

# B lymphocyte activation by contact-mediated interactions with T lymphocytes

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T cell dependent B lymphocyte activation requires interactions between numerous receptor–ligand pairs on the two cell types. Recently, advances have been made both in understanding how these various signals regulate B cell effector functions and in identifying many new receptor–ligand pairs that contribute to the regulation of B cell function by T lymphocytes.

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## Abbreviations

CD134 CD134 ligand

FLIP FLICE-inhibitory protein

TNF-R TNF receptor

TRAF TNF-R-associated factor

## Introduction: contact-dependent T cell mediated B cell activation

Although B lymphocytes express receptors that can bind many different soluble biologically active molecules, such as lymphokines and chemokines, the development of affinity maturation and a highly effective humoral memory response require receipt by the B cell of contact-mediated signals from an activated T lymphocyte. This requirement may be advantageous to the regulation of humoral responses in several ways. Although bystander polyclonal activation of B cells by activated T cells occurs, it is not widespread in the normal immune response. This suggests that cognate interactions between B cells that can present specific antigen and activated T cells that are specific for that antigen–MHC complex increase the efficiency of the delivery of noncognate signals between the two cell types. This possibility is supported by published reports [1–3]. At least three mechanisms could contribute to this increased efficiency. First, signaling through the BCR primes the B cell to become more responsive to T cell dependent activation signals and also stimulates enhanced expression of surface molecules contributing to antigen presentation. Second, the ability to interact directly through MHC–TCR binding can enhance the physical proximity of the two cell types, assisting in the delivery of contact-mediated signals as well as soluble molecules. Last, signals delivered to the B cell through engagement of MHC class II molecules have been shown to enhance both antigen presentation and B cell activation, and to cooperate with both BCR signals and other contact-dependent signals (see below).

Elimination of self-reactive T cell clones appears to be more complete than that of B cell clones, particularly in the presence of limiting amounts of autoantigen [4]. Thus, if a cognate antigen-specific T cell clone is not available, even an autoreactive B cell clone has little opportunity to become activated and produce high-affinity pathogenic autoantibodies. Consistent with this, the importance of contact-mediated signals in developing autoimmunity has been highlighted in recent reports [5•,6•]. It therefore seems that the requirement for contact-mediated signals in antigen specific B cell activation serves both to enhance the effectiveness of adaptive humoral responses and to decrease the potential for activation of autoreactive B cells.

Here we provide an overview of the various receptor–ligand pairs currently known to contribute to T cell contact mediated B cell activation, and highlight recent advances both in the understanding of how each receptor delivers its signals to the B cell and in the roles played by various receptors of T cell dependent signals in the process of B cell regulation (see Table 1).

## MHC class II

It was once widely believed that T cell cytokine production was both necessary and sufficient to mediate full activation of antigen specific B cells. Subsequently, when more stringently separated resting B cells were used, it was shown that, although cytokines clearly play important roles in B cell activation, contact-mediated signals from the T cell are likewise crucial (reviewed in [7]). The first such signal to be identified was MHC class II [8,9]. Engagement of class II molecules on B cells stimulates early biochemical signaling events as well as later effector functions, such as B cell proliferation and differentiation (reviewed in [10]), and both cytoplasmic and transmembrane domains of the molecule contribute to signaling events [11,12].

Studies of class II deficient mice have revealed that B cell activation can occur in the absence of class II expression [13]. However, class II engagement enhances both BCR and CD40 signaling [1], and may contribute to activation of CD40 deficient B cells [14], suggesting that, by enhancing the effectiveness of other B cell activation signals, class II signaling may promote the preferential activation by T cells of cognate antigen-presenting B cells. Consistent with this role is the recent finding that class II signaling may be partly regulated by two BCR co-receptors, CD19 and CD22 [15•].

Another recent finding of interest is that, after its engagement, class II localizes to cholesterol- and glycosphingolipid-enriched membrane microdomains [16•], which are thought to be sites where transmembrane signaling complexes assemble.

Table 1

**B cell transmembrane receptors and T cell ligands involved in contact-dependent regulation of B cell activation.**

B cell receptor	T cell ligand	B cell effector functions
Class II MHC	TCR; CD4	Cooperates with other activation signals to stimulate proliferation, differentiation and enhanced antigen presentation
CD11a–CD18/CD54	CD54/CD11a–CD18	Cell adhesion, enhanced antigen presentation and enhanced activation
CD72	CD100	Development of B-1 B cells, production of high-affinity IgG response and enhanced antigen presentation
CD40	CD154	Proliferation, differentiation, isotype switching, cytokine production, protection from apoptosis, and germinal center and memory response development
CD134L/OX40L	CD134/OX40	Stimulation and enhancement of IgG response
CD137L/4-1BBL	CD137/4-1BB	Stimulation of T cells through CD137
CD27	CD70	Differentiation into plasma cells
CD30/CD153	CD153/CD30	Inhibition of B cell responses, such as isotype switching and plasma cell differentiation
CD95/Fas	CD95L/FasL	Induction of programmed cell death (apoptosis)

CD40 has also been shown to localize to these microdomains in B cells [17••] and physical membrane association between the two receptors after their engagement on B cells has been reported recently [18•]. An important future direction in understanding the physiological role of class II signaling in B cell activation is to determine how physical interactions between class II and other transmembrane receptors affect the ultimate nature and strength of regulatory signals delivered to the B cell.

### Adhesion molecules

Both B and T lymphocytes express various transmembrane adhesion molecules, whose surface expression is increased after diverse activation signals. Both cell types express ICAM-1 (CD54) and LFA-1 (CD11a–CD18), which bind to each other and can thus mediate both homotypic and heterotypic adhesion. Enhanced adhesiveness between B cells and T cells can amplify activation signals delivered by one cell type to the other; however, the role played by direct signaling through either of these two adhesion molecules is less well understood, particularly signaling to the B cell. Early studies suggested that both LFA-1 and ICAM-1 can directly transmit activation signals to B cells [19,20] and it was subsequently shown that such signals can both contribute to enhanced B cell antigen-presentation [21] and cooperate with CD40 mediated signaling [22]. More recently, ICAM-1-mediated upregulation of class II expression on a B cell line has been shown to correlate with activation of the Src family kinase, Lyn, and the mitogen-activated protein kinases [23]. A clearer picture of the physiological circumstances in which LFA-1 and ICAM-1 signal to B lymphocytes, and of how these signals are both made and coordinate with other T cell mediated signals, will help to elucidate the role played by these membrane molecules in interactions between T cells and B cells.

CD81, a member of the tetraspanin family of molecules, has also been implicated in adhesion and in T cell dependent B cell activation [24,25]. But, to date, a natural ligand

for CD81 has not been isolated, therefore a clear understanding of how CD81 affects interactions between T cells and B cells awaits information revealing how CD81 signaling is triggered *in vivo*.

Another adhesion molecule capable of signaling to B cells is CD22, a member of the immunoglobulin superfamily that modulates BCR signaling (recently reviewed in [26]). Its natural ligands are *N*-linked oligosaccharides that contain terminal  $\alpha$ 2,6-linked sialic acids (reviewed in [27]). Thus, a large variety of cell-surface glycoproteins, glycolipids and gangliosides could potentially act as CD22 ligands, including many expressed on T lymphocytes; but which of these really act as physiologically important ligands *in vivo* remains unclear. As with CD81, key to understanding the role of CD22 signaling in interactions between B cells and T cells will be determining both the physiological circumstances in which CD22 is engaged and its biologically important ligands.

### CD72

It has been known for some time that antibody-mediated engagement of the CD72 molecule on B cells stimulates proliferation, enhanced B cell survival and upregulation of MHC class II expression (reviewed in [28]). However, the natural ligand for CD72 had proved elusive. New studies have identified this ligand as CD100, a member of the semaphorin family more commonly known for roles in neuronal regulation, and shown that it is expressed on both B cells and activated T cells [29••]. Engagement of CD72 by CD100 enhances B cell activation mediated by CD40, and blocking this interaction inhibits T cell dependent IgG production although IgM production is unaffected [29••].

Complementary studies in CD100-deficient mice show that CD100 expression is required for normal development of B-1 B cells, and is important for development of high-affinity IgG responses to T cell dependent antigens [30••], although T cell independent responses are intact. Results also indicate that CD100-mediated CD72 signals are important for antigen

presentation and potentially responsible for inducing the dissociation of the phosphatase SHP-1 from CD72 [29•,30•].

### The TNF and TNF-receptor family

Some of the most biologically important signals that the B cell receives from the cognate activated T cell are delivered through membrane-bound members of the TNF and TNF receptor (TNF-R) family of proteins (see below). The number of identified members of this family is rapidly increasing.

#### CD40

One of the first TNF family members shown to be of major importance in T cell dependent B cell activation is CD40, a TNF-R family member whose ligand, CD154, is expressed as a membrane-bound trimer on activated T lymphocytes. Engagement of CD40 on B lymphocytes promotes proliferation, antibody secretion, cytokine production, upregulation of various surface molecules involved in antigen presentation, isotype switching, development of germinal centers and a humoral memory response (recently reviewed in [31,32]). In addition, whereas dendritic cells are superior to B cells in antigen presentation in many situations, several recent studies have shown that the B cell can be an important antigen-presenting cell for T cell activation and that CD40 signaling to the B cell plays a critical role in this function [5•,33•].

The molecular mechanisms by which CD40 signals to B cells have not been established clearly. The carboxy-terminal domain of CD40 binds to a cytoplasmic family of adapter molecules, called 'TNF-R-associated factors' (TRAFs; [34]), which play important roles in CD40 signaling. But just how the TRAFs that associate with CD40 (i.e. TRAF-1, -2, -3, -5 and -6) regulate its function is not yet clear. Earlier studies in this area often used model systems in which both CD40 and candidate signaling molecules, such as TRAFs and/or various kinases, were highly and transiently overexpressed in transformed epithelial cells. But later studies in B lymphocytes and mice have not always recapitulated the results of the earlier, more artificial model systems. For a recent review on the molecular basis of CD40 signaling, see [35].

Previous studies have shown that although TRAF2 and TRAF6 can both promote CD40 activation of the transcription factor NF- $\kappa$ B, they seem to be redundant with respect to each other in this function. However, only TRAF2 appears to be necessary for CD40-mediated JNK activation and surface molecule upregulation (reviewed in [35]). Paradoxically, recent studies using TRAF2<sup>-/-</sup> mice bred onto a TNF-R1<sup>-/-</sup> background (which allows survival of the otherwise neonatally morbid TRAF2<sup>-/-</sup> mice) suggest that TRAF2 is required for CD40-mediated NF- $\kappa$ B activation, B cell proliferation and antibody production [36•]. However, recent studies show that TRAF2 can play an indirect role in CD40 mediated IgM secretion, possibly through association with TNF receptors [37••], as CD40 stimulates B cell TNF production, which can stimulate IgM secretion.

Thus, interpreting the findings in mice with abnormal TNF signaling throughout development is complicated. TRAF2 seems to have a more direct role in mediating isotype switching, a process in which other TRAFs may also be required [38•]. Overexpressing TRAF3 inhibits CD40 mediated IgM production but, as both full-length and truncated ('dominant-negative') versions of TRAF3 have this effect, it is likely that TRAF3 overexpression disrupts the stoichiometry of the signaling complex to inhibit CD40-mediated function [37••]. Thus, TRAF3 may also have positive regulatory roles in additional CD40 functions and these remain to be elucidated.

Recently, several studies have shown that TRAF6 is required for CD40-mediated IgM production, IL-6 secretion, surface molecule upregulation and isotype switching in B cells [38•,39•,40••]. The potential role played by TRAF1, which is the only TRAF lacking a zinc RING domain, remains obscure. The TRAF RING domain has been shown to be critical for the signaling functions of TRAF-2, -3 and -6 (reviewed in [35]) and has recently been found to have a role in the recruitment of TRAF2 to membrane rafts by CD40 [17••].

Overexpression studies have indicated that a variety of kinases can interact with either the TRAF domains or (less commonly but perhaps more importantly) the zinc-binding domains of TRAF molecules (reviewed in [35]). However, determining which kinases actually mediate initial CD40 signaling events at physiological levels in cells is an important future goal. Previous studies have shown that CD40 signaling in B cells stimulates the stress-activated protein kinases, JNK and p38 [35], although the CD40 effector functions in B cells that these kinases mediate have not been established. More recent work shows that CD40-induced phosphatidylinositol 3-kinase is important for CD40 signals that enhance B cell survival [41•].

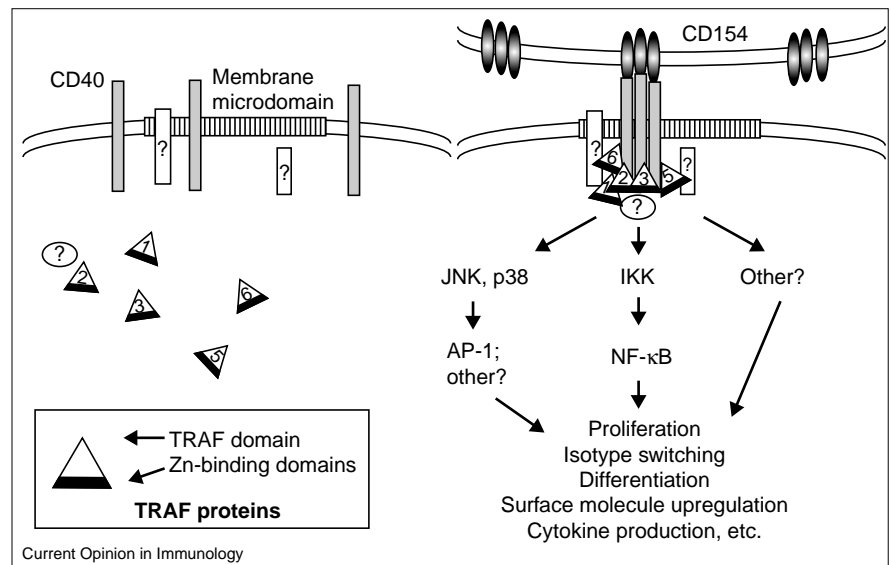
The mitogen-activated protein kinase, NIK, has been implicated by overexpression studies to participate in CD40 mediated I $\kappa$ B kinase phosphorylation [35], and a recent study showed that mice expressing a mutant NIK molecule have defective B cell activation [42•]. However, B cells from these mice failed to respond normally to any tested stimuli, including BCR and mitogen activation, so their defect in B cell activation is not specific to CD40 and may be at the level of B cell development. Studies in physiologically relevant systems linking CD40 engagement to kinase activation and transcriptional regulation are important to gain a better understanding of how CD40 activates normal B cells. Figure 1 presents a model of our current understanding of CD40 signaling, based upon events verified to occur in B lymphocytes.

#### CD134 ligand and CD137 ligand

Considerable interest has been shown recently in the role played by the TNF-R family molecule OX40/CD134 in T cell co-stimulation. However, earlier studies demonstrated that the CD134 ligand (CD134L), expressed on activated B cells, can

Figure 1

Model of CD40 signaling pathways in B lymphocytes. The left side of the figure depicts the resting B cell, in which TRAF proteins are distributed in the cytoplasm, and the majority of CD40 is outside the cholesterol-rich membrane microdomains. The right side of the figure illustrates initiation of CD40 signaling following trimerization of CD40 by CD154 (on T cells); the inset shows TRAF protein structure. TRAF-1, -2, -3, -5 and -6 bind via their TRAF domains to the cytoplasmic tail of CD40, and both CD40 and its associated TRAFs are rapidly recruited to membrane microdomains. Additional CD40-binding proteins (TRAFs or non-TRAFs, as indicated by the question marks within some of the shapes) may also remain to be discovered. Following recruitment to microdomains, the Zn-binding domains of the TRAFs associate with as yet undefined proteins that initiate activation of c-jun kinase (JNK) and p38, leading to the activation of the transcription factor AP-1 and perhaps others. Independently, the I $\kappa$ B kinase (IKK) complex is activated, leading to the nuclear translocation of NF- $\kappa$ B. Activation of NF- $\kappa$ B is required for CD40-mediated upregulation of certain surface molecules, proliferation, differentiation and isotype switching whereas other



CD40-mediated effector functions (upregulation of adhesion molecules and production of IL-6 and TNF) are partially or largely independent of increases in NF- $\kappa$ B (reviewed in [35]). The causal relationship between CD40 mediated

JNK/p38 activation and downstream effector functions has not yet been clearly defined. It is also likely that additional CD40-induced signaling cascades contributing to B cell function remain to be characterized.

itself send signals to B cells that promote proliferation and differentiation [43,44]. Recent data from CD134L-deficient mice reveal that T cell dependent IgM production is normal but that the production of switched immunoglobulin isotypes is reduced [45•]. In contrast to CD40 deficient mice, which lack germinal centers, the lack of CD134L signals in CD134L-deficient mice does not prevent germinal center formation [44], suggesting that these signals are more important for normal production of a strong secondary antibody response, rather than for memory B cell development. Another role for CD134L has been suggested by the finding that CD134 stimulation of B cells enhances the rate of IgG production stimulated through CD40, IL-4 and IL-10 [46•].

CD137 (4-1BB), which is expressed on activated T cells, interacts with its ligand CD137L (4-1BBL), which is expressed on B cells. This interaction has been shown to provide important co-stimulatory signals to the T cell (reviewed in [47]) but a clear role for CD137L in B cell signaling *in vivo* has not been demonstrated. Earlier studies showed that *in vitro* stimulation with CD137 enhances the B cell proliferative response to anti- $\mu$  antibody [48]; however, the primary and secondary humoral responses to a T cell dependent viral antigen are intact in CD137L deficient mice [49•], suggesting that the primary function of CD137L is to stimulate CD137 signaling in T cells, rather than to deliver critical signals to the B cell.

#### BCMA and TACI

Two recently identified TNF related proteins, APRIL ('a proliferation-inducing ligand' [50]), and TALL-1 [51] (also

referred to as BlyS [52] or BAFF [53]), can augment B cell growth and differentiation by signaling through either of the TNF-R family members BCMA or TACI (recently reviewed in [54]). Although potentially important in regulating humoral immune responses, the involvement of APRIL and TALL-1 in T cell contact dependent B cell activation remains to be proved. Myeloid cells seem to be the principal source of both APRIL and TALL-1. Both molecules are type 2 transmembrane proteins and may act in a cell contact dependent manner; however, each molecule is also likely to undergo proteolytic cleavage at the cell surface and thus may be more important as a soluble molecule.

#### CD27

CD27 is a TNF-R family member expressed by a subpopulation of B lymphocytes and has been proposed as a marker of memory cells (reviewed in [55]). CD70, a TNF family member and the ligand for CD27, is expressed by T lymphocytes relatively late in their activation [56]. CD27 signals seem to be particularly important in the terminal differentiation of B cells into antibody-secreting plasma cells [57–59]. Interestingly, CD27 is also expressed by many T lymphocytes, in which one of its roles may be to modulate the effects of CD70 on B cells by acting as a decoy receptor [60]. Like other TNF-R family members, signaling by CD27 is mediated, at least in part, by TRAF molecules (TRAF-2, -3 and -5) [34]. Although CD27 delivers some signals in common with CD40, such as activation of NF- $\kappa$ B and c-Jun kinase [61,62], additional unidentified signals and the timing of CD27 expression presumably contribute to its unique activities in B cell differentiation.

### CD30 and CD153

In contrast to the generally stimulatory effects of TNF-R family members such as CD27 and CD40, CD30 and its ligand CD153 (a TNF family member) have been proposed as negative regulators of humoral immune responses. Signaling through either molecule on B lymphocytes seems to inhibit isotype switching and may limit both the magnitude of an immune response and activation of low affinity or bystander B cells [63<sup>\*\*</sup>,64<sup>\*</sup>]. It should also be noted, however, that B cell homeostasis and humoral immune responses to vesicular stomatitis virus in CD30 deficient mice appear grossly normal [28], suggesting that additional redundant control mechanisms exist.

Like many of its relatives, CD30 mediates its signaling through TRAF proteins [34], and can stimulate NF- $\kappa$ B activation [65]. Presumably, CD30 also delivers unique signals to B cells that are not supplied by other TNF-R family members but these remain to be identified. Signal transduction through CD153 is even less well understood. CD153 engagement has been reported to inhibit the appearance of mRNA encoding Blimp-1 (B-lymphocyte-induced maturation protein 1), a transcription factor involved in the differentiation of plasma cells, and to enhance the binding of the BSAP (B-cell-specific activator protein) repressor to the immunoglobulin 3' enhancer [64<sup>\*</sup>]. The proximal signaling events leading to these phenomena have not been elucidated.

### CD95

During the activation of a humoral immune response, CD40 signals induce upregulation of CD95/Fas on the responding B lymphocytes, which then become increasingly susceptible to apoptosis induction by CD95L expressed on activated T lymphocytes. Defects in CD95 or its ligand *in vivo* cause marked dysregulation of humoral immune responses, resulting in hypergammaglobulinemia, splenomegaly, lymphadenopathy and autoimmunity.

Regulation of the immune response by CD95 is the subject of an extensive recent review [66] but a few recent findings deserve comment here. Considerable effort has been invested in understanding the mechanisms by which B lymphocytes avoid responding to CD95 signals during the earlier phases of an immune response. Assembly of the CD95 signaling complex at the plasma membrane seems to be one major site of regulation. Assembly of this complex can be disrupted or inhibited in at least two ways. BCR and CD40 signals can upregulate the expression of c-FLIP (FLICE-inhibitory protein), a proteolytically inactive homolog of caspase-8, the protease immediately downstream of CD95 [67<sup>\*</sup>,68<sup>\*</sup>]. In B cells with elevated levels of c-FLIP, CD95 engagement results in normal FADD recruitment to the receptor but the FADD-mediated recruitment of caspase-8 (FLICE) is markedly diminished and apoptosis inhibited or delayed. Recently, it has also been shown that BCR signaling can dramatically inhibit the recruitment of FADD to CD95 [69<sup>\*</sup>]. This inhibition is observed even if BCR and CD95

engagement are delivered simultaneously, is independent of new protein synthesis and perhaps contributes to the inhibition of apoptosis among antigen-specific cells until the antigen is cleared. The activation of phosphatidylinositol 3-kinase and Akt/protein-kinase-B can also inhibit CD95 mediated B cell apoptosis in some situations [70] although the potential sites of regulation appear to be downstream of assembly of the CD95 signaling complex (reviewed in [71]).

### Conclusions

It is increasingly evident that a large number of diverse receptor–ligand interactions contribute to the activation of B lymphocytes after contact with the activated T cell. Various types of molecules act as signal receptors for the B cell, including MHC molecules, adhesion molecules, co-stimulators and regulators of the BCR complex, and the rapidly growing TNF-R/TNF superfamily of molecules. For some of these B cell signal receptors, their natural ligands on T cells await identification — a key step to understanding where they fit in the regulation of B cell function.

For most of the receptor–ligand pairs that have been identified, information ranging from tantalizing hints (e.g. CD30–CD153) to extensive characterization of physiological function (e.g. CD40) has accumulated but many gaps remain in our understanding of even the best-studied molecules. It is clear that a complete understanding of both the molecular details of receptor signaling pathways and the physiological roles played by each particular receptor will require a multifaceted approach. Studying relevant receptors and signaling molecules at endogenous levels, in B cells themselves, will be key to identifying which pathways and molecular interactions are truly relevant *in vivo*. In addition, when an activated T cell interacts with a B cell, a large number of signals are delivered in concert and the spatial organization of various interacting receptors also has an important role in the outcome of their interactions.

Future goals thus need to include the study of how individual receptors interact physically, as well as how their induced signaling cascades interact, to determine the final outcome for the regulation of B cell activation.

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This study reports semaphorin CD100 to be the long-awaited natural ligand for CD72. CD100 is expressed on B and activated T cells, is CD40 inducible and synergizes with CD40 in B cell activation. Soluble CD100 can inhibit an *in vivo* T cell dependent antibody response.
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Mice deficient in expression of CD100, the natural ligand for CD72, have a decreased number of B-1 B cells but normal numbers of conventional B and T cells. The mice show defective T cell dependent IgG responses, however, as well as defects in antigen presentation. See also annotation to [29\*\*].
31. van Kooten C, Banchereau J: **CD40-CD40 ligand.** *J Leukoc Biol* 2000, 67:2-17.
32. Calderhead DM, Kosaka Y, Manning EM, Noelle RJ: **CD40-CD154 interactions in B-cell signaling.** *Curr Top Microbiol Immunol* 2000, 245:73-99.
33. Constant SL: **B lymphocytes as APC for CD4+ T cell priming ●● in vivo.** *J Immunol* 1999, 162:5695-5703.  
This study addresses the often-controversial question of whether B cells can present antigen effectively to naive T cells, using a new model system. Using an *in vivo* system in which mice transgenic for hen egg lysozyme (HEL)-specific BCRs are given soluble HEL-cytochrome-c conjugate, the author demonstrates that antigen-specific B cells can upregulate CD81 and present cytochrome c effectively to naive CD4+ T cells. This presenting capacity is dependent on BCR uptake of antigen; it is not seen if antigen is taken up by nonspecific endocytosis.
34. Arch RH, Gedrich RW, Thompson CB: **TRAFs – a family of adapter proteins that regulates life and death.** *Genes Dev* 1998, 12:2821-2830.
35. Bishop GA, Hostager BS: **Molecular mechanisms of CD40 signaling.** *Arch Immunol Ther Exp* 2001, in press.
36. Nguyen LT, Duncan GS, Mirtsos C, Ng M, Speiser DE, Shahinain A, ● Marino MW, Mak TW, Ohashi PS, Yeh W: **TRAF2 deficiency results in hyperactivity of certain TNFR1 signals and impairment of CD40-mediated responses.** *Immunity* 1999, 11:379-389.  
These authors found a way to obtain functional data from TRAF2 deficient mice, which are otherwise limited as a model system by their early mortality. Strains of TRAF2 deficient mice will survive past birth if bred onto mice deficient in either TNF or TNF-R1. The double-knockout mice show defective IgG

antibody responses to a virus, and splenocytes show defective *in vitro* proliferation and NF- $\kappa$ B nuclear translocation in response to anti-CD40 stimulation.

37. Hostager BS, Bishop GA: **Cutting edge: contrasting roles of TRAF2 and TRAF3 in CD40-mediated B lymphocyte activation.** *J Immunol* 1999, **162**:6307-6311.

Inducible overexpression of either full-length or truncated (minus the zinc-binding domains) TRAF3 in mouse B cells inhibits CD40-induced antibody production; this requires TRAF3-CD40 binding. In contrast, overexpression of full-length TRAF2 enhances, whereas truncated TRAF2 inhibits, CD40-mediated immunoglobulin secretion; however, the effects of TRAF2 do not require it to bind to CD40. These results demonstrate that TRAFs can influence CD40 signaling both directly and indirectly, and emphasize the importance of interactions between CD40 and other TNF-R family members in regulating B cell effector functions.

38. Leo E, Zapata JM, Reed JC: **CD40 mediated activation of Ig-C $\gamma$ 1 and Ig-C $\epsilon$  germ-line promoters involves multiple TRAF family proteins.** *Eur J Immunol* 1999, **29**:3908-3913.

The authors use a B cell line transfected with various CD40 cytoplasmic-domain mutants, as well as transient expression of various TRAF molecules, to demonstrate that CD40-mediated isotype switching requires and is regulated by various TRAFs.

39. Baccam M, Bishop GA: **Membrane-bound CD154, but not anti-CD40 mAbs, induces NF- $\kappa$ B independent B cell IL-6 production.** *Eur J Immunol* 1999, **29**:3855-3866.

Although most CD40 functions in B cells can be stimulated by agonistic monoclonal antibodies as a substitute for CD154, this study shows that, in both normal B cells and a B cell line, induction of IL-6 secretion requires cell membrane bound CD154 and is mediated at the transcriptional level. Surprisingly, although CD40 induces increased NF- $\kappa$ B production and the IL-6 promoter contains several NF- $\kappa$ B binding sites, induced IL-6 secretion is independent of increased NF- $\kappa$ B.

40. Jalukar SV, Hostager BS, Bishop GA: **Characterization of the roles of TRAF6 in CD40-mediated B lymphocyte effector functions.** *J Immunol* 2000, **164**:623-630.

Two complementary approaches—signaling through a CD40 mutant that cannot bind TRAF6 but binds other TRAFs normally and inducible overexpression of truncated mutant TRAF6—show that B cells require CD40-TRAF6 binding for normal CD40-mediated immunoglobulin secretion, CD80 upregulation and IL-6 production. Interestingly, TRAF6 is not required for NF- $\kappa$ B activation or c-Jun kinase stimulation, suggesting that it may serve redundant roles with TRAF2 in these particular CD40-mediated events.

41. Andjelic S, Hsia C, Suzuki H, Kadowaki T, Koyasu S, Liou H: **PI 3-kinase and NF- $\kappa$ B are at the divergence of CD40-mediated proliferation and survival pathways.** *J Immunol* 2000, **165**:3860-3867.

CD40 is known to be a strong stimulus in B cells for the nuclear translocation of NF- $\kappa$ B. This paper now shows that this event is dependent on the activation of both phosphatidylinositol 3-kinase and the kinase, AKT. The pathway is shown to be crucial for CD40-mediated B cell proliferation but not CD40 mediated antagonism of spontaneous apoptosis in normal B cells.

42. Garceau N, Kosaka Y, Masters S, Hambor J, Shinkura R, Honjo T, Noelle RJ: **Lineage-restricted function of NIK in transducing signals via CD40.** *J Exp Med* 2000, **191**:381-385.

This study presents the first specific examination of B and dendritic cell activation in *alymphoplasia (aly)* mice, which express a mutant version of the serine/threonine kinase, NIK. IL-2 production by *aly* homozygous splenic dendritic cells is normal or enhanced compared with that by heterozygous cells. Activation of NF- $\kappa$ B, surface molecule upregulation, proliferation and immunoglobulin production are reduced in *aly* homozygous B cells in response to CD40, lipopolysaccharide, phorbol myristate acetate or anti-IgM stimulation.

43. Stüber E, Neurath M, Calderhead D, Fell HP, Strober W: **Cross-linking of OX40 ligand, a member of the TNF/NGF cytokine family, induces proliferation and differentiation in murine splenic B cells.** *Immunity* 1995, **2**:507-521.

44. Stüber E, Strober W: **The T cell-B cell interaction via OX40-OX40L is necessary for the T cell-dependent humoral immune response.** *J Exp Med* 1996, **183**:979-989.

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The authors find that CD40 ligation induces OX40L/CD134L expression on both B lymphocytes and dendritic cells. Treating mice with a blocking anti-CD134L monoclonal antibody leads to suppression of memory T cell responses and antigen-specific IgG responses. Similar results are seen in CD134L<sup>-/-</sup> mice. The results suggest a biologic role for interactions between CD134 and CD134L in T cell mediated B cell activation.

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This study shows that stimulation of B cells through CD134L does not itself induce IgG production but can enhance IgG induced through CD40 and cytokine stimulation. Results complement those described in the annotation to [45\*\*].

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Experiments performed with 4-1BBL/CD137L<sup>-/-</sup> mice reveal specific defects in cytotoxic T cell responses to alloantigen and influenza virus; however, the response to a second virus, lymphocytic choriomeningitis virus, is normal, as are neutralizing antibody responses to a third virus, vesicular stomatitis virus. It may thus be that the primary role of CD137L is as a costimulus for weaker T cell responses.

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