest negative roles for Lyn in B lymphocytes. B cells from $Lyn^{-/-}$ mice have increased Ca^{2+} flux, spontaneous hyperactivity in the absence of antigen, increased mitogen-activated protein kinase (MAPK) activation, and increased proliferative response upon BCR engagement.[64,65] $Lyn^{-/-}$ mice are also characterized by splenomegaly and production of autoantibodies, supporting a critical inhibitory role for Lyn in BCR signaling. The negative contribution of Lyn is believed to be mediated via phosphorylation of the immunoreceptor tyrosine-based inhibitory motif (ITIM) found on CD22 (see discussion of CD22 below). These data support a multi-purpose role for Lyn in B lymphocytes.

Phosphorylation of tyrosines in the ITAM leads to recruitment of **Syk,** a member of the Syk PTK family that serves a crucial role in transduction of signals emanating from the BCR. Syk consists of two SH2 domains, separated by a unique linker region and a C-terminal catalytic domain.[66] The critical role of Syk in BCR signaling is evident in $Syk^{-/-}$ mice, which have a block in B-cell development at the pro-B to pre-B-cell transition.[67,68] These mice are also deficient in mature B cells, supporting a role for Syk in their maintenance. BCR engagement results in recruitment and association of Syk with the BCR complex.[69] The presence and phosphorylation of both conserved tyrosines within ITAM motifs is necessary for efficient recruitment of Syk via its SH2 domains.[70] Recent work in *Drosophila* S2 Schneider cells has challenged the necessity for both ITAM tyrosines to be phosphorylated for Syk recruitment, and suggests that Syk recruitment is independent of Lyn.[71] However, this needs confirmation in B cells. BCR recruitment of Syk leads to increased autophosphorylation at Y518/Y519, which ultimately enhances Syk catalytic activity.[70] Syk is itself tyrosine phosphorylated on multiple residues,[72,73] each of which might play different roles in Syk association with and disassociation from the BCR[74] or activation of

downstream signaling pathways.[74,75] Tyrosine phosphorylation enables Syk to phosphorylate downstream adaptor proteins, such as BLNK, which then serves as a docking site for phospholipase C-γ2 (PLC-γ2) and facilitates its recruitment to the plasma membrane. Once in the plasma membrane, PLC-γ2 is phosphorylated and activated by Syk and Btk.[76]

The Tec family of PTKs, predominantly Bruton's tyrosine kinase (**Btk),** also plays a positive role in BCR signaling.[77] Btk contains an N-terminal pleckstrin homology domain (PH), a proline-rich Tec homology (TH) region, SH3 and SH2 domains, and a C-terminal catalytic domain, which allow interaction of Btk with a variety of signaling molecules,[78] impacting multiple signaling pathways. Mutations in each of the domains have been reported to cause X-linked agammaglobulinemia (XLA) in humans.[79–81] Interestingly, Btk is the only protein known where mutations in the PH domain cause a disease. The PH domain is important for membrane localization of Btk via interactions with phosphatidylinositol (3,4,5)-triphosphate (PIP_3).[82] When recruited to the plasma membrane, Btk is brought in proximity to the BLNK-PLC-γ2 complex via the SH2-domain, which binds to tyrosine phosphorylated BLNK. This recruitment enables Btk to induce tyrosine phosphorylation of PLC-γ2 and initiates production of inositol-tris-phosphate (IP_3) and mobilization of Ca^{2+}.[76,83]

Mutations in the PH domain are detrimental to Btk function, by interfering with Btk binding to PIP_3. This is evident in humans with XLA[80,81] and in mice with X chromosome-linked immunodeficiency (XID).[84,85] XLA patients have reduced numbers of BCR-positive B cells because of a developmental blockade at the pre-B-cell stage, and low levels of Ig in the serum, and are therefore susceptible to bacterial infections.[86]
Interestingly, $Btk^{-/-}$ mice display a more similar phenotype to XID mice than XLA patients, suggesting a differential role for Btk among different species.[87,88] A point mutation in the PH domain (E41K) leads to increased membrane association, tyrosine phosphorylation, and, more importantly, transforming activity,[89] suggesting a role for Btk in oncogenesis, and the necessity of a negative regulatory mechanism. Recently, protein kinase C beta (PKCβ) and a novel inhibitor of Btk (IBtk) were shown to negatively regulate Btk activity.[90,91] PKCβ was shown to exert its inhibitory effects via serine-phosphorylation within the TH domain, affecting membrane translocation,[90] whereas IBtk binds to the PH domain and impedes the kinase activity, Ca^{2+} mobilization, and nuclear factor-κB (NF-κB) activation.[91] These data point to multiple regulatory mechanisms that ensure appropriate threshold and specificity of Btk-mediated events.

The central role that Btk plays in B lymphocytes is underscored by the similar XID phenotype observed in mice lacking other molecules that interact with or influence Btk activation, such as the p85α subunit of phosphatidylinositol-3 kinase (PI3K),[49,92] BLNK,[93,94] PKCβ,[95] PLC-γ2,[96] and Vav1/Vav2.[97,98] All these mice show defects in Ca^{2+} regulation, emphasizing the interdependence among these proteins.

B. Adaptor Proteins

Activated Lyn, Syk, Btk, and other PTKs phosphorylate additional signaling molecules, which leads to production of various second messengers and initiation of multiple intermediary signaling pathways. The link between PTKs and downstream signaling molecules is mediated via adaptor proteins, which do not possess inherent enzymatic activity, but serve as scaffolds by enabling interactions among various molecules via different domains. Adaptor proteins play an important role in subcellular localization, conformational rearrangement, and interaction among effector molecules, enabling a tightly and accurately regulated signaling network. This section will focus on BLNK, Vav, and Cbl.

BLNK was originally identified as a 70-kDa tyrosine phosphoprotein preferentially expressed in B lymphocytes.[99,100] Fu et al. demonstrated that BCR engagement leads to Syk-induced tyrosine phosphorylation of BLNK, creating docking sites for downstream effector molecules.[101] By bringing multiple effector molecules in close proximity to each other (i.e., Btk, PLC-γ2),[83] BLNK enables interactions among them, as well as the necessary cross-talk among various signaling pathways. Using gene targeting to generate BLNK-deficient B cells, Ishiai et al. show that BLNK is

necessary for the recruitment of PLC-γ2 to the plasma membrane, where it can exert its effects on its substrate phosphatidylinositol 4,5-biphosphate (PI 4,5-P_2) and induce Ca^{2+} mobilization. In addition, the Rac1-JNK pathway is dependent upon BLNK expression.[102]

The crucial role of BLNK in B-cell development is evident in mice lacking BLNK, in which B-cell development is blocked at the pre-B and immature-B-cell stages.[93,94] There is a dramatic decrease in peripheral B cells in the spleen and lymph node and a lack of B-1 cells. Functional analysis of BLNK-deficient B cells revealed a role for BLNK in the proliferative responses to BCR, CD40, and mitogen, since both these and Ca^{2+} mobilization are defective when compared to wild-type cells. However, the Ca^{2+} response is not completely abolished, pointing to other BLNK-independent pathways regulating Ca^{2+} mobilization.[93,94] Confirming the central role of BLNK in B-cell signaling, these mice also show defects in IgM and IgG3 responses to TI and TD antigens.[93] Recent examination of $BLNK^{-/-}$ mice, 9–14 weeks of age, demonstrates the presence of solid tumors, consisting exclusively of pre-B cells, and splenomegaly, pointing to a novel role for BLNK as a tumor suppressor.[103]

One of the molecules that interacts with BLNK is the guanine-nucleotide exchange factor **Vav** (reviewed in Ref. 104). Structurally, Vav proteins (Vav-1, Vav-2, and Vav-3) contain several protein-interaction domains, including a calponin-homology domain, a diffuse B-cell lymphoma-homology domain, a PH domain, a Zinc-finger domain, a proline-rich region, and two SH3 domains separated by an SH2 domain.[104] The presence of multiple domains allows Vav to interact with various signaling molecules. Yeast-two hybrid experiments as well as *in vitro* studies using anti-BCR stimulated cells show that Vav interacts with Syk; this interaction is dependent on the activity of Syk and the SH2 domain of Vav.[105] Vav activation also leads to activation of the Rho-family GTPases,[106] which serve as molecular switches for downstream signaling molecules, including c-jun kinase (JNK) and p38.[107] CD19 is also able to activate and synergize with the BCR in Vav tyrosine phosphorylation, and, consequently, Ca^{2+} mobilization and JNK activation[108] (see CD19 discussion below).

Genetic studies have been used to dissect the role of Vav proteins in B-lymphocyte activation. Mice deficient for Vav-1[109,110] or Vav-2[97,98] display normal B-cell development in the bone marrow, partial decrease in Ca^{2+}mobilization, and BCR-induced proliferation, suggesting a redundant role for these molecules. $Vav1^{-/-}Vav2^{-/-}$ mice display a more profound defect, evident in the complete abrogation of Ca^{2+}mobilization, a dramatic decrease in absolute number of splenic B cells, and impaired B-cell maturation.[97,98] However, additional data suggest that Vav-1 and Vav-2 have distinct, non-overlapping roles in B lymphocytes. $Vav1^{-/-}$, but not $Vav2^{-/-}$ mice, lack B1 cells, whereas only $Vav2^{-/-}$ mice show defects in response to TI-2 and TD antigens, isotype switching, and germinal center formation.[98] The phenotype of $Vav1^{-/-}Vav2^{-/-}$ mice resembles the XID phenotype associated with other components of the "signalosome" (see above discussion), underscoring the crucial role of a properly assembled signaling complex.

The **c-Cbl** proto-oncogene was initially identified in mice infected with a retrovirus that caused pre- and pro-B lymphomas.[111] The comparison of v-Cbl and c-Cbl sheds light on important structural requirements for tumorigenesis, as well as on the function of Cbl.[112] The members of the Cbl family are ubiquitously expressed[113,114] and are notable for the RING domain, which endows Cbl proteins with ubiquitin ligase properties.[115] Engagement of the BCR leads to Lyn-dependent tyrosine phosphorylation of c-Cbl.[116] Phosphorylation of c-Cbl creates docking sites for other signaling proteins, such as the p85 subunit of PI-3K[117] and Btk,[118] thereby affecting their function. Using $Cbl^{-/-}$ DT40 B cells, Yasuda et al. show a negative role for Cbl in regulating the PLC-γ2 pathway by interfering with the association of BLNK with PLC-γ2.[119] Cbl-deficient B cells display hyperphosphorylation of PLC-γ2, perhaps because of increased association with BLNK and enhanced IP_3 and Ca^{2+} responses.[119] Interestingly, another member of the Cbl family, Cbl-b, plays a positive role in BCR signaling by enhancing the interaction of PLC-γ2 with Btk and BLNK, resulting in a sustained Ca^{2+} response.[120]

Analysis of Cbl-deficient mice has revealed a role for Cbl-b in regulating the signaling threshold of antigen receptors and preventing develop-

ment of autoimmunity.[121,122] Cbl-b$^{-/-}$ mice are characterized by multiple organ infiltration of B and T cells. Whereas loss of Cbl-b does not affect development and selection of B cells, the cells are hyper-responsive to anti-BCR and anti-CD40 signals, supporting a negative role for Cbl-b in these pathways.[121] Current data raise interesting questions about whether Cbl proteins play positive[120] or negative[119,121] roles in B-lymphocyte activation and suggest that there are distinct, nonoverlapping roles played by each of the Cbl family members.

C. Protein Tyrosine Phosphatases

The duration and strength of the signals generated upon BCR engagement depend on the balance between positive and negative factors. Excellent reviews address the crucial role that the tyrosine phosphatases CD45[54,123] and SHP-1[124,125] play in B-lymphocyte activation. Here we will provide a brief discussion of the functions of these molecules.

CD45 expression is required for normal B-cell development, particularly for the transition from the immature to the mature stage.[126] CD45-deficient mice are unresponsive to anti-BCR signals.[126–128] Data reveal that CD45 exerts its effects on BCR signaling by dephosphorylating the C-terminal inhibitory tyrosine of Lyn, ensuring its optimal activation.[123] Interestingly, when CD45$^{-/-}$ mice are back-crossed to mice carrying the Ig transgene hen egg lyzozyme (HEL), negative selection of the HEL-binding B cells is impaired, and these cells are positively selected.[129] These data support a role for CD45 in setting the signaling threshold for the BCR and, ultimately, B-cell tolerance.

The critical role that **SHP-1** tyrosine phosphatase plays in lymphocyte regulation became evident from a study of the spontaneous mouse mutants (point mutations resulting in anomalous splicing), motheaten or viable-motheaten.[130,131] Lack of SHP-1, or a catalytically inactive SHP-1, leads to profound defects in B-cell differentiation, proliferation, and survival (reviewed in Refs. 124, 125, 132). There is an overall decrease in the size of the B-cell population, and the cells are hyper-responsive to BCR stimulation, displaying amplified Ca^{2+} mobilization and increased MAPK activity. Mice also exhibit high autoantibody titers and immune complex deposition. The overall exaggerated responses in the absence of a functional SHP-1 show that SHP-1's role is to downregulate BCR signaling. This occurs via the association of SHP-1 with inhibitory receptors, such as CD22 (discussed below).

D. Lipid Metabolizing Enzymes

In addition to proteins, BCR utilizes phosphorylation and dephosphorylation of membrane lipids to transmit signals to the cytoplasm and nucleus. PI3K and PLC-γ are two critical enzymes that utilize membrane lipids to generate second messenger molecules important for the activity of downstream signaling proteins.

The **PI3K** family consists of three classes and multiple isoforms.[133] We focus on class I PI3Ks, which are the most important for B-lymphocyte responses. Class I PI3Ks consist of a p110 catalytic subunit (p110α, p110β, and p110δ isoforms), and a p85 regulatory subunit (five isoforms). The regulatory subunit contains SH2 domains, which enables recruitment of PI3K to tyrosine phosphorylated proteins in the membrane.[133] PI3K is activated upon BCR engagement in a PTK-dependent manner.[49] It may also be recruited to the membrane via the cytoplasmic tail of CD19 (see below). Recently, BCAP (B-cell adaptor for PI3K) was shown to be phosphorylated by Syk and Btk upon BCR engagement, and facilitates recruitment of the p85 subunit of PI3K to the membrane.[134] However, B cells from BCAP$^{-/-}$ mice show no significant decrease in PI3K activity, although BCR signaling is impaired.[135] These data raise questions about the role of BCAP in PI3K regulation and suggest the involvement of other molecules.

PI3K activation leads to generation of $PI(3,4)P_2$ and PIP_3, which serve as docking molecules in the plasma membrane for PH-containing cytosolic proteins,[136] such as Btk,[137] PLC-γ2,[138] Akt,[139] and Bam32.[140] By recruiting and bringing in close vicinity multiple signaling molecules, PI3K is able to directly and indirectly affect Ca^{2+} mobilization, Akt activation, transcriptional regulation, cell growth, and survival.[136,141]

The critical role of PI3K in B-lymphocyte development and activation is evident in mice lacking the p85α regulatory subunit,[92,142] or lacking or expressing an inactive form of the p110δ catalytic subunit.[143,144] The development of B cells in such mice is impaired at the pro-B-cell stage; B cells display defective responses to polyclonal B-cell activators and mice are not able to mount a normal humoral response to TI and TD antigens. The $p110\delta^{-/-}$ mice also lack germinal centers in the spleen, lymph node, and Peyer's patches, and develop a mild inflammatory bowel disease.[143]

Control and attenuation of PI3K-mediated signals is dependent on SH2 domain-containing inositol phosphatase (**SHIP**), which converts PIP_3 to $PI(3,4)P_2$ and $Ins(1,3,4,5)P_4$ to $Ins(1,3,4)P_3$,[145–147] limiting the PIP_3 available for recruitment of signaling molecules. Bolland et al. demonstrated that Btk recruitment to the plasma membrane is dependent on the available PIP_3; SHIP deficiency leads to increased Btk association with the membrane and consequently increased Ca^{2+} response.[148] A similar mechanism is observed for SHIP-mediated Akt inhibition,[149,150] which utilizes its PH domain to be recruited to the plasma membrane. SHIP's inhibitory activities are dependent upon its recruitment to CD32, (FcγRIIB)[151](see discussion below).

Phospholipase Cγ (**PLC-γ**) is another BCR stimulated enzyme that utilizes phosphatidylinositols to generate two important second messengers, inositol 1,4,5-bisphosphate (IP_3) and diacylglycerol (DAG) (reviewed in Refs. 152, 153). Engagement of the BCR leads to PLC-γ1 and PLC-γ2 activation, but PLC-γ2 is more predominant in BCR signaling, which requires tyrosine phosphorylation and relocalization from the cytosol to the plasma membrane.[154–156] PLC-γ contains SH2 domains, an SH3 domain, and a PH domain, which allow it to associate with a diverse range of molecules.[152,153] BCR-activated BLNK binds and recruits PLC-γ2 to the membrane,[157] making it available to Syk and Btk for phosphorylation and activation. The products of PLC-γ2 activation, IP_3 and DAG, induce Ca^{2+} mobilization and PKC activation, respectively.[141] PLC-γ2–deficient mice are characterized by defects in the B-lymphocyte population: There is a decrease in mature B cells, due to a block in pro-B-cell differentiation, a disruption of the Ca^{2+} response, and decreased proliferation in response to mitogenic stimuli.[96] Overall, the phenotypic abnormalities in these mice resemble those of Btk and BLNK-deficient mice,[88,94,102] which supports a role for the interactions among these molecules for proper B-cell responses to antigen receptor engagement.

E. Serine-Threonine Kinases

Activation of PLC-γ and PI3K leads to the generation of second messengers, which are involved in the activation of the protein kinase C (**PKC**) family of serine/threonine kinases. The PKC family can be divided into three subfamilies, depending on the second messengers required. The conventional PKCs (PKC-α, β, β_{II},γ) utilize both DAG and Ca^{2+} for their activation, relying primarily on the PLC-γ pathway. The activation of novel PKCs (PKC-δ, ε, η, ϕ) is DAG-dependent and Ca^{2+}-independent. The atypical PKCs (PKC-ζ, λ/ι) are not activated by either DAG or Ca^{2+} and have been shown to be downstream of PI3K.[158]

More recently, the PKD family has been described, with PKC-μ/PKD as its main member.[159] B lymphocytes express PKC-α, β, γ, δ, ε, ζ, η, and μ isoforms,[160,161] and BCR engagement has been shown to induce their activation, as measured by translocation of PKC from the cytosol to the plasma membrane.[162] PKC activation has been shown to lead to activation of extracellular-regulated kinase (ERK), NF-κB, cyclic AMP response element binding protein (CREB), and Elk-1 (reviewed in Ref. 163).

Generation of mice deficient for PKC-β demonstrate a critical role for PKC in B-cell development and activation.[95] B cells lacking PKC-β display impaired BCR-mediated proliferation. Examination of downstream signaling showed that PKC-β is required for the recruitment of the IκB kinase (IKK) complex into rafts and, consequently, NF-κB-mediated survival signals.[164,165] Recent studies have supported a crucial role for PKC-δ in maintaining B-cell tolerance and autoimmunity.[166,167] Mice lacking PKC-δ show an expansion in the B-lymphocyte population, enlarged spleens and lymph nodes, germinal center formation in the absence of stimulation, increased IL-6 production, and circulating au-

toantibodies. These data show that PKC-δ, in contrast to PKC-β, plays a negative role in BCR signaling. Analysis of PKCζ$^{-/-}$ mice has also revealed an important role for this isoform in B-lymphocyte activation, albeit in different processes.[168,169] Although the overall splenic structure of PKCζ$^{-/-}$ mice is preserved, the marginal zone is anomalous, manifested by smaller B-cell follicles. Similar abnormalities are observed in peripheral and mesenteric lymph nodes and Peyer's patches, because of impaired segregation of B- and T-cell populations. B cells from these mice show impaired survival and proliferation in response to anti-BCR stimulation, impaired ERK and NF-κB activation, and defects in the ability to mount a humoral response to TI and TD antigens.[168,169]

Mitogen-activated protein kinases (**MAPK**s) are a family of serine-threonine protein kinases that regulate various cellular activities, including many in B lymphocytes. BCR engagement leads to activation of the extracellular signal–regulated kinases (**ERK**s), the c-Jun N-terminal kinases (**JNK**s)/stress-activated protein kinases (SAPK), and **p38** kinases,[163,170] members of the MAPK family.

Elegant studies using B-cell lines deficient in specific protein tyrosine kinases have helped to delineate the pathways and molecules involved in activation of these kinases.[171,172] These studies show that Syk and Btk, but not Lyn, are required for ERK2 and JNK activation. Interestingly, p38 activation is abrogated only in Lyn/Syk double-deficient cells.[171] Downstream of PTKs, ERK activation is dependent on BCR-induced activation of the RasGTP-Raf1-MKK1-Erk signaling cascade.[163] BCR ligation results in the activation of the oncoprotein Ras by increasing the amount of Ras bound to GTP.[173–175] The crucial role of Ras in B-lymphocyte activation is evident in mice transgenic for a dominant-negative form of Ras, which display a block in B-cell development that is restored by expression of an active Raf.[176,177] Interestingly, expression of an activated Ras is sufficient to drive the progression of recombinase activating gene (RAG1)-deficient pro-B cells in the absence of μ chain expression, underscoring the importance of Ras in differentiation and survival processes.[178] The activation of Ras-Raf-MKK culminates in ERK activation which links BCR ligation to various transcription factors including Ets-1, Ets-2, Elk-1, Fos, Egr-1, CREB, and NFAT.[163] Recent work has shown that ERK activation serves as a convergence point for PI3K, PLC-γ, and Ras pathways.[179] Interestingly, upon BCR engagement, ERK is primarily cytosolic, where it can phosphorylate cytosolic kinases, such as p90^{rsk}, whereas CD40 engagement leads to its nuclear localization.[180]

The mechanisms involved in JNK and p38 activation upon BCR engagement are not well understood. Similar to ERK, JNK and p38 are part of signaling cascades characterized by consecutive phosphorylation of various kinases.[170,181] In B lymphocytes, several molecules have been incorporated in MAPK, SAPK, and p38 activation, including Syk,[171] Btk, Rac1, PLC-γ2,[182,183] Bam32,[184] SHP-1, Nck,[185] Grb2, Ras,[172] BLNK,[102] PKCs,[186] MKK7,[187] and SEK1.[188] Activation of these MAPKs leads to their translocation to the nucleus where they can phosphorylate and activate their respective substrates, including many transcription factors, which are important for gene expression.[76,163] The induction of the various transcription factors requires temporal, quantitative, and qualitative regulation, which is accomplished by the intricate integration of various signaling cascades, depending on the differentiation stage and the signals B lymphocytes receive.

III. BCR CO-RECEPTORS

A. CD19

CD19, an ~95-kDa transmembrane member of the Ig superfamily, is expressed on B cells from early in development to the plasma cell stage of differentiation. CD19 was initially identified as a B-cell–specific antigen[189] and considered as a potential BCR co-receptor because it was found to co-modulate with the BCR.[190] The potential physiologic significance of CD19 became appreciated when it was discovered that its co-ligation with the BCR substantially lowers the threshold for BCR-mediated B-cell activation.[191] It was subsequently learned that CD19 functions as a signal receptor via a PTK-dependent pathway.[192] Since the late 1980s, a number of laboratories have contributed considerable information on both the signaling mechanisms and biologic role of CD19 in the humoral response. Here we review some of the highlights

and most recent findings; a number of excellent recent reviews provide more detailed and comprehensive coverage of earlier studies.[193–195]

CD19 is normally associated at the cell membrane in a complex with the complement receptor CD21 and the tetraspanin CD81; it has been suggested that association with this complex via CD19 extracellular (EC) and transmembrane (TM) domains is important for CD19 signaling,[196,197] although the CY domain is directly responsible for signal transduction.[198,199] Mice deficient in CD81 show impaired CD19 surface expression,[200] but CD21 deficiency does not detectably reduce CD19 function,[201] suggesting that ligation of the complement receptor is not essential for CD19 signals. This is consistent with the ability of agonistic CD19-specific Abs to stimulate signaling events *in vitro*.[202] However, a natural ligand for CD19 has never been convincingly identified, so the relative roles of CD19 as an individual receptor versus in a complex with CD21 and CD81, the natural *in vivo* state, remain unclear.

What are the important physiologic roles of CD19 in the humoral response? The aforementioned early finding that CD19-BCR co-ligation lowers BCR-mediated activation thresholds suggests that one role of CD19 is to enhance the effectiveness of a primary B-cell response by increasing the magnitude of the signal delivered by antigen, similar to the enhancement of T-cell receptor-mediated activation by CD4/CD8. Because the affinity of the initial IgM produced upon first exposure to a particular antigen is lower, co-signaling through CD19 may serve as a mechanism for increasing the probability that antigen binding will deliver a strong enough signal to initiate activation and clonal expansion. However, as CD19 functions can be complement receptor-independent, other roles and mechanisms of action are also likely.

Although expression of CD19 does not appear to be important for development of "conventional," B2 B cells, CD19-deficient mice show decreased numbers of B1[203,204] and marginal zone[205,206] B cells. The role of CD19 in response to TI antigens is inconsistent between studies and is thus currently unclear. In different reports, the response of $CD19^{-/-}$ mice to TI-1 antigens is either inhibited[203] or normal,[204] and to TI-2 antigens, it has been reported to show a decrease,[206] an increase,[207] or no change.[204] Developmental affects of CD19, in addition to inbred mouse strain background differences in response to distinct antigens, may contribute to the difficulties in interpretation. However, responses of CD19-deficient mice to TD antigens are quite consistent in showing a marked decrease. In all studies to date, the IgG response to TD antigens is considerably compromised by the absence of CD19.[203,204,206,207] In addition, normal germinal center (GC) development in response to immunization with model antigens is compromised, and although GC are formed in response to viral infection, normal B-cell memory does not develop.[208] It is thus clear that CD19 plays an important role in the development of a normal adaptive humoral response.

Much work has been done on the signaling mechanisms used by CD19. Tyrosine-containing motifs in the relatively long CY domain of CD19 enable association with a variety of SH2 domain-containing signaling molecules. One of the most prominent of these is Lyn, the Src family kinase that has been reported to initiate CD19 signaling.[209] However, it has also been reported that CD19 signaling is independent of Lyn activation.[210] Additional PTKs with which CD19 associates include Fyn and c-Abl,[211] and these may serve redundant roles with Lyn in CD19 signaling. The CY tail of CD19 also binds PI-3K,[212,213] PLC-γ2,[194] and adapter proteins such as Vav.[214,215] CD19 can cooperate with the BCR and other receptors to activate MAPK, ERK, and SAPK pathways as well (reviewed in Refs. 193, 194). This enhancement may be facilitated by the ability of CD19, like the BCR, to localize to cholesterol-enriched membrane rafts.[216]

Because CD19 can associate with a wide variety of signaling proteins, determining which signaling events are crucial to its biologic function in B-cell activation has been challenging. Adding to the challenge is uncertainty about how closely ligation with anti-CD19 mAb, the easiest way to initiate signaling *in vitro*, mimics initiation of CD19 signaling *in vivo*. The CY domain of CD19 contains 9 tyrosine residues, which are evolutionarily conserved, suggesting potentially important functions for each. Different Y residues have been shown to regulate binding to distinct intracellular proteins—Y330 to Grb2,[217] Y391 to

Vav,[217] Y421 to PLC-γ2,[217] Y403/Y443 to Lyn and Fyn, and Y482/Y513 to PI-3K.[212] This suggests that each of these associations plays distinct and important roles in CD19 signaling. However, recent studies in which CD19 transgenes containing various Y mutations were introduced into $CD19^{-/-}$ mice revealed an essential role only for Y482/Y513 in restoring many CD19-dependent functions. These include development of B1 and marginal zone B cells, as well as IgG responses to TD antigens, and normal GC development.[206] Interestingly, however, certain *in vitro* BCR-mediated signals are not completely restored by Y482/Y513. More in This could indicate that *in vitro* ligation with anti-BCR and anti-CD19 may not always reflect *in vivo* biology, but could also suggest a secondary, more subtle role for additional, PI-3K-independent signaling pathways in CD19's various roles in the humoral response.

B. CD21

CD21 is the complement receptor type 2 for iC3b, C3dg, and C3d. CD21 is expressed on B lymphocytes and follicular dendritic cells (FDC), and, together with CD35, is one of the alternatively spliced products produced by the *Cr2* locus.[218–220] CD21 also binds Epstein-Barr virus (EBV) via its viral glycoprotein gp350/220.[221] A 145-kDa type I membrane glycoprotein, CD21 consists of an extracellular domain of 15–16 short consensus repeat domains, a transmembrane region, and a short cytoplasmic tail.[222] Early evidence of a role for CD21 in B-lymphocyte activation showed that CD21 engagement synergizes with the BCR,[223] and that antibodies interfering with CR2 binding to its ligand abrogate the immune response to TD[224] and TI[225] antigens. Later work showed that CD21 is part of a B-cell surface signaling complex that also contains CD19 and CD81.[226] The role of CD21 in this complex is to increase the association of the complex with the BCR, rather than transmit any signals, which are provided by CD19.[227] In this manner CD21 is able to link the complement system to the humoral immune response. More recent work has suggested that the CD19/CD21 complex is able to enhance the BCR response by enhancing and prolonging the association of BCR with lipid rafts.[228]

The phenotype of mice with a disrupted *Cr2* locus underscores the importance of CD21/CD35 in generation of a humoral response.[229–231] CD21/CD35 deficient mice have reduced numbers of B1 cells and GC, and, consequently, an impaired humoral response. Fischer et al. demonstrated a critical role of CD21/CD35 for survival of B cells in GC independent of the affinity of the antigen receptor.[232] Notably, the levels of IgG2a, IgG2b, and IgG3 are significantly reduced.[229–231] $CD21/CD35^{-/-}$ show increased susceptibility to *S. pneumoniae* infection, supporting a role for CD21/CD35 in linking innate and adaptive immunity to bacterial challenge.[231] Because CD21 is expressed on both B cells and dendritic cells, the contribution of each of the cell types was tested in reconstitution experiments.[233–235] These studies showed that expression of CD21 by B cells is critical for the initial humoral response and Ig class-switching, whereas expression on dendritic cells is important for the maintenance of antibody response, affinity maturation, and memory induction.

To address the role of CD21/CD35 in the maintenance of self-tolerance, $Cr2^{-/-}$ mice were bred with mice transgenic for soluble hen egg lysozyme (sHEL), and B-cell negative selection was examined.[236] Interestingly, $Cr2^{-/-}$ HEL-binding B lymphocytes fail to undergo negative selection and accumulate in the spleen and lymph node. These data support an important role for CD21 in lowering the threshold for negative selection of autoreactive cells. In addition, *lpr/lpr* mice deficient in CD21/CD35 display exacerbated autoimmunity, evident in splenomegaly, glomerulonephritis, and increased antinuclear antibody titers, compared to *lpr/lpr* mice. These studies suggest a potential role for complement binding to CD21/CD35 in the generation of autoimmunity. Indeed, Boackle et al. were able to identify an altered *Cr2* allele in the murine autoimmune susceptibility locus in NZM2410 mice.[237] The *Cr2* gene product in these mice is structurally different from the normal product and results in enhanced glycosylation of CD21/CD35. The single nucleotide change observed in these mice introduces a new glycosylation site in the ligand binding domain, which leads to decreased ligand binding, diminished signaling in B cells (as measured by the Ca^{2+} response), and impaired humoral response to TD antigens.[237]

C. CD22

CD22 is a sialoadhesin expressed exclusively on B cells, and originally named Lyb8. Its expression, structure, function, and signaling pathways have been the focus of several comprehensive reviews in recent years.[193,238,239] Here we attempt to concisely summarize past findings and discuss more recent data pertaining to CD22's role in the regulation of BCR signaling. Mouse and human CD22 are 62% homologous in protein sequence. Both consist of an extracellular region of seven Ig domains, a TM domain, and a CY domain; the latter has six tyrosine residues in both species (reviewed in Ref. 193). As a sialoadhesin, CD22's initial postulated function was as an adhesion molecule, particularly since its ligand is glycoconjugates containing α2, 6-linked sialic acid, predominantly NeuAcα2,6Galβ1-4GlcNac (reviewed in Ref. 238). It has been found that cell types of hematopoietic origin, especially T and B lymphocytes, express greater amounts of CD22 ligands than do non-hematopoietic cells,[193,238] suggesting that one function of CD22 is to target B cells to environments where they are likely to interact with T cells. However, it subsequently became clear that CD22 also provides important regulatory signals for BCR-mediated B-cell activation.

It was observed a decade ago that the CY tyrosines of CD22 become phosphorylated in B cells stimulated through the BCR.[240] As described in an earlier section, this process is dependent upon the Src kinase Lyn, and the phosphorylated tyrosines are within an ITIM motif. Subsequent studies showed that CD22 physically associates with the BCR complex,[241] to which it can recruit the tyrosine phosphatase SHP-1 (previously referred to as HCP/PTP-1C),[242] as well various kinases,[243,244] PLC-γ2,[243] and the nucleotide exchange factor Vav.[245] However, very recent work has shown that the relationship between BCR and CD22 described above does not exist if the BCR is IgG, rather than IgM or IgD. Ligation of surface IgG does not stimulate CD22 phosphorylation or SHP-1 recruitment.[246] A variety of complementary experimental approaches, including detailed cellular biochemistry as well as the analysis of CD22-deficient mice, revealed that CD19 (discussed above) and CD22 provide counterbalancing regulatory signals to the BCR, and CD22 signaling has an overall negative role (reviewed in Refs. 193, 238, 239, 247). Thus, $CD22^{-/-}$ mice show features of hyper-responsiveness to BCR signals,[248,249] and a lack of CD22 phosphorylation contributes to the phenotypic features of the $Lyn^{-/-}$ mouse.[250] Recruitment of SHP-1 to the BCR complex permits this phosphatase to suppress activation of the MAP kinases ERK2, p38, and JNK, counteracting their potential activation by CD19 (reviewed in Ref. 193). It has also been consistently observed that CD22 signals decrease Ca^{2+} flux stimulated by the BCR,[251,252] possibly through inhibition of the phosphorylation of PLC-γ. This inhibition does not occur if an IgG BCR is providing the signal,[246] which may allow isotype-switched B cells to escape this method of negative regulation of B-cell activation. Interestingly, it has also been reported that *in vitro* signaling to B cells via MHC class II molecules can be reciprocally regulated by CD19 and CD22 signals,[253] although the role of this regulation *in vivo* is not yet clear. Although the preponderance of evidence points to a negative regulatory role for CD22 signals, more remains to be learned about this interesting signaling receptor, and it is possible that it also delivers important positive signals to the B cells.

Because CD22 does not have a single, well-defined, or easily isolated ligand, *in vitro* studies of CD22 as a signaling receptor have relied almost exclusively upon the use of agonistic anti-CD22 mAbs as a stimulus. Whereas it is clear that much has been learned using this approach, it is desirable to verify important signaling events and learn more about the physiologic interactions of CD22 with its natural ligands. Several recent studies have addressed the knowledge gap of the role of the EC domain of CD22 in receptor function. The high degree of sequence conservation of this region suggests an important role, but its nature has been unclear. Jin and colleagues studied the signaling function of CD22 mutants with defects in the putative sialic acid–binding domains and learned that inability to bind sialic acid impairs CD22-mediated downregulation of BCR-mediated Ca^{2+} flux, particularly the early rise in Ca^{2+} from extracellular sources.[254] Using the complementary approach of high affinity sialic acid analogs that inhibit binding of sialic acid to CD22, Kelm et al. found that CD22 binding to this ligand is

required for the receptor to inhibit BCR-mediated Ca^{2+} increases.[255] These studies introduce promising new approaches to a more physiologic initiation of the CD22 signaling pathway and begin to explore the role and nature of CD22 ligand binding.

D. CD32

CD32 (FcγRIIB) is an inhibitory receptor expressed on various immune cells, including B cells, macrophages, dendritic cells, mast cells, and neutrophils.[256,257] FcγRIIB is a single-chain glycoprotein that contains an ITIM sequence in its CY tail, which endows it with inhibitory properties. The 13 amino acid sequence AENTITYSLLKHP was shown to be necessary and sufficient for inhibition of the BCR-induced Ca^{2+} response and cellular proliferation.[258] Similar ITIM motifs are found in various other inhibitory receptors, and data show a crucial role for the regulation of immune responses by counteracting positive signals generated by **ITAM** -containing receptors.[259]

The inhibitory effects of FcγRIIB are threefold, two of which are ITIM-dependent.[260] Coengagement of BCR and FcγRIIB leads to phosphorylation of the tyrosine in the ITIM motif by Lyn kinase and, thereby, generation of a binding site for SHIP.[258] SHIP recruitment attenuates the PI3K pathway by hydrolyzing PIP_3, thereby interfering with the association of PH-containing molecules, like Btk and PLC-γ, and, consequently, blocking the Ca^{2+} response. Additionally, SHIP has been shown to recruit the RasGAP-binding protein $p62^{dok}$, which is critical for FcγRIIB inhibition of cell proliferation.[261,262] FcγRIIB is not able to inhibit proliferation in Dok-deficient B cells, while Ca^{2+} influx inhibition is intact,[262] supporting the existence of two distinct ITIM-dependent inhibitory pathways. In addition, FcγRIIB displays ITIM-independent inhibitory activity, which is evident upon homoaggregation of the receptor. FcγRIIB engagement has been shown to induce apoptosis,[263] and this effect requires an intact TM domain rather than the ITIM motif, is dependent on Btk, and is blocked by SHIP.[264]

Selection of memory cells in the GC is dependent on BCR recognition of immune complexes presented on dendritic cells. The balance of positive and negative signals generated by BCR and FCγRIIB, respectively, could lead either to stimulation, inhibition, or apoptosis. This tightly regulated balance ensures appropriate immune responses and elimination of self-reactive cells. Any disruptionto this process could contribute to the development of autoimmunity. The current model suggests that upon the encounter of B lymphocytes with immune complexes, FcγRIIB is necessary to counteract signals emanating from the BCR. Such negative regulation is important for preventing the development of autoimmunity.[257] This is evident in the study of mice deficient for FcγRIIB. The humoral immune response to TI and TD antigens is elevated in $FcγRIIB^{-/-}$ mice.[265] More importantly, mice with certain genetic backgrounds are are more susceptible to autoimmune disease, which is evident by the presence of autoantibodies to nuclear antigens and autoimmune glomerulonephritis.[266] In addition, an FcγRIIB deficiency exacerbates other autoimmune diseases, such as type II collagen-induced arthritis[267]and Goodpasture's syndrome.[268]

IV. T-DEPENDENT B-CELL ACTIVATION

Stimulation of the B cell via its BCR and coreceptors, summarized in Figure 1, provides crucial signals to the process of antigen-specific B-cell activation. However, the development of an effective humoral memory response requires the B cell to receive contact-mediated signals from the activated T lymphocyte. This requirement exerts important regulatory control over B-cell activation in a number of ways. In normal individuals, polyclonal activation of B cells by contact with T cells is very limited, although the two cell types are capable of stimulating one another through a variety of nonpolymorphic receptor-ligand pairs (see below). This suggests that cog-

nate interactions between antigen-presenting B cells and activated T cells increases the efficiency of delivery of non-cognate signals between the two cells, an hypothesis supported by several studies.[18,269,270] A number of mechanisms could provide this increased efficiency. BCR signals enhance B-cell responsiveness to T-dependent activation signals and induce increased expression of surface molecules contributing to antigen presentation. Direct interaction via MHC–T-cell receptor binding can also increase B-cell–T-cell proximity, amplifying delivery of both contact-mediated signals as well as soluble molecules. Signals delivered to the B cell through ligation of MHC class II molecules have also been shown to enhance both antigen presentation and B-cell activation, and to cooperate with both BCR and T-cell–derived signals (discussed below).

Regulation of contact-mediated B-cell activation may be important, not just to promote desirable activation events but also to prevent autoimmunity. It has been demonstrated that deletion of self-reactive T-cell clones appears more rigorous than that of autoreactive B-cell clones, especially if the amount of autoantigen is limiting [[CORRECT?]].[271] If a cognate autoantigen-specific T-cell clone does not exist, an autoreactive B-cell clone has few opportunities to become activated and produce high-affinity pathogenic autoantibodies. The importance of contact-mediated signals in the development of autoimmunity has been highlighted in several published studies.[272,273] Thus, contact-mediated signals in antigen-specific B-cell activation both increase the effectiveness of adaptive humoral responses and decrease the potential for activation of self-reactive B cells. Below we discuss the current state of knowledge about key signals delivered to B cells through contact with activated T cells.

A. MHC Class II

An earlier paradigm held that T-cell lymphokine production, alone or in combination with BCR signals, is sufficient to account for the contribution of T cells to TD B-cell activation.[274,275] However, when more stringently separated resting B cells were studied, it was revealed that although soluble factors play key roles in B-cell activation, contact-mediated signals from the T cell are also critical (reviewed in Ref. 276). The first of these signals to be identified was MHC class II. Ligation of B-cell class II molecules induces early biochemical signaling events as well as subsequent effector functions, including proliferation, differentiation (reviewed in Ref. 277), and enhanced antigen presentation.[278–281] Both CY and TM domains of the molecule have been demonstrated to contribute to signaling events.[282,283] Although TD B-cell activation can occur in the absence of class II expression,[284] class II signaling enhances both BCR and CD40 signals[269] and may contribute to the activation of CD40-deficient B cells.[285] Additionally, it has been shown that class II signals can inhibit CD95-mediated B-cell apoptosis,[286] an interaction that may promote the survival of B cells in the germinal center. Thus, by enhancing the effectiveness of other B-cell activation signals, class II signaling may serve a regulatory role by preferentially promoting the TD activation of cognate antigen-presenting B cells rather than bystander B cells. Consistent with this role is a report that class II signaling may in part be regulated by two BCR coreceptors, CD19 and CD22,[253] and may even utilize components of the BCR signaling complex, Ig-α and Ig-β, in its signaling pathway.[287] Additionally, it has been shown that, following its engagement, class II localizes to cholesterol and glycosphingolipid-enriched membrane microdomains or 'rafts',[288] potential sites of assembly of membrane signaling complexes. CD40 also localizes to membrane rafts following its ligation in B cells,[289] and physical association between CD40 and MHC class II subsequent to their engagement on B cells has been demonstrated.[290] Understanding how physical interactions between class II and other transmembrane receptors affect the ultimate nature and strength of regulatory signals delivered to the B cell is important for understanding the physiologic role of class II signaling in TD B-cell activation.

B. Adhesion Molecules

The expression of a number of adhesion molecules is increased on B cells as a result of initial activating signals, and enhanced expression of these

molecules amplifies B–T interactions and B-cell activation. Both B and T cells express ICAM-1 (CD54) and LFA-1 (CD11a/CD18), which bind each other and can thus mediate both homotypic and heterotypic adhesion. Enhanced B cell–T-cell contact can optimize activation signals delivered during TD B-cell activation, and adhesion molecules can also retain B cells in specialized environments in which they receive important regulatory signals.[291] Potential roles played by direct signaling to the B cell via adhesion molecules is less clear. Earlier studies suggested that both CD11a/CD18 and CD54 can directly provide B-cell activation signals,[292,293] and it has been shown that such signals could contribute to enhanced B-cell antigen presentation.[9] Signals via adhesion receptors can also interact with other B-cell signal receptors. It was shown that such signals cooperate with CD40-mediated activation,[294] and it was recently reported that CD54 signals can synergize with BCR signals to upregulate the costimulatory molecule CD80.[295] Additionally, CD54-mediated upregulation of B-cell class II expression was shown to correlate with activation of the Src family kinase Lyn and MAPKs.[296] A clearer understanding of how and in what physiological circumstances adhesion molecules can signal to B lymphocytes, and how these signals coordinate with other TD signals, will help to fill in the entire picture of how T–B interactions can regulate B-cell activation.

C. CD72

Earlier studies reported that antibody-mediated engagement of the CD72 molecule on B cells induces upregulation of MHC class II expression, proliferation, and prolonged B-cell survival, and it has been shown that these positive signals utilize the MAPK pathway.[297] However, for quite a few years the natural ligand for CD72 proved elusive. More recent studies identified this ligand as CD100, a member of the semaphorin family expressed on both B cells and activated T cells, and known to participate in neuronal regulation.[298] Engagement of CD72 by CD100 enhances B-cell activation mediated by CD40, and blocking this interaction inhibits T-dependent IgG production, although IgM production is unaffected.[298] Complementary studies in CD100-deficient mice show that CD100 expression is required for the normal development of B1 B cells, as well as for development of high-affinity IgG responses to TD but not TI antigens.[299] Results also implicate CD100-mediated CD72 signals as important to antigen presentation and potentially responsible for inducing the dissociation of the phosphatase SHP-1 from CD72.[298,299] Recent studies suggest that CD72 expression, when CD100 is not present, inhibits BCR-mediated Ig-α/Ig-β activation via its association with SHP-1.[300] Thus, the emerging picture of CD72 suggests a molecule whose expression itself provides negative regulation of BCR signaling, but when engaged by its ligand effects BCR signals positively.

D. Members of the TNF-R Superfamily

The tumor necrosis factor receptor (TNF-R) family of molecules is a large, diverse group of molecules that participates in the regulation of cellular activation, development, and programmed cell death.[301] B lymphocytes express a number of members of this family, which have been shown to participate in and regulate B-cell activation in a variety of ways. The receptors and their known functions in B-cell regulation are discussed in the following section.

1. CD40

CD40 was initially characterized as a potential tumor antigen on a bladder carcinoma.[302] Almost a decade later, the physiological roles played by CD40 in B-lymphocyte activation became clear when it was discovered that defects in the CD40 ligand, CD154, cause the rare X-linked human immunodeficiency disease Hyper-IgM Syndrome (HIGM)[303–305] by blocking delivery of CD40 signals. HIGM patients suffer profound defects in humoral immunity despite the presence of normal numbers of peripheral B cells. The term HIGM refers to the normal or abnormally high serum IgM levels seen in such patients. Although antibody responses to TI antigens are intact, responses to immunization with TD antigens and the production of "switched"

isotypes of Ig are greatly decreased.[306] During the same time period, CD154 was found to be the factor in activated T-cell membrane preparations responsible for inducing a variety of B-cell activation events.[307–310]

Subsequently, CD40 and CD154-deficient mice were produced using gene targeting technology. The phenotypes of both strains of mice are quite similar to each other and to HIGM patients, indicating a nonredundant receptor–ligand pair.[311–313] Human HIGM is a rare disorder, so the availability of such patients for detailed study is quite limited. The manifestation of the disease early in childhood additionally limits the amount of biological material available for study, as do ethical considerations. Thus, the mouse model systems have proven quite valuable in allowing more rapid accumulation of information on the physiologic roles of CD40–CD154 interactions. Studies in mice, as well as work performed *ex vivo* with freshly isolated B cells and B-cell lines, demonstrated that CD40 signals induce enhanced expression of surface molecules involved in T–B collaboration (costimulatory molecules, adhesion molecules, and others). Defective CD40 signals thus result in defects in antigen presentation by B cells, macrophages, and dendritic cells, contributing to deficiencies in cell-mediated as well as humoral immunity.[24,314–319]

Studies with CD40 and CD154-deficient mice revealed the importance of CD40 signals in TD B-cell activation. However, CD40 is also expressed on macrophages and dendritic cells, for which its signals **that enhance** antigen presentation are critical,[314,315,317,320–323] so data interpretation in the knockout mice can be complex. In this regard, more simplified *in vitro* model systems have been helpful in determining the specific direct effects of CD40 signals on B-cell activation. Although TI antigen stimulation can induce normal B-cell expansion in CD40-deficient mice, *in vitro* studies demonstrated that the CD40 signal can directly induce B-cell proliferation, and can synergize with signals through the BCR and/or the IL-4 receptor.[269,324,325] Although TI antigens can induce IgM production in mice or humans lacking CD40 signals, CD40 signaling can strongly promote B-cell IgM production.[269,324,325] Recently, it has been shown that various soluble factors induced by CD40 signals, including IL-6 and TNF-α, contribute to this IgM production.[326–329]

In addition to the aforementioned lymphokines, CD40 ligation on B cells can induce their production of lymphotoxin-alpha,[330–332] IL-10,[333] IL-12,[334] and chemokines.[335] These factors can regulate B-cell isotype switching (discussed below), migration, and antigen presentation capacity.

What are the molecular mechanisms by which CD40 signals to B lymphocytes? Because CD40 has, to date, been much more extensively studied than additional members of the TNF-R family **that contribute** to B-cell activation (discussed below), we will discuss the CD40 signaling pathway in detail; the events discussed in the text are summarized in Figure 2. However, many of these events are being revealed to have parallels in B-cell signaling via other TNF-R family receptors.

Members of the TNF-R superfamily, as well as certain other receptors, utilize distinct but overlapping sets of cytoplasmic adapter proteins, called TRAFs (TNF-R–associated factors), to deliver signals to cells. The first TRAF found to associate directly with CD40 was TRAF3, initially referred to as "CD40 binding protein." Subsequently, CD40 was also found to directly bind TRAFs 2 and 6, and to associate with TRAF1 principally via heterodimerization with TRAF2 (for a recent review, see Ref. 336). It has also been reported that CD40 binds TRAF5,[337] but a parallel report reached an opposite conclusion.[338] TRAF5$^{-/-}$ mice show modest alterations in CD40 signaling,[339] but since CD40 binding to TRAF5 has not yet been demonstrated in B cells, it is unclear if this phenotype is a direct or indirect result of the lack of TRAF5 in all cells and tissues of this mouse. With the exception of TRAF1, all TRAF molecules contain a zinc-binding RING finger domain at the N-terminus, and removal of this domain renders the TRAF unable to promote signaling and able to inhibit normal TRAF function as a "dominant negative" (DN).[340]

Many studies have sought to understand the physiologic roles of TRAFs in CD40 functions in B cells by asking which TRAFs contribute to these functions. A number of early studies approached this question by transiently overexpressing both CD40 and specific TRAFs (Wt or DN), together with various reporter gene constructs, in the easily

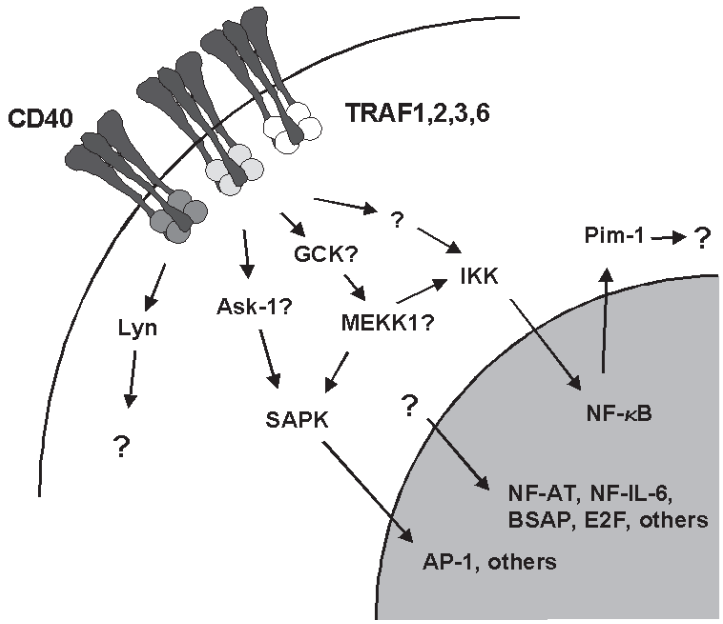

FIGURE 2. CD40-mediated signaling pathways. It has been clearly demonstrated that NF-κB and stress-activated protein kinases (e.g., p38 and JNK) are activated by CD40, but how interaction with TRAF molecules leads to these events is still unclear. Potential intermediary steps are outlined. Multiple transcription factors are activated by CD40, but, in most cases, the events leading to their activation have not been determined.

transfectable epithelial cell line, 293. However, some major caveats apply to the interpretation of data obtained by this approach. CD40 binds at least 4 distinct TRAFs, and 3 of these (TRAFs 1, 2, and 3) have overlapping binding sites.[336] Thus, overexpression significantly alters receptor interaction with all of the TRAFs binding near this location. Additionally, it has been recently shown that CD40-TRAF binding defined in such systems does not necessarily reflect binding requirements seen at normal protein levels in B cells.[341] Finally, physiologically important functions of CD40 specific to B cells cannot be assessed in other cell types. For these reasons, we will restrict our discussion in this review to information gained about CD40–TRAF function in studies that actually examined B lymphocytes.

Studies of B cells expressing structural mutants of CD40 revealed that CD40 effector functions are regulated by several different structural motifs in the CD40 CY domain.[342–345] Several of these motifs were ultimately found to correspond to TRAF binding sites, indicating that TRAFs 2 and 6 play important positive roles in CD40-mediated B-cell activation.[346–350] Interestingly, binding of the known TRAFs cannot account for all the CD40 signaling motifs found, suggesting that additional CD40 binding proteins remain to be discovered. To attempt to directly investigate the causal role of different TRAFs in CD40 signals, mice genetically deficient in individual TRAFs were created. However, TRAFs interact with many members of the TNF-R superfamily, playing a variety of roles in normal physiology and development.[340] It is perhaps thus not surprising that mice made deficient in TRAFs 2, 3, and 6 die *in utero* or shortly after birth, and have severe abnormalities in multiple organs and tissues.[351–353] This largely precludes their use in obtaining clearly interpretable information on TRAF roles in CD40 signaling to mature B cells. To attempt to circumvent this limitation, alternate approaches have been used. As mentioned above, CD40 molecules with targeted mutations in TRAF binding sites have been studied to assess TRAF dependence of

particular CD40 signals in B cells; this approach has been widely used and provided considerable information.[342,343,345,346,354] The approach has also been adapted to *in vivo* models, by inserting mutant CD40 transgenes into $CD40^{-/-}$ mice.[355–357] However, although this approach allows potential study of the roles of individual TRAFs in *in vivo* B-cell responses, discordant conclusions about such roles in distinct studies [356,357] illustrate that this model also has technical caveats. A number of factors may have contributed to complications in data interpretation and different conclusions in the studies cited above. These include using transgenic receptors with the external domain of human CD40 (which has lower binding affinity for mouse CD154 than does mouse CD40), significant variation in levels of CD40 expression between various Wt and mutant molecules (overexpression of partially-defective CD40 molecules could obscure defects in signaling), and differences in the specific antigens studied in immunization experiments (CD40 contributions may not be the same for responses to all TD antigens). Additionally, assumptions made about the TRAF binding characteristics of each mutant transgene need to be verified in the transgenic B cells themselves. For example, a particular point mutant in which the T residue of the PXQXT motif in the CD40 CY tail has been changed to an alanine has been widely believed to lack the ability to bind either TRAF2 or TRAF3, on the basis of data obtained with *in vitro* binding methods, and overexpression studies in epithelial cells. However, when we examined this mutant CD40 molecule expressed in B cells, we were surprised to discover that its binding to TRAF2 is impaired but not eliminated, and its binding of TRAF3 is not detectably different from that of Wt CD40.[341] Thus, it is not valid to use this mutant to draw conclusions about the TRAF3 independence of particular CD40 functions, and its ability to deliver a particular signal also does not indicate complete independence from TRAF2. Finally, even in the best-designed CD40 structure–function studies, data interpretation cannot completely eliminate the possibility that, in perturbing binding of a known TRAF or TRAFs, there is also altered binding of additional, unknown proteins, or inadvertently permitted binding of a factor that does not associate with Wt CD40. Structure–function approaches using CD40 mutants have thus provided many valuable clues to TRAF function, but they cannot by themselves provide definitive information.

To produce alterations in TRAFs directly, while circumventing the low viability of $TRAF^{-/-}$ mice, investigators have exogenously expressed DN TRAF molecules in B-cell lines[347,350,358,359] or mice.[360] These studies have also provided useful clues about TRAF function. However, as noted above, overexpressing a Wt or DN TRAF molecule does not just inhibit the binding of its corresponding endogenous TRAF; it also alters the entire stoichiometry of the CD40 signaling complex, and thus this approach also yields complications in data interpretation. Recently, we have used a method of homologous recombination-based gene targeting in somatic cells to produce $TRAF^{-/-}$ B-cell lines. Initial studies of these B cells has revealed that TRAFs 2 and 6 serve both redundant and unique roles in CD40 signals to B cells. This approach allows transfection of the cells with both mutant CD40 molecules as well as Wt and mutant TRAFs and, thus, has the potential to provide valuable additional information about the roles of TRAFs in CD40 signaling to B cells.[361] The picture emerging from all these various complementary approaches is that TRAFs 2 and 6 provide important, and partially redundant, roles in CD40 functions in B cells. The roles of TRAF3 may be both positive and negative, and the roles of TRAF1 are not at all well understood; further investigation is needed to determine these roles more precisely.

How does CD40 regulate TRAF function in B lymphocytes? Following CD40 ligation, TRAFs 2 and 3 are rapidly recruited to the cell membrane,[362] which allows localization of TRAFs to cholesterol-rich membrane microdomains, or lipid rafts.[289] Definitive determination of the absolute requirement of raft localization for TRAF function has been difficult, because commonly used cholesterol-depleting reagents were found to compromise membrane integrity and activate stress-activated protein kinases (SAPKs),[289] one of the first steps of CD40's signaling pathway in B cells (reviewed in Ref. 363). Following raft localization, association with CD40 in B cells induces degradation of both TRAFs 2 and 3, but not TRAFs 1 or 6.[289,364] The degradation of both TRAF2 and TRAF3 is largely dependent upon TRAF2 binding to

CD40,[364] requires the TRAF2 RING domain, and is dependent upon ubiquitination.[365] The amplified and sustained B-cell signaling induced by CD40's virally encoded oncogenic mimic, latent membrane protein 1 (LMP1), correlates with a defect in LMP1's ability to induce TRAF degradation,[364] whereas blocking CD40-mediated TRAF2 degradation leads to LMP1-like changes in the magnitude and duration of CD40 signals to B cells.[365] Taken together, these observations suggest that TRAF degradation is an important means by which CD40 regulates TRAF function and signaling. Understanding how the degradation of TRAFs 2 and 3 is initiated and how CD40 regulates TRAFs 1 and 6 are areas of interest for further investigation.

TRAFs themselves have no known enzyme activity; they serve as adapter proteins in signaling. TRAF aggregation, initiated by trimerization of CD40 that occurs upon engagement by CD154, leads to interaction of TRAFs with downstream signaling molecules. At least several distinct CD40 signaling pathways appear to be initiated by TRAFs. Two lead to the activation of SAPKs, JNK, and p38, and a third leads to NF-κB activation. Other kinases may be involved in these pathways, and/or additional pathways. Germinal center kinase (GCK)[366] and related enzymes[367] can interact with the TRAF domain of TRAF2 in B cells and may contribute to CD40-induced JNK activation. The mitogen-activated protein kinase family may also contribute to CD40 signaling pathways. GCK can bind MEKK1, an MAPK upstream of JNK,[368–370] and TRAF2 may also interact with the MAPK, ASK-1, which could induce activation of JNK and p38.[371] However, these latter interactions have yet to be confirmed in B cells.

Additional kinases may contribute to CD40 signal transduction. CD40 signals to B cells can activate the Src family kinase Lyn and induce the phosphorylation of both phosphatidylinositol-3-kinase and phospholipase Cγ2 in human B cells.[372] Recently, CD40 signals were also shown to activate the serine-threonine kinase Pim-1 in mouse B cells.[373]

Which kinase or kinases initiate(s) the NF-κB pathway in CD40 signaling? The activation of NF-κB by TRAF2 and TRAF6 was initially credited to NIK,[374,375] an MAPK family member. NIK can phosphorylate and activate the IκB kinase (IKK) complex, which phosphorylates inhibitors of NF-κB (IκB) proteins, thus leading to their ubiquitination and ultimate degradation.[376] Although overexpressed NIK enhances NF-κB activation in 293 epithelial cells, there is no direct evidence that it specifically mediates CD40-mediated NF-κB activation in B lymphocytes. Alymphoplasia (*aly*) mice, expressing a mutant form of NIK,[377] show certain defects in B-cell activation. However, these defects are not specific to CD40 responses; B cells from 3*aly* mice also fail to respond normally to LPS and BCR signals.[378] Additionally, mice completely deficient in NIK show defects in response to the TNF family member lymphotoxin β, but do respond to CD40 signals.[379] Thus, the role played by NIK in CD40 responses may be indirect. Interestingly, MEKK1 has also been shown able to phosphorylate and activate the IKK complex.[380] It is also important to note that the interactions of TRAFs with MEKK1, NIK, and other kinases have almost exclusively been demonstrated under nonphysiological conditions, in which both TRAFs and candidate kinases are transiently overexpressed in epithelial cell lines. It thus remains to be determined which, if any, of the potential TRAF-interacting kinases, when present at physiological levels, are important players in CD40 signals to B cells.

Several other kinases have also been posited to contribute to CD40 signal transduction. CD40 engagement has been shown to activate the Src family kinase Lyn, and to induce the phosphorylation of both phosphatidylinositol-3-kinase and phospholipase Cγ2 in human B cells.[372] Recently, CD40 signals to B cells were also shown to activate the serine-threonine kinase Pim-1.[373] However, it is unclear how these enzymes fit into the overall mechanism of CD40 signal transduction, a question of great interest.

CD40 signaling to B cells induces production of many types of proteins that play roles in the immune response. These include lymphokines and chemokines, immunoglobulins, and cell membrane receptors/ligands, and other B-cell activation molecules, such as MHC class II and CD70 (see below). To date, these increases have all been shown to correlate with enhanced mRNA expression. Most of the earlier studies on CD40-induced

transcriptional regulation in B cells have focused on activation of members of the Rel/NF-κB family of transcription factors. The absence in mice of the NF-κB subunit RelA causes embryonic lethality,[381] whereas mice deficient in RelB have severe abnormalities in hematopoietic development and widespread inflammation of multiple organs.[382] $p52^{-/-}$ mice have defects in the organization of their lymphoid tissues.[383] It is therefore difficult to use any of these mice to clearly define the roles of NF-κB in CD40 signaling to normal B cells. Mice deficient in p50 or c-Rel subunits survive to adulthood and have relatively normal numbers of hematopoietic cells. However, both strains have defects in antibody production,[384,385] and p50-deficient mice have defective CD40-mediated NF-κB activation.[386] However, B cells from these mice have developed in an abnormal environment, because NF-κB–mediated transcriptional regulation participates in so many cellular functions. A complementary approach that avoids this problem is to inducibly inhibit NF-κB activation in B cells, using inducible expression of a form of IκBα that cannot be phosphorylated and degraded. When CD40-mediated NF-κB activation was blocked in several mouse B-cell lines using this technique, it was found that NF-κB activation is critical for some, but not all, CD40 effector functions.[387] Enhanced production of CD80/B7-1 is highly dependent upon NF-κB activation, upregulation of CD23, CD95, and CD54 is partially NF-κB–dependent, and upregulation of CD11a does not require NF-κB. CD40-mediated Ig production is present but markedly diminished when NF-κB translocation is inhibited.[387] The activation of JNK by CD40 is independent of the activation of NF-κB,[387] but CD40-mediated Pim-1 kinase induction appears to require NF-κB activation.[373] Whereas CD40-mediated IL-6 production requires TRAF6 association, it is independent of CD40-mediated increases in nuclear NF-κB,[350,388] although basal levels of NF-κB are required.[389] CD40-mediated transcriptional regulation thus involves transcription factors in addition to, and/or working cooperatively with, NF-κB.

The factors BSAP and Stat6 can also be activated in B cells by CD40 ligation[390,391] and may promote transcription of the germline ε gene that precedes class switch recombination to IgE.[392,393] Stat6 and NF-κB may also interact, potentially contributing to the synergy between CD40 and IL-4 signals in the induction of germline ε transcription.[394] CD40 signaling to B cells can stimulate activation of AP-1, NF-AT,[395] and E2F.[396] However, the functional roles of these factors in CD40-mediated B-cell functions have not yet been explored. Because CD40-mediated induction of IL-6 gene expression and production in B cells does not require increased NF-κB activation,[388] this promoter provides an attractive model for exploring the involvement of additional transcription factors in CD40 function. We have recently found that the transcription factors AP-1 and NF-IL-6 both appear to play important roles in CD40-mediated activation of the IL-6 gene, as well as subsequent IgM production in B cells.[389] It has also been suggested that both the germline ε and CD23 promoters contain yet unidentified regulatory elements specific to CD40-mediated gene expression.[397,398] Additional transcriptional regulation of target genes by CD40 remains to be characterized.

2. CD120b (TNFR2)

The cytokine TNF has been shown to promote antibody production by B cells,[399–402] although earlier studies did not determine whether CD120a (TNFR1) or CD120b (TNFR2), or both, were responsible for delivering B-cell activation signals. More recent findings show that B cells express little or no CD120a, so CD120b is primarily responsible for delivering TNF signals.[329] Of particular interest, CD40 signals induce both human and mouse B cells to produce TNF,[329,331] and this TNF makes a significant contribution to CD40-induced IgM production.[329] This TNF-mediated signal was shown to require the binding of TRAF2 to CD120b.[329] Further studies are needed to more completely elucidate the roles played by CD120b in B-cell activation, and the molecular mechanisms used by this receptor.

3. CD137L (4-1BBL)

CD137L (4-1BBL), expressed on B cells, interacts with CD137 (4-1BB) expressed on activated T cells. CD137 signaling has been shown to provide im-

portant costimulatory signals to the T cell (reviewed in 403), but whether CD137L has an *in vivo* role in B-cell signaling is still unclear. Earlier *in vitro* studies showed that ligation of CD137L enhances the B-cell proliferative response to anti-μ antibody,[404] but the primary and secondary antibody responses to a TD viral antigen are intact in CD137L-deficient mice.[405] However, a more recent study of CD137L$^{-/-}$ mice showed a reduction in IgG2a and IgG3 produced in response to the model antigen KLH.[406] Thus, while it currently appears that the primary function of CD137L is to stimulate CD137 signaling in T cells, this receptor may also regulate TD B-cell activation in particular situations.

4. CD134L (OX40L)

In recent years, considerable interest has been shown in the role of the TNF-R family molecule CD134 (OX40) in T-cell costimulation. However, earlier studies showed that the ligand for CD134, which is expressed on activated B cells, can itself send signals to B cells that promote proliferation and differentiation.[407,408] Studies of CD134L-deficient mice reveal that TD IgM production is normal, but there is reduced production of switched Ig isotypes.[409] However, in contrast to CD40$^{-/-}$ mice, which lack GC, GC formation can proceed in the absence of CD134L signals.[408] This suggests that these signals play their major role in production of an effective secondary antibody response, rather than in development of memory B cells. An additional role for CD134L was suggested by the finding that CD134 stimulation of B cells enhances the rate of IgG production stimulated via CD40, IL-4, and IL-10.[410]

5. CD27 and CD70

CD27, expressed by a subpopulation of peripheral human B lymphocytes and germinal center B cells, has used in recent years as a marker of memory B cells (reviewed in Refs.411, 412). The ligand for CD27, CD70, is expressed by T lymphocytes relatively late in their activation,[413] and has also been found on activated B cells[414]; CD40 signals can participate in its upregulation.[415] CD27 signals appear to be particularly important in the terminal differentiation of B cells into antibody-secreting plasma cells[416–418] and are thought to be important for IgG, but not IgM, production.[414,419] Interestingly, CD27 is also expressed by many T lymphocytes where one of its roles may be to modulate the effects of CD70 on B cells by acting as a decoy receptor.[419] Like other TNF-R family members, signaling by CD27 is mediated, at least in part, by TRAF molecules (TRAFs 2, 3, and 5).[420] Although CD27 delivers some signals in common with CD40, such as activation of NF-κB and JNK,[420,421] additional unidentified signals and the timing of CD27 expression presumably contribute to its unique activities in B-cell differentiation.

6. CD30/CD153

The contribution of CD30 and its ligand CD153 to T-cell–dependent B-cell activation is not well understood. CD30 appears to be expressed at low levels on resting B and T lymphocytes and can be upregulated by immunological stimuli.[422] CD30 can be cleaved from the cell surface, and elevated levels of the soluble form have been detected in sera from patients with autoimmune diseases, viral infections, and various types of cancer.[422] Unlike other soluble monomers of the TNF-R family, soluble CD30 retains a reasonable affinity for its ligand, suggesting that it may have some type of activity in ongoing immune responses.[423] CD40 signals upregulate CD30 expression on B lymphocytes, but the expression can be downregulated by signaling through the B-cell antigen receptors, IL-6 or IL-12.[424] One important role of CD30 signals in B cells may be in suppressing CD40-stimulated Ig isotype switching when a specific antigenic stimulus is absent, hence the negative regulation of CD30 expression by BCR.[424] The CY domain of CD30 potentially interacts with TRAF1, TRAF2, TRAF3, and TRAF5,[425] and in at least some cell types is able to stimulate the activation of NF-κB, JNK, and p38.[426–428] Like CD40, CD30 signaling may result in the degradation of TRAF molecules, potentially altering signaling by other members of the TNF-R family, such as CD40 or the TNF receptors.[429] Although CD30 appears to play negative regulatory roles in B cells, signaling through CD30 in

$CD4^+$ T cells has been shown to be a costimulus for cell proliferation[430] and cytokine production,[431] including the production of IL-13.[428]

Interestingly, CD153 signaling in B cells also appears to inhibit isotype switching.[432] Although the proximal signaling events initiated by CD153 remain uncharacterized, signals from this receptor appear to inhibit CD40 signaling and transcription of mRNA encoding Blimp-1 (a transcription factor involved in the development of plasma cells), and enhance the binding of the B cell-specific activator protein (a repressor of Ig transcription) to the immunoglobin 3× enhancer.[432]

7. BAFF and APRIL

Recently, two novel TNF family members were identified and have been shown to contribute to the establishment of humoral immune responses. One of these proteins, termed BAFF (alternatively, BlyS, THANK, zTNF4, or TALL-1), is the subject of an extensive recent review.[433] Expressed by macrophages and dendritic cells, the primary function of BAFF appears to be in preserving the viability of immature B cells in the spleen during their maturation from the transitional type 1 stage to type 2. BAFF also contributes to the maintenance of the mature B-cell population. BAFF transgenic (overexpressing) mice exhibit B-cell–mediated autoimmune diseases, perhaps because of the rescue of autoreactive clones that would otherwise die during development. BAFF may also help to prevent apoptosis of B cells involved in an ongoing immune response.[434] Potential receptors for BAFF include BCMA, TACI, and the more recently identified BAFF-R. The function of BCMA is not yet understood, but BAFF activity is not impaired in BCMA-deficient mice. TACI-deficient mice display defects in humoral responses to TI-2 antigens, but this effect is likely related to disruption of signaling initiated by APRIL (see below). Like BAFF-deficient mice, BAFF-$R^{-/-}$ mice have virtually no mature B cells, illustrating the important roles for both BAFF and BAFF-R in B-cell development. This also illustrates that even though BAFF can potentially bind BCMA and TACI, these receptors are unable to substitute for the function of BAFF-R.

Although BAFF is able to bind TACI and BCMA, at least one other ligand, APRIL, exists for these two receptors. APRIL appears not to bind BAFF-R, but may have yet another uncharacterized receptor.[435,436] Together, APRIL transgenic mice[435] and TACI-deficient mice[437,438] indicate that one biological role of this ligand-receptor pair is in the promotion of humoral responses to TI-2 (polysaccharide) antigens. TACI also appears to contribute to the regulation of B-cell homeostasis, since TACI-deficient mice display expanded B-cell compartments. One might expect therefore that APRIL would reduce B-cell numbers when injected or expressed from a transgene. However, this is not the case,[435,439] suggesting that APRIL may not contribute to TACI-mediated B-cell homeostasis.

8. CD95 (Fas)

CD95/Fas-mediated regulation of the immune response has been discussed in general by several recent reviews.[440,441] Here, we focus on the specific effects of CD95 on the activation of B lymphocytes. One of the ways in which B-cell responses are normally limited involves CD95-mediated apoptosis. During the latter stages of CD40-mediated B-cell activation, CD40 signals induce CD95 upregulation on the responding B lymphocytes, which then become increasingly susceptible to apoptosis induction by CD95L expressed by activated T lymphocytes.[442–445] Defects in CD95 signaling or expression of CD95L result in dramatic dysregulation of antibody responses *in vivo*, resulting in hypergammaglobulinemia, splenomegaly, lymphadenopathy, and autoimmunity.[446–448] However, effective B-cell activation requires that cells be resistant to CD95-mediated apoptosis until an effective antibody response, Ig isotype switching, and somatic hypermutation have taken place. Additional signals provided to B cells during their interaction with antigen and T lymphocytes appear pivotal in this ability to avoid apoptosis during the active phases of an antibody response.[286,449–454] Signals provided by follicular dendritic cells also appear to play an important role in the regulation of apoptosis of B cells.[455–457] How can such signals prevent or rescue B cells

from CD95-induced apoptosis? One site of early intervention may be during the assembly of the CD95 signaling complex at the cell membrane. Assembly of the death-inducing signaling complex (DISC) can be disrupted or inhibited in several ways. BCR and CD40 signals have been shown to increase expression of c-FLIP (FLICE-inhibitory protein), a proteolytically inactive homologue of caspase-8.[458,459] Caspase 8 is the first cysteine protease to be activated in B cells by CD95 ligation. In the presence of elevated levels of c-FLIP, CD95 ligation results in normal Fas-associated death domain protein (FADD) recruitment, but the subsequent recruitment of caspase-8 is substantially decreased and apoptosis is inhibited or delayed. Relevant to this mechanism, it has been demonstrated that BCR signaling is able to block recruitment of FADD to CD95.[460] This inhibition is observed even if BCR and CD95 signals are delivered simultaneously, is independent of *de novo* protein synthesis, and may contribute to the inhibition of apoptosis in B cells responding to specific antigen, until the antigen is cleared. This could be important in the generation of long-lived memory B cells in the germinal center. The activation of PI3K and Akt/PKB can also inhibit CD95-mediated B-cell apoptosis in some situations,[461] although the potential sites of regulation appear to be downstream of the assembly of the CD95 signaling complex (reviewed in Ref. 440). Events that occur later, requiring *de novo* gene expression and protein synthesis, cannot rescue B cells long-term from the irreversible effects of caspase-mediated DNA cleavage,[462] but can preserve cell viability until effector functions have been performed. For example, it has been shown that both BCR and CD40 signals enhance expression of the antiapoptotic protein bcl-x_L, and ectopic expression of this protein can inhibit CD95-mediated death.[454,463–465] Another transcriptionally regulated mechanism can operate through regulatory factors that downregulate CD95 expression.[466] Further study should reveal greater details about the multiple means B cells can use to regulate CD95-mediated apoptosis, and the role of such programmed death in control of B-cell activation.

V. IMMUNOGLOBULIN ISOTYPE SWITCHING

One of the most important components of the fully effective antibody response is the ability of B lymphocytes to change the class or isotype of Ig produced, while retaining the antigen-binding specificity of the antibody. The isotype of Ig molecules is contributed by their constant (C) region genes, which endow each class of Ig with specialized properties, allowing function to be tailored to be most effective in different situations. IgM is an excellent isotype for an initial response, because its pentameric structure permits greater clustering even if its affinity for antigen is not especially high. However, "switched" isotypes are designed for optimal function in subsequent stages of the humoral response. For example, IgG, the most abundant serum Ig, is highly effective at neutralizing bacterial and viral toxins, whereas IgA binds to an additional component that permits it to be transported across mucosal surfaces.[467] The presence of receptors for distinct Ig constant regions (Fc receptors) on various cells of the immune system is another way in which distinct Ig isotypes perform specialized regulatory roles.[468] The severe clinical problems associated with defective isotype switching in HIGM patients, discussed above, demonstrate the importance of isotype switching in the effective defense against pathogens.

Most isotype switching involves cooperation between contact-mediated TD signals and those delivered via lymphokine receptors, with the type of lymphokine dictating the isotype preference (reviewed in Ref. 469). IL-4 and IL-13 preferentially stimulate switching to IgG1/IgG4 and IgE,[470–472] interferon-γ to IgG2,[473] and IL-5, IL-10, or TGF-β to IgA.[474,475] A key mechanism by which lymphokines exert their effect on isotype selection appears to be the promotion of the production of the unrearranged, "germline" transcript of the constant region gene for the selected isotype, and TD signals enhance this event.[476–480] It is not yet clear how germline C gene transcription promotes isotype switching, but a commonly held theory is that this transcriptional activity leads to a more open chromosomal configuration,[481,482] allowing greater accessibility

of the DNA to recombinases that perform deletional switch recombination.

Prior to the realization that TD signals, such as CD40, play an important role in normal isotype switching, *in vitro* systems successfully used bacterial products, such as lipopolysaccharide (LPS), in combination with lymphokines to induce Ig isotype switching.[471,476] This raises the possibility that perhaps any mitogenic stimulus could combine with a lymphokine signal to induce B cells to undergo isotype switching. Indeed, the process of isotype switching has been shown to be closely linked to regulation of B-cell division.[483–485] However, the severely defective isotype switching seen in HIGM patients, who are regularly exposed to bacterial products, suggests that although microbial stimuli may indeed induce some TI isotype switching *in vivo*, TD signals predominate. Of these, CD40 provides a major signal to cooperate with lymphokines in inducing isotype switching *in vivo*.[306,311,486] CD40 ligation can itself stimulate germline C gene transcription, in addition to synergizing with lymphokine signals in this function,[487] and a distinct CD40-responsive transcriptional element has been identified in the germline Cε gene.[397] This particular role of CD40 appears to be mediated via its NF-κB–dependent pathway,[488] which may in part explain the isotype switching defect in $p50^{-/-}$ mice,[384] as well as the requirement for p65 in mediating switching to IgG3.[489] Activation of NF-κB mediated by CD40 may cooperate with Stat6-activated transcription induced by IL-4 receptor signaling.[394] There is evidence that additional transcription factors also play roles,[490–493] including BASP, which regulates both CD40 and IL-4 receptor signals,[393,494] and T-bet, which is required for normal production of IgG2a, IgG2b, and IgG3.[495]

Other interactions involving TNF-R family receptors and ligands also participate in the regulation of Ig isotype switching. Mice lacking TNF and lymphotoxin-α show defects in switching to IgG,[496] and lymphotoxin also appears to be important in switching to IgE.[497] In addition to providing both lymphokine and contact-mediated signals promoting isotype switching, T cells can also provide signals that inhibit this process, via CD30-CD153 interactions, as discussed above.[498] Interestingly, the negative signal delivered by CD30 may also target transcription of the germline C region genes, using a binding site distinct from those used by CD40 or IL-4 receptor signals.[499] Recently, it has been shown that dendritic cells can also regulate isotype switching, via the BAFF and APRIL molecules (discussed above).[500]

Although germline transcription and cell division are key components of the isotype switching process, switching cannot occur without deletional recombination, to juxtapose a new C region gene with the antigen-binding portion of the Ig molecule. The process of switch recombination is multifactorial and complex, and has itself been the subject of many comprehensive reviews. Thus, for a more detailed discussion of switch recombination, the reader is referred to several recent reviews.[501–504] We summarize here the major steps in the process, including recent information and unanswered questions.

It has become clear that two distinct processes are used in the recombination that generates V(D)J recombination in Ig genes, and that which generates switch recombination. V(D)J joining does not require germline gene transcription[505] and is dependent upon the activity of the recombinases RAG-1 and RAG-2.[506,507] However, class switch recombination (CSR) can still occur in $RAG^{-/-}$ B cells.[508] In contrast, isotype switching requires activity of a cytidine deaminase, AID (activation-induced deaminase), whereas V(D)J recombination does not.[509] Ig C region genes are preceded by repetitive 1-10 kb sequences unique to each C region, called switch (S) regions, and CSR occurs when an upstream S region undergoes nonhomologous recombination with that of a downstream C gene, deleting the intervening DNA.[503,504] It is unclear what signals initiate CSR and the S region joining. As mentioned above, germline C gene transcription has been proposed to open chromatin for access to recombinases, but this transcription may also have a more direct role in triggering recombination itself.

Because CSR involves nonhomologous joining of the S regions, roles of various proteins involved in DNA break repair have been investigated for potential participation in CSR. The Ku subunits of DNA protein kinase are required for V(D)J joining, and their absence leads to a defect in production of switched Ig isotypes.[510,511] This, together with the findings that stimuli that promote isotype switching upregulate Ku expres-

sion, and Ku may associate with CD40,[512,513] indicate that Ku may play a role in CSR. This conclusion is tempered, however, by the proliferation defects found in Ku-deficient B cells, since cell division is also an important component of isotype switching. It has also been found that mismatch repair enzymes are likely to play a role in CSR.[502,514,515]

A finding in recent years of great interest was the discovery that a cytidine deaminase, called AID, is required for both class switching and Ig somatic hypermutation (see below) in mice and humans.[516,517] Because AID also promotes mutation, it is likely that this activity is key to its function, an idea supported by the recent finding that AID can also mutate an actively transcribed gene expressed in a fibroblast cell line.[518] It remains to be discovered at which step of CSR AID is specifically required, and its precise mode of action.

Another interesting protein recently implicated in isotype switching is SWAP-70, found in a screen of proteins that promote recombination in a cell-free model system. SWAP-70, like AID, is induced upon switch-associated stimuli and is expressed in locations where switched B cells are found.[519] Also like AID, a deficiency in SWAP-70 results in defective isotype switching, but the effect is restricted to IgE.[520] How SWAP-70 mediates its effects on switching to IgE is still an unsolved mystery; it was recently discovered to associate with IP_3 and promote guanine nucleotide exchange to Rac, as well as localize to membrane ruffles.[521] It appears likely that additional players in the isotype switch process await discovery.

VI. B-CELL MEMORY

Antigen engagement of B cells *in vivo* can lead to a number of possible outcomes: B cells activated by antigen and T-cell help can directly differentiate into IgM-secreting plasma cells, or the B cells can enter the germinal center reaction, with those surviving ultimately emerging as either plasma cells or long-lived memory cells (reviewed in Refs. 522–524). In the last few years, several important findings concerning B memory cells have been published and are reviewed here.

Memory B cells exhibit a number of distinguishing characteristics. Because they have survived antigen-driven selection in the germinal center reaction, they are characterized by point mutations in the antigen-binding portions of the BCR (reviewed in Ref. 525). They may have BCR of switched isotype, although human memory B cells include a large population of IgM-positive memory cells.[526] Subpopulations of human tonsillar B cells have been defined using many surface markers, including CD19, CD20,CD38, and IgD, and the memory B cells are identified as the population that is $CD19^+$,CD20+, $CD38^-$, and IgD^-,[522,527] although there is a small population of IgM^-IgD^+ cells that have undergone somatic hypermutation.[528] More recently, CD27 has also been identified as a marker of human memory B cells.[528,529] The definition of these subsets has greatly facilitated the study of human memory B cells.

In contrast to the human system, markers of mouse memory B cells are less well-defined. Mouse germinal center cells are CD38 low, PNA-binding high, and GL7 high,[530,531] but CD38 is increased on memory cells, and PNA binding declines from the germinal center levels.[532] Mouse memory cell studies often use cells with switched isotype. CD27 has not proven useful as a marker of mouse memory B cells.

Memory B cells appear to be optimized to respond positively to signals through the BCR. **Feldhahn et al.[534]** compared naïve, GC, and memory subsets of human B cells using serial analysis of gene expression, reverse transcriptase-PCR, and flow cytometry. They showed that mRNAs associated with BCR signaling, including BLNK, Btk, Igα, Igβ, and Syk, were increased in memory B cells compared to naïve B cells.[533] Furthermore, the mRNAs encoding inhibitors of BCR-mediated activation—SHP-1, SHIP, Csk and Cbl—were all higher in naïve than in memory B cells.[533] The cytoplasmic tail of IgG also appears to confer a burst-enhancing effect, increasing the number of memory B cells and plasma cells produced in response to antigen.[534]

Memory B cells express high levels of costimulatory molecules, including CD19, CD21, CD27, CD40, CD74, CD80, and CD86, which

facilitate their interactions with T cells and accessory cells.[528,533,535] Memory B cells, unlike germinal center B cells, express the adhesion receptors L-selectin, $\alpha_4\beta_7$, and cutaneous lymphocyte antigen.[536] They also can respond with chemotaxis to CXCL12, CXCL13, and CCL19, which allows a shift in the tissue-specific homing of memory B cells compared to naïve B cells.[536,537]

Cytokines appear to play an important role in directing the fate of memory B cells. Arpin et al. utilized an *in vitro* system to analyze the requirements for directing memory B cells to either proliferate or differentiate into antibody-secreting cells.[538] These investigators showed that in the presence of cell-bound CD154, the cytokines IL-2 and IL-10 would drive human GC cells to acquire a $CD38^{-}CD20^{+}$ phenotype characteristic of memory cells and continue to proliferate, while in the absence of CD154, or in the presence of blocking antibodies, the cells would become $CD38^{+}$ $CD20^{-}$ plasma cells. In this system, IL-10 apparently acts by up-regulating expression of the high-affinity IL-2 receptor,[539] a response that is greater in memory than naïve B cells.[540] Interestingly, another group has described a similar *in vitro* system in which treatment of human tonsillar GC cells with soluble CD154, in the presence of a follicular dendritic cell line, gives rise to memory cells in the presence of IL-4 and IL-2, and plasma cells in the presence of IL-10 and IL-2.[541,542] Reconciling the differences in response to IL-10 in the two systems may lie in closer analysis of the form and strength of the signal to the B cells through CD40, since variation in the CD40 signal has been suggested to affect the fate of B-cell differentiation.[543,544]

Memory B cells have higher levels of IL-4 receptors than their naïve counterparts,[533] and treatment of memory B cells with IL-4 results in downregulation of a large group of immunoglobulin superfamily members, which cooperate negatively in signaling through the BCR.[533] Human memory B cells have elevated levels of the BAFF receptor BCMA, as detected by flow cytometry, as well as elevated levels of mRNA-encoding BAFF-R.[533] The importance of BAFF in maintaining the memory B-cell population has not been explored.

Although memory B cells appear to be poised to respond to specific antigen, antigen is not required for their maintenance.[545,546] Recent work from Lanzavecchia's group suggests that antigen-specific memory B cells and antibody titers may rely on non-specific memory B-cell stimulation from T cells stimulated in a noncognate fashion, or by polyclonal activators. They demonstrated that naïve $CD27^{-}$ human B cells do not proliferate well in response to unmethylated CpG DNA *in vitro*, even in the presence of bystander T-cell help, or to T-cell help alone, unless first stimulated through the BCR.[547] In contrast, memory $CD27^{+}$ B cells, either IgM^{+} or of switched isotype, responded robustly to CpG DNA or CpG DNA + bystander T-cell help or to T-cell help alone without costimulation through the BCR, resulting in both the proliferation of memory cells as well as their differentiation into plasma cells.[547] Furthermore, IgD^{-} B cells of switched isotype isolated from human peripheral blood respond with greater proliferation to the polyclonal activator *Staphylococcus aureus* Cowan A strain + IL-2 and IL-10.[548]

Toll-like receptors (TLR) are involved in the innate immune response to a variety of microbial products. The response of both mouse and human B cells to unmethylated CpG DNA is mediated by TLR9.[549,550] Bernasconi et al. have published RT-PCR data showing that naïve human B cells do not express TLR9 unless first stimulated through the BCR, whereas memory B cells express TLR9 constitutively, as well as TLRs 6, 7, and 10.[551]

Two transcriptional repressor molecules have been postulated to hold the plasma cell terminal differentiation program in check: BSAP, encoded by the Pax5 gene, and **Bcl-6**(reviewed in Ref. 552). Most GC B cells are positive for the negative transcriptional regulatory protein Bcl-6, which in overexpression models functions to inhibit the plasma cell differentiation program through blocking expression of Blimp1.[553] After leaving the GC, memory cells appear to maintain a low level of expression of Bcl-6 mRNA,[554] as well as Pax5 mRNA,[533,551] but lack Blimp1.[555] Fearon et al.[556] postulated that continued Bcl-6 expression maintains the memory cells as a self-renewing population, a "stem-cell" for continual production of plasma cells. It is not yet clear whether the levels of **Bcl-6** and Pax5

expression in memory cells is sufficient to prevent plasma cell differentiation, or whether other mechanisms may be required to maintain B cells in the memory cell stage.

VII. CONCLUSIONS

The topic of antigen-specific, T-dependent B-cell activation covers a huge area of research, to which many investigators have and continue to contribute. To preclude exorbitant length, we have only been able to cover highlights of the current state of knowledge of the various steps in the process; these are summarized in Figure 3.

It is now abundantly clear that the ultimate outcome of the binding of antigen to the BCR is dependent upon the balance between a variety of regulatory signals delivered via coreceptors that send **positive, negative, or** both (depending upon other factors) messages to the cell. The BCR itself utilizes Ig-α and Ig-β to deliver activating signals. These, however, are modulated by receptors such as CD19, CD21, CD22, and CD32, with the balance between these auxiliary signals determining how the B cell perceives the initial stimulus. Experiments described above have clearly demonstrated that a loss of any of these signaling pathways results in abnormal B-cell responses to activating signals.

The ability of the B cell to process and present the antigen bound to its BCR allows it to engage in cognate interactions with an activated T cell. This interaction results in the delivery of a large number of contact-mediated signals between the two cells (detailed in the preceding text and illustrated in Fig. 1). Many of these receptor–ligand pairs have only recently been identified, and more surely await discovery, as does a more detailed understanding of the particular roles of each. Interestingly, some of these receptors serve dual purposes in both signaling and additional functions (e.g. adhesion molecules, MHC class II), and in some cases both receptor and ligand can also be found on activated B cells, allowing further amplification of signals initially delivered by T cells (see above).

The outcomes of antigen-specific, T-dependent B-lymphocyte activation are also varied, and each contributes to a fully functioning im-

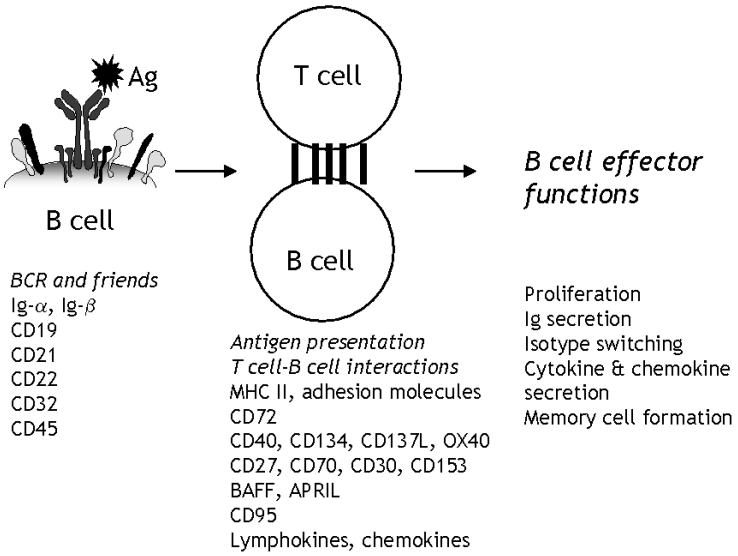

FIGURE 3. Overview of antigen-specific B-cell activation. Each of the steps and molecules involved is discussed in this review. Activation is initiated by the binding of specific antigen to the BCR. A variety of co-receptors serve to regulate the nature of the signal delivered by this binding. Antigen is internalized, processed, and presented to the activated T lymphocyte, and the interaction between T cell and B cell delivers numerous additional activating and regulatory signals to both cells. The ultimate outcome of this complex process is B-cell effector functions, which are summarized in the figure.

mune system. To be effective, a specific clone of B cells must expand by proliferation to a size sufficient to counteract a microbial threat. To interact effectively with T lymphocytes and receive their many costimulatory signals, the B cell must upregulate a number of surface molecules that enable it to be more effective in antigen presentation, including adhesion molecules, MHC molecules, and costimulators (such as CD80, CD81, CD27, and others).

The unique and most critical function of the B lymphocyte is the production of antibodies, and thus full B-cell activation must result in antibody production and secretion. Additionally, because particular immune responses optimally require distinct Ig isotypes, the responding B cell must be capable of receiving and responding to signals specifying Ig-isotype switching. In concert with these processes, B cells also serve as a source of production of a large variety of lymphokines and chemokines, which serve to regulate the activity of B cells themselves and other cell types with which they interact. As detailed above, important cooperating signals in isotype switching, development of memory, and other B-cell activation events, are provided by soluble factors. However, a full discussion of the roles of lymphokines and chemokines in B-cell activation is beyond the scope of this article.

The development of the humoral memory response, although critical to normal mammalian health, has long been mysterious and its mechanisms elusive. While the above discussion reveals that much remains to be understood, considerable progress has been made in identifying memory B cells in the human, and in understanding the signals and environmental cues required for the development of B-cell memory.

Thus, in all the events and stages of B-cell activation, many of the important details and requirements have been revealed over the past decade. However, many questions remain to be answered. In particular, how each of the individual cues received by B cells are integrated to produce an effective, well-regulated response, is of great interest. Emerging tools, techniques, and experimental approaches will help to address these new areas of investigation.

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