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An essential role for tyrosine kinase in the regulation of Bruton's B-cell apoptosis

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ABSTRACT **Mutations of the *Bruton's tyrosine kinase* (*btk*) gene cause X-linked agammaglobulinemia (XLA) in humans and X-linked immune deficiency (Xid) in mice. To establish the BTK role in B-cell activation we examined the responses of wild-type and Xid B cells to stimulation through surface IgM and CD40, the transducers of thymus independent-type 2 and thymus-dependent activation, respectively. Wild-type BTK was necessary for proliferation induced by soluble anti-IgM (a prototype for thymus independent-type 2 antigen), but not for responses to soluble CD40 ligand (CD40L, the B-cell activating ligand expressed on T-helper cells). In the absence of wild-type BTK, B cells underwent apoptotic death after stimulation with anti-IgM. In the presence of wild-type but not mutated BTK, anti-IgM stimulation reduced apoptotic cell death. In contrast, CD40L increased viability of both wild-type and Xid B cells. Importantly, viability after stimulation correlated with the induced expression of bcl-x_L. In fresh *ex vivo* small resting B cells from wild-type mice there was only barely detectable bcl-x_L protein, but there was more in the larger, low-density ("activated") splenic B cells and peritoneal B cells. *In vitro* Bcl-x_L induction following ligation of sIgM-required BTK, was cyclosporin A (CsA)-sensitive and dependent on extracellular Ca^{2+}. CD40-mediated induction of bcl-x required neither wild-type BTK nor extracellular Ca^{2+} and was insensitive to CsA. These results indicate that BTK lies upstream of bcl-x_L in the sIgM but not the CD40 activation pathway. bcl-x_L is the first induced protein to be placed downstream of BTK.**

In humans, any of several mutations of the *Bruton's tyrosine kinase* (*btk*) gene cause X-linked agammaglobulinemia (XLA) (1, 2). In mice, a point mutation of *btk* causes X-linked immune deficiency (Xid) (3, 4). In XLA, B-cell development is blocked at the time of transition from pro-B to pre-B cells and very few B cells are produced (5). In mice with Xid, mature B cells are generated, but they have multiple functional defects (reviewed in ref. 6): a reduced number of B cells; decreased production of M and G_3 immunoglobulins; holes in the immunoglobulin repertoire including a lack of natural autoantibodies; loss of the B cells that are responsible for the production of these antibodies ($CD5^+$ or B-1a cells); and, while they make antibodies in response to thymus-dependent (TD) antigens they make none to thymus independent-type 2 repeating unit (TI-2) antigens, such as polysaccharides and their haptenated derivatives. Xid B cells do not proliferate *in vitro* in response to anti-immunoglobulin, which, like TI-2 antigens, activates by ligating and cross-linking surface Ig.

The role of BTK in normal B cells is unknown, hence the pathogenesis of XLA and Xid remain obscure. The enzyme belongs to a small family of cytoplasmic tyrosine kinases that have pleckstrin homology domains. This family includes the T-cell enzyme Tsk (or Itk) and the enzyme TecII, (reviewed in ref. 7). Whereas several of the *btk* mutations found in humans eliminate kinase activity, the Xid form of BTK retains *in vitro* activity (4). Significantly, deletion of the kinase domain from murine BTK still results in the Xid phenotype (8, 9); that is, the difference between the human and murine phenotypes is not due to difference in the mutations. Normal BTK function requires functioning kinase and pleckstrin domains. Deletional mutation of *btk* in a chicken cell line blocks B-cell receptor-mediated activation of phospholipase C and the Ca^{2+} flux (10).

To define the role of BTK in the activation of murine B cells we examined the responses of B cells from wild-type and Xid mice to activation via sIgM or CD40. Activation through sIgM by $F(ab')_2$ fragments of anti-IgM is a model for T independent type II stimulation, as occurs with polysaccharide antigens (11). Activation through CD40 with a soluble fusion protein containing CD40 ligand (CD40L) and CD8 provides a signal normally transmitted by activated T-helper cells (12). We were interested to see that wild-type and Xid B cells differed greatly in their responses to sIgM, but not CD40 signaling. Further experiments were conducted to establish the basis for the observed differences.

MATERIALS AND METHODS

Cells. Small resting splenic B cells were purified from CBA/HHW, CBA/HHW.IgH^b, CBA/HHW.xid, and CBA/HHW.IgH^b.xid as described (13). To measure proliferation, cells were seeded at a density of 1×10^6 cells/ml in 96-well culture plates. Polyclonal goat anti-mouse IgM $F(ab')_2$ μ specific antibody (Jackson ImmunoResearch) was used at 10 μg/ml, CD40L:CD8 fusion protein produced as a hybridoma supernatant was added as described (13) at a final dilution of 1:2, and rIL-4 was added at 200 units/ml. In the final 16 hr of culture, 5 μCi/ml (1 Ci = 37 GBq) of [^{3}H]thymidine (ICN) was added. The cells were harvested and the level of [^{3}H]thymidine incorporation determined. Cell viability was determined by the exclusion of propidium iodide. Propidium iodide was added to samples to a final concentration of 1 μg/ml, and the uptake of propidium iodide by B cells was determined on a Becton Dickinson FACScan using LYSYS II software and analyzed with WINLIST (Verity Software House, Topsham, ME) software. Cell cycle analyses were performed by propidium iodide staining of DNA (14). Data were acquired on a Becton Dickinson FACScan as described previously.

Western Blot Analysis of bcl-x_L. Small high-density resting- and low-density activated-splenic B cells and peritoneal B cells were isolated and stimulated as described (13). For stimulation, 7×10^6 small resting splenic B cells were used for each treatment, seeded into 25-cm^2 culture flasks at 1×10^6/ml. Phorbol 12-myristate 13-acetate (PMA), ionomycin, and thapsigargin (Sigma) were used at 30 nM, 1 mM, and 30 nM, respectively. Following stimulation (at 17 hr except where indicated), dead cells were removed by centrifugation through Lympholyte M for 10 min at $600 \times g$. B-cell progenitors were obtained from bone marrow by sorting on a flow cytometer and selecting for $CD45(B220)^+$, $sIgM^-$ cells. Live cells (eosin dye excluding) were counted on a

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Abbreviations: BTK, Bruton's tyrosine kinase; Xid, X-linked immune deficiency; TD, T-cell dependent; TI, T-cell independent; CsA, cyclosporin A; CD40L, CD40 ligand; XLA, agammaglobulinemia.

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hemocytometer then lysed with 60 μl/10^6 cells of lysis buffer (1.2% Nonidet P-40/150 mM NaCl/25 mM HEPES/5 mM NaF/0.5% deoxycholate/1 mM phenylmethylsulfonyl fluoride) for 10 min on ice. The lysates were spun for 10 min at 12,000 × *g* to remove debris, and the supernatants taken and stored at −20°C. For Western blot analysis SDS/15% PAGE gels were prepared and 20 μl of cell lysate (equivalent to 3×10^5 live cells) in 20 μl of reducing sample buffer was run per lane. The gels were blotted onto nitrocellulose (Bio-Rad), and the blots blocked with Dulbecco's phosphate-buffered saline (DPBS) containing 5% dried milk. They were probed with 1:500 anti-bcl-x_L (Transduction Laboratories, Lexington, KY) in DPBS/0.1% BSA, followed by a $1:10^4$ goat anti-mouse H + L-HRP conjugate (Boehringer Mannhiem), and a signal was obtained using the Amersham chemiluminescence kit. Each Western blot shown is representative of at least three experiments.

RESULTS

Small resting B cells from wild-type mice proliferated in response to either $F(ab')_2$ anti-IgM or CD40L (Fig. 1*a*). Proliferation in response to the combination of anti-IgM and CD40L was the same or modestly greater than with either alone (Fig. 1*a*). In contrast, B cells from Xid mice responded to CD40L and to CD40L plus anti-IgM, but not to anti-IgM alone (Fig. 1*c*). Thus, mutation of BTK did not prevent proliferation induced via CD40. Other studies have reported similar results (9, 15, 16), but this is not a consistent finding (17). A proliferative response to CD40L was not unique to CBA/HHW.xid mice because we obtained similar results in experiments with cells from CBA/J.xid and CBA/N mice (data not shown). To determine whether the proliferation-inducing element in the CD40L–CD8 fusion protein containing supernatant was in fact CD40L we did additional experiments in which we found that an anti-CD40L antibody blocked proliferation, that supernatants from nontransfected plasma cells failed to induce proliferation, and that proliferation of wild-type and Xid B cells was induced by antibody to CD40 (data not shown).

We confirmed that Xid B cells demonstrate proliferation in response to anti-IgM when stimulated in the presence of IL-4 (18) (Fig. 1*d*). Because IL-4 by itself induced no thymidine incorporation, but allowed Xid B cells to respond to anti-IgM, it appears that in Xid B cells sIgM transduces at least a portion of the signals necessary for the transit of S. As reported, mutation of BTK still permits some signaling through sIgM (19, 20) enabling Xid B cells to enter G_1.

Xid B cells that failed to incorporate thymidine in response to stimulation with anti-IgM could either be dead or arrested in cycle. Indeed, a Xid-associated deficit of bcl-2 and an increase in apoptosis has been reported (15). We compared the viability of wild-type and Xid B cells in culture using the ability to exclude propidium iodide as the criterion of viability. We found that stimulation with anti-IgM improved the viability of wild-type cells (Fig. 2*a*), whereas the same treatment allowed, or increased, Xid B-cell death (Fig. 2*d*). CD40L and CD40L plus anti-IgM increased the viability of both normal and wild-type B cells. Our interpretation is that anti-IgM-treated wild-type B cells are rescued from death, whereas similarly treated Xid B cells die unless rescued by IL-4 (21) or CD40L. According to this view, following an activation-inducing event an arrest of entry into a death pathway is necessary for cells to transit S. We cannot rule out the alternative possibility that it is arrest in cell cycle that induces programmed cell death of these cells. However, for reasons addressed below we do not think this is likely.

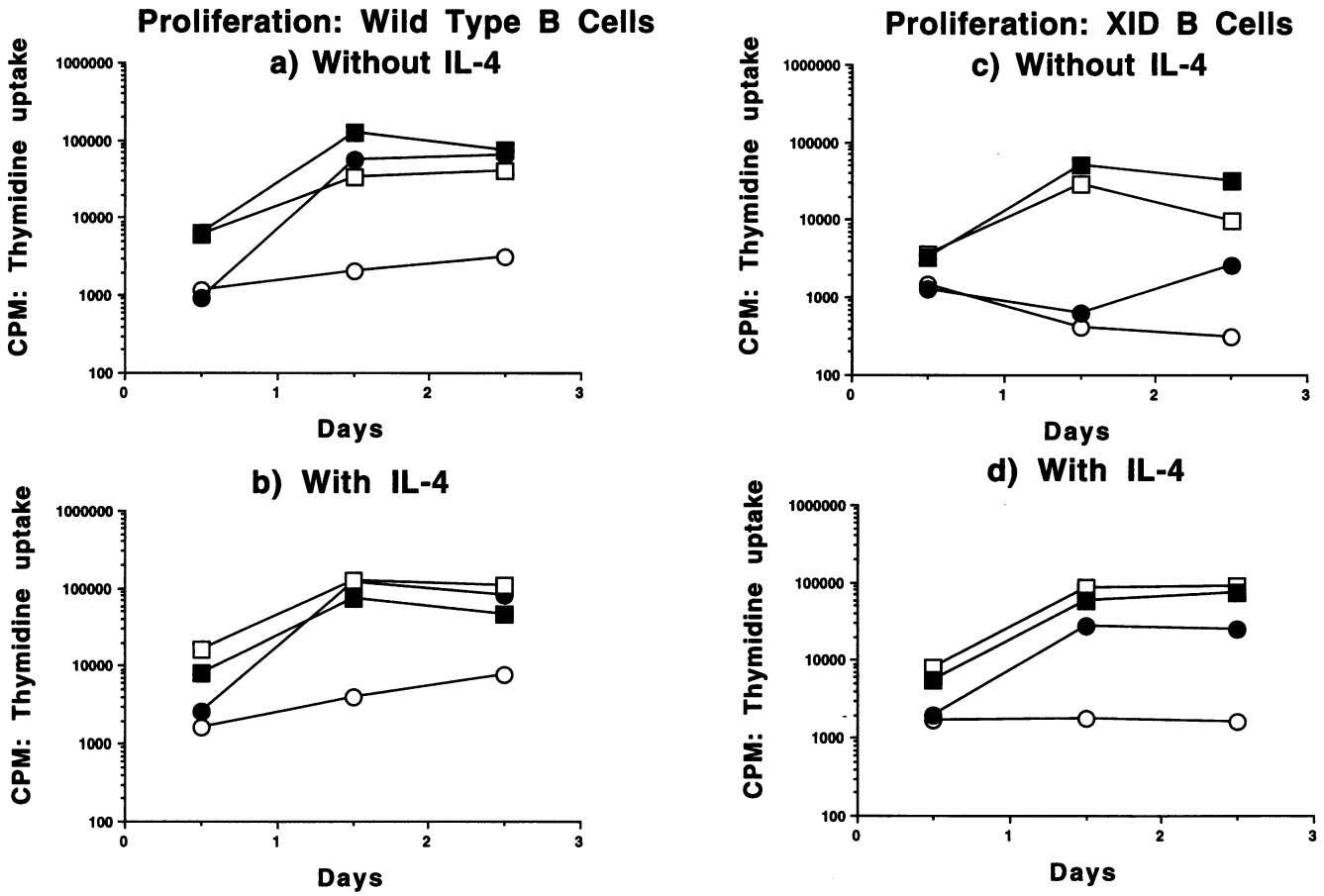

FIG. 1. B-cell proliferation in response to TI-2 or TD type stimuli in the absence and presence of rIL-4. (*a* and *b*) Wild-type B cells in the absence and presence of rIL-4, respectively. (*c* and *d*) Xid B cells in the absence and presence of rIL-4, respectively. (○), medium control; (●), anti-IgM; (□), CD40L; (■), anti-IgM plus CD40L. Each data point is the log mean of triplicate wells and the figures are representative of at least three experiments. Standard errors were less than ×/÷ 1.5.

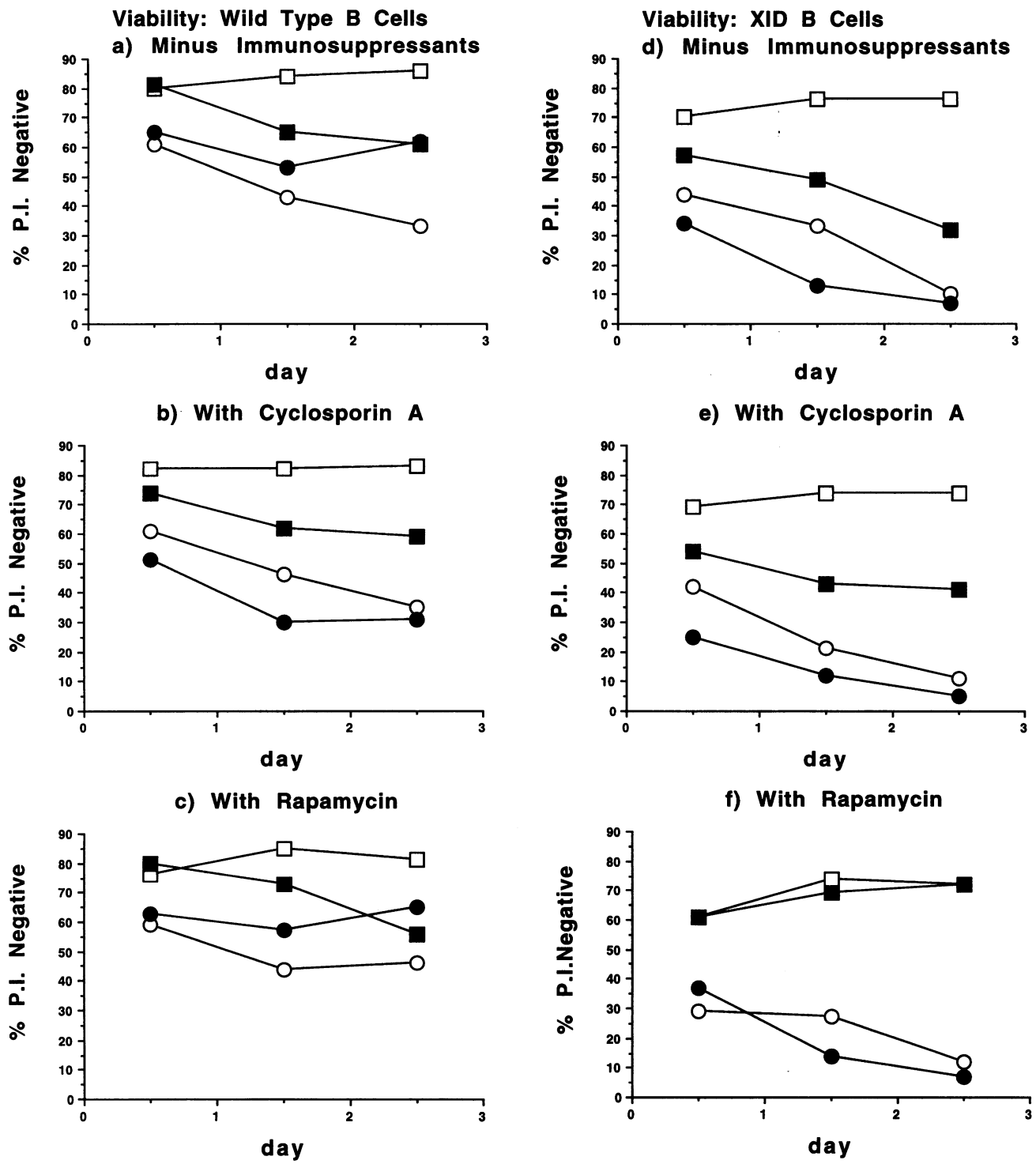

FIG. 2. B-cell viability following TI-2 or TD type stimulation in the absence or presence of CsA or rapamycin. (*a–c*) Wild-type B cells. (*d–f*) Xid B cells. *a* and *d* are in medium, *b* and *e* in the presence of CsA, *c* and *f* in the presence of rapamycin. (○), medium control; (●), anti-IgM; (□), CD40L; (■), anti-IgM plus CD40L. Small resting B cells were stimulated as described either alone or with CsA or rapamycin at 3 nM and 3 ng/ml, respectively (13).

Previously, we found that CsA blocked the anti-IgM-induced proliferation of wild-type B cells and that CD40L-induced proliferation was less sensitive to this reagent (13). We asked whether CsA also blocked the activation-induced rescue from death. With wild-type cells treatment with anti-IgM in the presence of CsA increased death (Fig. 2*b*), but no decrease (or increase) in dead cells was seen with Xid B cells treated in the same fashion (Fig. 2*e*). CsA did not adversely affect the marked increase in viability induced by CD40L (Fig. 2 *b* and *e*). In experiments not shown we found that in a medium containing 0.3 mM EGTA B cells would proliferate normally in response to CD40L, but not at all to anti-IgM. Addition of 0.1 or 0.03 mM bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetra-acetate, an intracellular chelator of Ca^{2+} (22), was equally effective at blocking anti-IgM and CD40L-induced proliferation. Therefore, there are at least two surface receptor-mediated pathways to increased viability; one is initiated by ligation of sIgM, is dependent on BTK and extracellular Ca^{2+}, and is sensitive to CsA, whereas the other is triggered by ligation of CD40, is independent of BTK and extracellular Ca^{2+}, and is CsA resistant. Unlike CsA, rapamycin did not cause increased

death of either wild-type or Xid B cells regardless of culture conditions (Fig. 2 *c* and *f*). However, rapamycin did cause cell cycle arrest. At the higher dose (3 ng/ml) rapamycin was more effective at blocking entry into S but still did not cause increased death (Fig. 3 and Table 1). Therefore, in the presence of rapamycin, primary B cells that are arrested in cycle remain viable. Either cell cycle arrest caused by rapamycin (23–25) is not a sufficient signal for entry into a death pathway or the death pathway is also blocked by this reagent.

To assess whether death occurred by apoptosis we fixed B cells at various times after activation, stained the DNA with propidium iodide, and examined the cells by flow cytometry. Cells were determined to be apoptotic (i.e., to have sub-G_0 amounts of DNA), to be at G_0–G_1, or to be in S–G_2–M. The analytic histograms obtained at 42 hr of culture are shown in Fig. 3 and a summary of the apoptosis and cell cycle analyses at 16, 28, and 42 hr is presented in Table 1. Anti-IgM induced wild-type cells to enter S (Fig. 3 top row), but increased the fraction of apoptotic Xid cells (Fig. 3 second row). CD40L reduced the fraction of apoptotic cells and increased cells in S regardless of the strain of origin (Fig. 3 first and second rows). There was more apoptosis, as well as entry into S, when anti-IgM was used in combination with CD40L than with CD40L alone. The increase in apoptosis was greater for the Xid cells. Rapamycin reduced the fraction of cells in S–G_2–M and increased the numbers in G_0-G_1. Fig. 3 third and fourth rows contains the DNA histograms at 42 hr for wild-type and Xid B cells treated with 3 ng/ml, and the data are presented in Table 1.

From these experiments we concluded that the induction of DNA synthesis in B cells was associated with an increase in viability and a decrease in apoptosis. We thought it possible that this correlation could be explained if activation induced the expression of bcl-2 or bcl-x_L, molecules that are known to block programmed cell death (26, 27). Expression of Bcl-2 itself is reported to be inversely correlated with T- and B-cell activation (28). We were therefore particularly interested in bcl-x_L, a protein that is both capable of blocking apoptosis and known to be expressed in activated T cells (29) and human B cells (30). We found that in fresh *ex vivo* small resting B cells there was only barely detectable bcl-x_L protein, but in the larger, low-density ("activated") splenic B cells and peritoneal B cells more bcl-x_L was found (Fig. 4*a*). Bcl-x_L was markedly elevated in pre-B cells of both wild-type and Xid mice (Fig. 4*b*). Thus, *in vivo*, elevated bcl-x_L correlated with proliferation or activation.

Bcl-x_L levels in wild-type B cells increased markedly in response to either anti-IgM or CD40L (Fig. 4*c*, lanes 2 and 3), but only CD40L induced this increase in Xid B cells (Fig. 4*c*, lane 5). By itself IL-4 caused no change in the amount of bcl-x_L, but used in combination with anti-IgM, IL-4 induced elevated amounts of bcl-x_L, particularly in Xid B cells (Fig. 4*d*).

Rapamycin did not block the induction of bcl-x_L by either anti-IgM or CD40L, but 0.7 mM EGTA blocked its induction

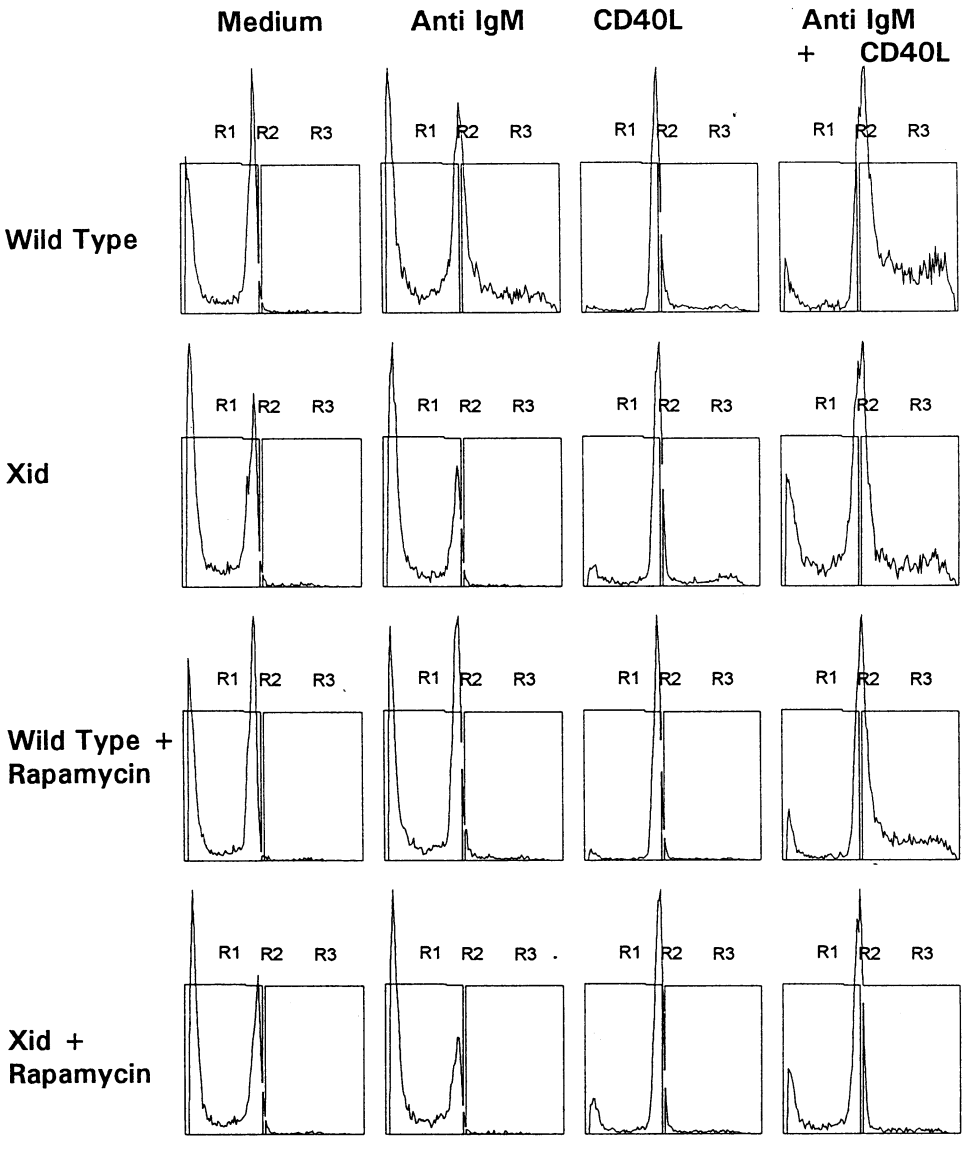

FIG. 3. Cell cycle analysis of B cells following TI-2 or TD type stimulation in the absence or presence of rapamycin. The top and third rows represent wild-type B cells in the absence and presence of 3 ng/ml of rapamycin, respectively. The second and bottom rows are Xid B cells in the absence and presence of rapamycin respectively. For all rows the first column is medium control, the second anti-IgM, the third CD40L, and the fourth anti-IgM plus CD40L. Regions R1, R2, and R3 encompass sub G_1, G_0–G_1 and S–G_2 cells. B cells were stimulated as above.

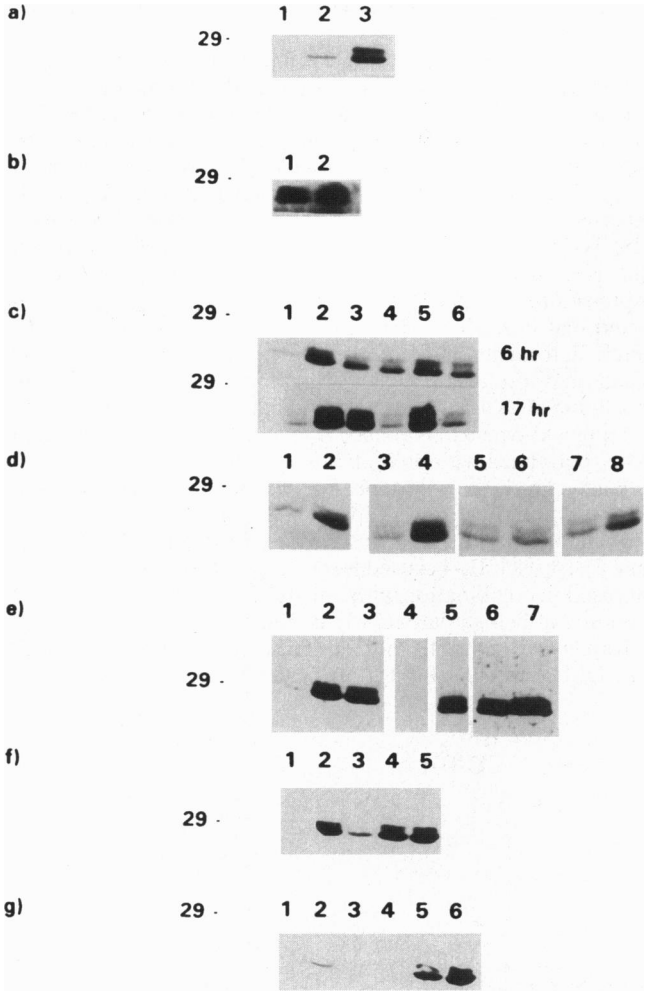

FIG. 4. Western blot analysis of bcl-x_L expression in B cells. (*a*) Untreated *ex vivo* CBA B cells. Lanes: 1, small resting B cells; 2, activated B cells; 3, peritoneal B cells. (*b*) Untreated *ex vivo* cells. Lanes: 1, CBA pre-B cells; 2, Xid pre-B cells. (*c*) Lanes 1–3, CBA B cells and lanes 4–6, Xid B cells. Lanes: 1 and 4, medium control; 3 and 6, anti-IgM; 2 and 5, CD40L. (*d*) Lanes 1–4, CBA B cells and lanes 5–8, Xid B cells. Lanes: 1 and 5, medium control; 2 and 6, anti-IgM; 3 and 7, IL-4; 4 and 8, anti-IgM plus IL-4. (*e*) CBA B cells. Lanes: 1, medium control; 2, anti-IgM; 3, CD4OL; 4, anti-IgM plus EGTA; 5, CD40L plus EGTA; 6, anti-IgM plus rapamycin; 7, CD40L plus rapamycin. (*f*) CBA B cells. Lanes: 1, medium control; 2, anti-IgM; 3, anti-IgM plus CsA; 4, CD40L; 5, CD40L plus CsA. (*g*) CBA B cells. Lanes: 1, medium control; 2, PMA; 3, ionomycin; 4, thapsigargin; 5, PMA plus ionomycin; 6, PMA plus thapsigargin.

by anti-IgM (Fig. 4*e*). CsA blocked induction of bcl-x_L by anti-IgM, but not CD40L (Fig. 4*f*). Only a small increase in bcl-x_L was seen in cells treated with phorbol ester alone. Phorbol ester used in combination with either ionomycin or thapsigargin, agents that induce a capacitive calcium flux, induced large amounts of bcl-x_L protein (Fig. 4*g*).

DISCUSSION

Wild-type B cells responded to either anti-IgM or CD40L by expressing increased amounts of bcl-x_L protein and entering into DNA synthesis. In Xid B cells CD40L induced bcl-x_L and DNA synthesis, but anti-IgM failed to induce bcl-x_L or DNA synthesis, and apoptotic cells appeared with the same frequency. Our interpretation is that wild-type BTK lies in the sIgM signal pathway and is therefore necessary for bcl-x_L induction via sIgM. CD40 ligation triggers an alternative pathway that does not require BTK.

Alternatively, there is the possibility that BTK function is necessary at a critical junction of B-cell development. Absent this function, a lineage of B cells is created that can signal effectively through CD40 but not sIgM. We think it is likely that BTK is important in the signal pathway because it is phosphorylated in response to sIgM-mediated signals (31, 32). Other evidence supporting this role for BTK is that deletion of BTK from a transformed mature chicken B cell line blocks activation through the B-cell receptor (10). Furthermore, this lineage hypothesis does not readily explain the observations of Tullia Lindsten and Craig Thompson (personal communication) who found that Xid mice transgenic for bcl-x_L have increased numbers of B cells with the typical wild-type phenotype of low IgM and high IgD. Critically, these B cells proliferate in response to anti-IgM. This observation contrasts with published experiments of Woodland *et al.* (33) who found that anti-IgM stimulated B cells of bcl-2 transgenic Xid mice do not proliferate.

Table 1. Cell cycle progression and apoptosis in response to stimulation

	Wild type, %			Xid, %		
Treatment	Sub G_0	G_0–G_1	S–G_2–M	Sub G_0	G_0–G_1	S–G_2–M
Medium, 16 hr	29 (22)	70 (77)	1 (1)	42 (50)	53 (47)	5 (4)
Anti-IgM, 16 hr	22 (21)	74 (76)	5 (2)	44 (46)	52 (50)	4 (3)
CD40L, 16 hr	6 (5)	88 (92)	6 (3)	17 (15)	72 (80)	11 (6)
CD40L and anti-IgM, 16 hr	6 (7)	82 (88)	13 (5)	15 (18)	66 (75)	19 (7)
Medium, 28 hr	37 (43)	62 (56)	1 (2)	65 (56)	28 (42)	2 (3)
Anti-IgM, 28 hr	35 (40)	60 (57)	5 (2)	72 (55)	24 (43)	2 (2)
CD40L, 28 hr	8 (8)	80 (87)	12 (4)	15 (18)	65 (80)	7 (2)
CD40L and anti-IgM, 28 hr	10 (10)	44 (74)	46 (16)	31 (26)	59 (71)	12 (4)
Medium, 42 hr	51 (55)	46 (44)	3 (2)	65 (64)	33 (34)	2 (2)
Anti-IgM, 42 hr	44 (51)	30 (45)	26 (4)	72 (74)	26 (24)	1 (2)
CD40L, 42 hr	7 (9)	78 (88)	15 (4)	15 (20)	72 (75)	13 (5)
CD40L and anti-IgM, 42 hr	12 (12)	25 (44)	64 (43)	31 (29)	36 (63)	33 (8)

Percent of total B cells found to be sub G_0 (apoptotic), in G_0 and/or G_1 or in S–G_2–M. Data in parentheses represent values obtained from cells cultured in the presence of 3 ng/ml of rapamycin. One of two experiments with similar results.

The sIgM, BTK-mediated pathway of bcl-x_L induction requires external calcium and is CsA sensitive but rapamycin insensitive. This strongly suggests that the pathway is calcineurin dependent. In contrast, the CD40 pathway requires neither external calcium nor BTK and is CsA insensitive.

While IL-4 by itself was not capable of inducing bcl-x_L it did supplement a BTK-independent portion of the pathway activated by anti-IgM to allow induction, showing that a T-cell generated interleukin can rescue Xid B cells. This is interesting because when Xid mice are deprived of T cells, by surgery (34) or coexpression of the mutation *nude* (35), B-cell development is blocked at the pre-B cell stage of development, as in human XLA. Deletional mutation of bcl-x_L also restricts murine B-cell development at the pre-B cell stage (36). It is therefore possible that in *btk* mutant mice a T-cell dependent factor or ligand allows induction of bcl-x_L expression in pre-B cells, but in humans with comparable mutations this rescue path is not available. This would account for the difference in phenotype between Xid in mice and XLA in humans.

In vivo, bcl-x_L levels are elevated in B cells known to be activated and/or proliferating (pre-B cells, large low-density cells, germinal center B cells, and peritoneal B-1a cells) but little, if any, bcl-x_L was seen in small high-density "resting" B cells. In contrast, bcl-2 levels are reported to be high in resting

B cells (26, 27). With each of the several variables we examined (*btk* allele, ligand, addition of IL-4, sensitivity to CsA, response to phorbol ester, ionomycin and thapsigargin, and requirement for extracellular Ca^{2+}) induction of bcl-x_L was correlated with viability and proliferation. Taken together these results suggest that the ability of *ex vivo* B cells to survive during periods of activation depends on their ability to generate bcl-x_L.

Mice with deletional mutations of *vav* resemble Xid mice in that they fail to generate B-1a cells, are deficient in IgM and IgG_3, and have B cells that respond to CD40L but not anti-IgM (37, 38). It will be interesting to see whether B cells from these animals express elevated levels of bcl-x_L in response to either signal. Other studies show that newly arising wild-type B cells fail to activate the *src* family kinases FYN and FGR in response to sIgM crosslinking (39). These newly emergent cells are also induced to enter programmed cell death following sIgM ligation (40, 41). In both respects these newly arising B cells differ from more mature B cells. Again there is the possibility that these kinases (and possibly BTK and VAV) lie along a bcl-x_L induction path. That is, the induction of bcl-x_L and transit through the cell cycle might be separate branches of the activation pathway (42). Proliferation and progression might require the integrity of both branches.

Our results suggest the possibility that following an activation signal primary B cells do not transit S unless they express bcl-x_L. While it is also possible that it is the failure to enter or transit S in response to an activation signal that commits a cell to programmed cell death, we do not think that this is likely. First, we see increased levels of bcl-x_L as early as 6 hr after stimulation, but B cells do not enter S until at least 16 hr after stimulation (20). Second, rapamycin treatment causes B-cell arrest prior to S (see refs. 23–25) without increasing the amount of cell death or apoptosis (Fig. 3 and Table 1). Also, rapamycin-induced cell cycle arrest blocked neither the induction of bcl-x_L (Fig. 4) nor apoptotic death (Fig. 3 and Table 1). Together, these results support the idea that in primary B cells expression of bcl-x_L is required for entry into S. It will be interesting to see if this is true. The reciprocal expression of bcl-2 and bcl-x_L in resting and proliferating lymphocytes (see refs. 27 and 28) raises the provocative possibility that whereas both molecules can act to prevent apoptosis, they differ in function such that expression of the latter is necessary for cells to progress following activation.

We consider it likely that because Xid B cells produce bcl-x_L in response to activation by T-helper cells (through CD40), they are able to mount effective TD responses. In contrast, because they do not produce bcl-x_L in response to sIgM ligation, Xid B cells are deleted following this form of stimulation. We propose that by this mechanism TI-2 type antigens induce apoptosis and clonal deletion of Xid B cells. We further propose that in wild-type mice it is autoantigenic stimulation via sIgM that induces autoantibody producing, $CD5^+$, B-1a B cells. This is supported by our earlier observation that CD5 expression can be induced on wild-type B cells by sIgM but not CD40 ligation (13). According to this schema, the absence of B-1a cells and natural autoantibodies in Xid mice is a consequence of the lack of bcl-x_L expression and the resultant apoptotic deletion of cells stimulated by autoantigen ligation of sIg. Although this hypothesis remains to be tested and bcl-x_L might be necessary but not sufficient for cell cycle progression (43) our finding that the induced expression of bcl-x_L lies downstream of BTK provides the first demonstration of a specific role for this kinase.

Note. After submission, Choi *et al.* (44) reported abonormalities of bcl-x_L expression in Xid B cells and Grillot *et al.* (45) described a *bcl-x* transgenic mouse.

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