CD27 and CD40 Inhibit p53-independent Mitochondrial Pathways in Apoptosis of B Cells Induced by B Cell Receptor Ligation*

Hidenori Hasé‡, Yumiko Kanno‡, Hidefumi Kojima‡, Chikao Morimoto§, Ko Okumura‡, and Tetsuji Kobata‡

Received for publication, September 4, 2002, and in revised form, September 23, 2002
Published, JBC Papers in Press, September 24, 2002, DOI 10.1074/jbc.M209050200

B cells in the germinal center are known to undergo apoptosis after B cell receptor (BCR) ligation, a process relevant to immunological tolerance. Human CD27 is a B cell co-stimulatory molecule. The aim of this study was to compare the effects of CD27 and CD40 signals on BCR-mediated apoptosis of B cells. BCR ligation activated mitochondrial apoptotic pathways including down-regulation of Bcl-XL, dissipation of mitochondrial transmembrane potential, release of cytochrome c, and activation of caspase-9. Each of these effects was significantly inhibited by CD27 and CD40. Bik expression was weakly but significantly down-regulated by CD27 but up-regulated by CD40. BCR ligation resulted in p53 activation including its phosphorylation at Ser15, nuclear translocation, and target gene p53AIP1 induction. CD27 and CD40 clearly suppressed these processes. Analyses that used dominant-negative p53 variants revealed a low but still substantial level of BCR-mediated apoptosis and intact mitochondria-mediated apoptotic pathway. These pathways were further inhibited by CD27 and CD40, although the cells showed no p53 phosphorylation or p53AIP1 expression. Our results suggested that, at the mitochondrial level, CD27 and CD40 co-stimulatory signals regulated the p53-amplified apoptotic pathway in B cells through the inhibition of p53-independent apoptotic pathway primarily induced by BCR ligation.

B cell fate is controlled by signals through the B cell receptor (BCR) on the cell surface. BCR signaling initiates receptor editing, apoptosis, anergy, and immune response (1). The nature of the antigen (Ag)-induced response depends on the developmental stage of the responding cell, the avidity of the Ag-BCR interaction, and the availability of co-stimulatory signals from helper T cells. This differential responsiveness to Ag signals is important to the functional repertoire and results in immunological tolerance (2). The human Burkitt lymphoma cell line, Ramos, expresses surface IgM as BCR, and engagement of this BCR with an anti-IgM antibody (Ab) induces apoptotic cell death. Because of these features, Ramos cells have been used extensively as a model of clonal deletion of B cells in the germinal center, and Ramos cells facilitate the dissection of molecular and biochemical pathways that lead to cell death (3, 4).

Accumulating evidence suggests that BCR ligation activates the mitochondria-mediated apoptotic pathway (5–8). General agreement exists that alterations in the ratio of proapoptotic to antiapoptotic members of the Bcl-2 family can protect against the initiation or progression of apoptosis by modulating mitochondrial membrane permeability (9). Previous studies have reported that overexpression of antiapoptotic protein Bcl-XL prevents BCR-mediated apoptosis (10, 11) and that BCR ligation induces proapoptotic protein Bik (12).

On the other hand, a growing number of gene products have been identified as components of the machinery that leads to cell death. Among these, the tumor suppressor gene p53 is of particular interest. The p53 protein plays important roles in the control of cell death as well as cell-cycle inhibition (13, 14). The precise molecular mechanism(s) through which p53 exerts these effects is not clear but seems to depend on the ability of p53 protein to act as a transcription factor. Recently one of the important p53 transcriptional target genes was found to be p53-regulated apoptosis-inducing protein 1 (p53AIP1) (15). p53AIP1 is localized within the mitochondria and plays a direct role in the mediation of p53-dependent apoptosis. Although it was reported that in the murine B lymphoma cell line WEHI-231, p53 and the p53 target gene, cyclin-dependent kinase inhibitor p21WAF1/CIP1, are involved in BCR-mediated apoptosis (16), whether BCR-mediated signaling primarily activates p53 remains unresolved.

Among a number of cell surface molecules involved in T-B cell cooperation and subsequent B cell fate, the B cell molecule CD40, a member of the TNFR family, mediates a co-stimulatory signal and plays a pivotal role in B cell proliferation, memory B cell differentiation, and B cell survival (3, 17, 18). Previous studies have shown that Bcl-2 and Bcl-XL are involved in CD40-mediated rescue from BCR-mediated apoptosis (17–20). CD27 is also a member of the TNFR family, and we have previously reported that this molecule mediates a co-stimulatory signal for B cell proliferation and plasma cell differentiation in humans (21, 22). Although CD27 signaling, in addition to CD40 signaling, induces activation of nuclear factor κB (NF-κB) (18, 23), the role of CD27 signaling in BCR-mediated apoptosis and p53 activation is still obscure. In the present study we offer evidence for the crucial roles of both CD27...
and CD40 signals in human BCR-mediated apoptosis. Our results showed that p53 is secondarily activated and amplifies the apoptotic cascade that is regulated by both CD27 and CD40 signals at the mitochondrial level.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—The following Abs were used in this study: Bax (Medical & Biological Laboratories, Nagoya, Japan), Bcl-XL, caspase-3, cytochrome c, poly(ADP-ribose) polymerase (PARP), p53 (BD Pharmingen, San Diego, CA); caspase-9, phospho-p53 (Ser15) (Cell Signaling Technology, Beverly, MA); NBK (Bik), Bcl-2, histone H4 (Santa Cruz Biotechnology, Santa Cruz, CA); cytochrome oxidase type IV (COV IV; MorphoSys, Munich, Germany); p53AIP1 (Abcam); phospho-p53 (Ser15) (Cell Signaling Technology); caspase-9, phospho-p53 (Ser15) (Cell Signaling Technology); and CD40L (R&D Systems). All other Abs were purchased from Human Science Research Resources Bank (Osaka, Japan) and grown in RPMI 1640 medium supplemented with 25 mm Hepes, 10% fetal calf serum, 2 mm l-glutamine, 1 mm sodium pyruvate, 5.5 × 10^{-2} mm 2-mercaptoethanol, 100 units/ml penicillin, and 100 units/ml streptomycin (all from Invitrogen). Cells were treated with anti-IgM Ab (Sigma) or control Ab (goat IgG, Sigma) to a final concentration of 10 μg/ml. For co-culture with cells transfected with human CD154 gene, CD70 gene, or vector alone (22), 1 × 10^{8} Ramos cells were preincubated with varying amounts of transfectants treated with mitomycin C (Sigma) in 96-well, round-bottomed plates. After incubation for 30 min, anti-IgM Ab was added to the culture and cells were harvested at 24 h. The caspase inhibitors zVAD-fmk and zLEHD-fmk were obtained from Calbiochem. For treatment with caspase inhibitors, 1 × 10^{8} Ramos cells were preincubated with serial dilution of zVAD-fmk or zLEHD-fmk in 96-well, round-bottomed plates. After the incubation for 30 min, anti-IgM Ab was added to the culture and cells were harvested at 24 h.

**Detection of Apoptotic Cells by Using Fluorescein Isothiocyanate-labeled Annexin V**—To detect apoptotic cells, phosphatidylserine exposure was measured by using an annexin V-fluorescein isothiocyanate apoptosis detection kit according to the instructions provided by the manufacturer (BD Biosciences). Propidium iodide was added just before analysis on a FACSCalibur (BD Biosciences).

**Subcellular Fractionation and Immunoblot Analysis**—Nuclear protein extraction was performed as described previously (15) except for the use of digitonin instead of Nonidet P-40. Briefly, cells washed in phosphate-buffered saline were resuspended in cold TKM10-10°C (10 mm Tris-HCl, pH 7.6, 10 mm KCl, and 5 mm MgCl2) with protease inhibitors. For whole cell extractions, the cell pellets washed in phosphate-buffered saline were resuspended with 0.5% SDS solution and boiled for 5 min. Proteins (4 μg) were separated on a 7.5% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk, and immunoblotted with arbitrary Abs as described previously (15). The thermal cycling conditions included 50°C for 2 min and 95°C for 10 min followed by 40 cycles of amplification at 95°C for 15 s and 60°C for 1 min for denaturing and anneal-extension, respectively.

**Construction of Transfected Cell Lines**—The p53 dominant-negative construct, in which cysteine at position 135 in the p53-binding site is mutated to tyrosine (C135T), abrogates the endogenous p53 wild-type function (25). To construct a p53DN-expression vector, a cDNA that encoded full-length p53DN protein was subcloned from pCMV-p53mt135 vector (Clontech, Palo Alto, CA) into pBluescript SK (+) (Stratagene, La Jolla, CA) and finally into the pBCMGSneo expression vector (26) at the XhoI and NotI sites, generating a clone termed pBCMGS-p53DN. pBCMGS-p53WT was also constructed. Ramos cells were electroporated with pBCMGS-p53DN, pBCMGS-p53WT, or pBCMGSneo alone. The human CD70 and CD154 and the vector alone (mock)-transfected cells in the murine pre-B 300-19 cell line have been described previously (22).

**Statistical Analysis**—The paired Student’s t test was used to determine the statistical significance of the data. Values of p < 0.05 were considered significant.

**RESULTS**

**CD27 and CD40 Inhibit the Mitochondria-mediated Apoptotic Pathway Activated by BCR Ligation**—BCRs on Ramos cells ligated with anti-IgM Ab displayed their apoptotic figures as annexin V-positive cells. Apoptotic cells started to appear at 6 h. These cells constituted approximately 40% of the cells at 24 h and approximately 50% at 48 h (Fig. 1A). We then compared the effects of CD27 and CD40 signals on BCR-mediated apoptosis. Ramos cells were mixed with increasing numbers of transfected cells that expressed CD70 (CD2TL) or CD154-expressing Ramos cells.
(CD40L), both of which are expressed by activated T cells, in the presence of anti-IgM Ab. CD27 ligation as well as CD40 ligation inhibited BCR-mediated apoptosis in a dose-dependent manner (Fig. 1B). When the ratio of transfectant cells to Ramos cells was 20%, CD70- and CD154-transfected cells exhibited a significant inhibition of apoptosis of Ramos cells stimulated with anti-IgM Ab compared with mock-transfected cells (70%, \( p < 0.05 \) and 75%, \( p < 0.01 \), respectively). The extent of inhibitory effect by CD27 ligation was almost comparable to that by CD40 ligation (no significant difference). These inhibitory effects were specifically abrogated by the addition of anti-CD70 or anti-CD154 Ab (data not shown). These results suggest that both CD27 and CD40 signals are involved in antiapoptotic effects for B cell survival.

In agreement with previous reports that BCR ligation activates the mitochondria-mediated apoptotic pathway (5–8), the initial cleavage of caspase-9, caspase-3 and PARP started to appear at 12 h and was evident at 24 h (Fig. 2A). In addition, Bik was strongly induced by 6 h, whereas Bcl-X\(_l\) was gradually down-regulated to a barely detectable level at 48 h (Fig. 2B). The expression of Bcl-2 was absent in unstimulated Ramos cells and was induced weakly at a relatively late phase. CD27 ligation suppressed the activation of caspase-9, caspase-3, and the cleavage of PARP with the same efficacy as that observed for CD40 ligation (Fig. 2A). As previously reported (17–20), CD40 ligation up-regulated Bcl-2 and Bcl-X\(_l\) as much as 9.7- (\( p < 0.01 \)) and 6.8-fold (\( p < 0.01 \)), respectively (Fig. 2, B and C). Under similar conditions, CD27 ligation also up-regulated Bcl-2 and Bcl-X\(_l\) as much as 5.0- (\( p < 0.01 \)) and 3.9-fold (\( p < 0.01 \)), respectively. Interestingly, proapoptotic Bik was weakly down-regulated (1.3-fold decrease by CD27 ligation, \( p < 0.05 \)), whereas CD40 ligation resulted in a 1.6-fold increase in Bik expression (\( p < 0.01 \)) (Fig. 2, B and C).

Cytochrome \( c \) release from the intermitochondrial space into the cytosol was detectable even after 6 h and increased by 48 h (Fig. 3A). BCR ligation altered the mitochondrial \( \Delta \psi \)m (Fig. 3C). These results confirm that BCR ligation activates the mitochondria-mediated apoptotic pathway. As expected, CD27 ligation strongly reduced cytochrome \( c \) release after BCR ligation, in which the inhibitory level was almost the same as CD40 ligation (Fig. 3B). Likewise, CD27 ligation significantly inhibited the dissipation of mitochondrial \( \Delta \psi \)m, similar to CD40 ligation (Fig. 3C). These results clearly indicate that CD27 and CD40 signals regulate BCR-mediated apoptosis at the mitochondrial level through the alteration of the balance among Bcl-2 family members to regulate mitochondrial permeability.
CD27 and CD40 Inhibit p53 Activation—p53 can directly engage apoptotic pathways in the cell and stimulate mitochondrial perturbations including cytochrome c release (13, 14). To assess the functional activation of p53 in response to BCR ligation, we examined p53 phosphorylation at Ser\textsuperscript{15}. p53 phosphorylation at Ser\textsuperscript{15} represents a response to cellular stress such as DNA damage (27). As shown in Fig. 4A, phospho-p53 was identified at 24 h after BCR ligation, and a steady increase in phosphorylation level was observed. Phosphorylation of p53 at Ser\textsuperscript{15}, Ser\textsuperscript{20}, or Ser\textsuperscript{46} was not detected (data not shown). Recent studies have demonstrated that p53 phosphorylation at Ser\textsuperscript{15} decreased its nuclear export activity and resulted in accumulation of p53 in the nucleus (28). On the basis of this finding, we next examined the location of p53 after BCR ligation. The intracellular distribution of p53 was found to be biased toward the nucleus (Fig. 4C). Recent studies demonstrated that, among the identified target genes of p53, p53AIP1, an indispensable mediator of the p53-dependent apoptotic pathway, was induced directly by p53 in response to DNA damage and that p53AIP1 was responsive to functional p53 by phosphorylation at Ser\textsuperscript{15} and Ser\textsuperscript{46} residues (15). In the present study, the p53AIP1 gene was clearly induced in Ramos cells at 24 h after BCR ligation compared with unstimulated cells (Fig. 4E). Compared with a mock control at 24 h after BCR ligation, CD27 and CD40 ligation inhibited p53 phosphorylation at Ser\textsuperscript{15} (p < 0.01) (Fig. 4, A and B), its translocation into the nucleus (p < 0.05 and p < 0.01, respectively) (Fig. 4, C and D), and p53AIP1 expression induced by BCR ligation (p < 0.05) (Fig. 4E). These results suggest that CD27 and CD40 signals inhibit p53 activation.

p53 Activation Occurs Downstream of Mitochondria and Is Inhibited by CD27 and CD40 Signals—It is assumed that BCR-mediated signaling primarily activates p53 downstream, which in turn executes the apoptotic process. However, the above finding of p53 phosphorylation at Ser\textsuperscript{15} and p53AIP1 induction on BCR ligation could also be interpreted to mean that p53 activation was secondarily induced in response to cellular stress such as DNA damage. To examine the direct involvement of p53 activation in BCR-mediated apoptosis, stably transfect Ramos cells with p53DN or p53WT were prepared. Western blot analysis showed high levels of both p53DN and p53WT expression, and fluorescence-activated cell sorter analysis showed no difference in surface IgM expression between these transfectants (data not shown). As shown in Fig. 5, A and B, overexpression of p53WT caused Ramos cells to become hypersensitive against BCR ligation, demonstrating rapid cell death by 6 h (p < 0.01) and magnified p53AIP1 gene expression (p < 0.01) compared with transfected Ramos cells with vector alone. On the other hand, p53DN still showed a substantial level of BCR-mediated apoptosis after 24 h incubation compared with vector control in the presence of anti-IgM Ab (p < 0.05) despite the fact that p53AIP1 gene expression at 12 h after BCR ligation was almost completely suppressed by p53DN to a basal level compared with p53DN in the presence of control Ab. After BCR ligation, p53DN only slightly suppressed phosphorylation of p53 at Ser\textsuperscript{15}, the cleavage of PARP, and cytochrome c release compared with vector control (no significance except for PARP cleavage (p < 0.05), whereas p53WT strongly amplified these (p < 0.01) (Fig. 5, C and D, and Fig. 6, A and B). Interestingly, although no significant difference was found in Bcl-X\textsubscript{L} expression among these transfectants, up-regulation of Bax and Bik was observed in p53WT compared with vector control in the presence of anti-IgM Ab (p < 0.01) (Fig. 6, C and D). Consistent with the above results, whereas p53WT
strongly magnified the alteration of mitochondrial Δψm, p53DN still showed a substantial level of mitochondrial Δψm dissipation compared with vector control after BCR ligation (Fig. 6E). These results suggest that p53-independent and mitochondrial apoptotic pathways may operate in BCR-mediated apoptosis. In addition, p53 may be activated secondarily and subsequently drive apoptosis at the mitochondrial level through p53AIP1, because p53AIP1 is located in the mitochondria and its overexpression causes dissipation of mitochondrial Δψm (15).

Finally, we examined the effects of CD27 and CD40 signals on BCR-mediated apoptosis of p53DN cells to clarify whether their signaling inhibits p53-independent apoptotic pathway. Compared with vector control after BCR ligation, CD27 and CD40 signals significantly inhibited apoptosis of p53DN cells (p < 0.05) (Fig. 7A), up-regulated Bcl-XL and Bcl-2 expression (p < 0.01) (Fig. 7, B and C), and suppressed cytochrome c release into the cytosol (p < 0.01) (Fig. 7, D and E). Thus, these results suggest that CD27 and CD40 signals regulate the p53-independent apoptotic pathway at the mitochondrial level and consequently regulate the p53-amplified pathway.
DISCUSSION

In this report we compared the effects of CD70/CD27 and CD154/CD40 interactions that belong to the TNF/TNFR family on human T cell-dependent B cell survival after Ag stimulation. For this purpose, we used Ramos cells as a model for germinal center B cells and mimicked provision of T cell help through the selective use of transfectant cells that express the ligands CD70 and CD154 for the receptors CD27 and CD40 on B cells, respectively. The potential physiologic relevance of these TNF/TNFR family molecules in B cell activation, differentiation, and survival has yet to be fully proved. However, it is likely that multiple surface molecules are involved in the fine tuning of T cell-dependent B cell responses and fate and that different molecules are functionally implicated in each maturation stage of B cells. In addition, to evaluate the role of p53 in BCR-mediated apoptosis, we developed and used p53DN and p53WT Ramos cells.

First we found that both CD27 ligation and CD40 ligation each clearly inhibited BCR-mediated apoptosis (Fig. 1B). Of note, these results are in contrast to those of Prasad et al. (29) who reported that CD27 signaling itself induced apoptosis of Ramos cells. The reason for the opposite results is that CD27 might provide different intracellular signals to B cells under different culture conditions, for instance the presence or absence of Ag stimulation as observed with other TNF receptors. For example, Fas (CD95) provides co-stimulatory signals to resting T cells but triggers apoptosis of activated T cells (30). Thus, CD27 could provide proapoptotic signals for B cells in the absence of Ag stimulation or low-affinity Ag recognition but could provide survival and/or co-stimulatory signals for activated B cells with high-affinity Ag stimulation.

Our results also showed that both CD27 and CD40 ligation altered the expression of the Bcl-2 family molecules, mitochondrial transmembrane potential, cytochrome c release, p53 phosphorylation at Ser15, nuclear translocation of p53, and p53AIP1 induction (Figs. 2–4). These findings clearly indicate that CD27 and CD40 signals exhibit their regulatory effects on BCR-mediated apoptosis at least at the mitochondrial level. It should be noted that Bik was weakly down-regulated by CD27 ligation, whereas it was up-regulated by CD40 ligation (Fig. 2, B and C). Thus, it is possible that CD27 signals regulate BCR-mediated apoptosis at the mitochondrial level in a manner different from CD40 signals through the alteration of the balance among Bcl-2 family members to regulate mitochondrial permeability. In addition to Bax, one of the target proteins for p53, Bik might be involved in the p53-dependent apoptosis pathway. Because previous studies have demonstrated that BCR ligation increased the amount of Bik associated with Bcl-XL during apoptosis (12), Bik may contribute at least in part to the high apoptotic sensitivity of p53WT together with Bax to antagonize the function of Bcl-XL.

We also examined the role of p53 in BCR-mediated apoptosis and the role of CD27 and CD40 signals on this mechanism. Previous studies indicated that DNA damage could induce p53 phosphorylation at Ser15 as well as p53AIP1 expression (15, 17). In addition, p53 activation seems to occur at a relatively late phase after BCR ligation when compared with the appearance of annexin V-positive cells and cleavage of caspases and other molecules...
PARP (Figs. 1A, 2A, and 4A). After BCR ligation, p53DN still revealed a detectable level of cytochrome c release, cleavage of PARP, p53 phosphorylation, down-regulation of Bcl-X_L, and up-regulation of Bik to levels almost comparable to those observed in vector control and showed a substantial level of apoptosis despite the failure of induction of p53AIP1 (Figs. 5 and 6). In addition, although the exact time of activation of the executioner, caspase-3, after BCR ligation was not clear, we were able to detect DNA fragmentation even after 6 h (data not shown). When we used z-VAD-fmk, a broad-spectrum caspase inhibitor, we observed a complete inhibition of both DNA fragmentation and p53 phosphorylation at Ser15 during a 24-h culture period after BCR ligation (data not shown). Taken together, these results strongly suggest that 1) BCR ligation initiated a p53-independent apoptotic pathway that resulted in DNA damage, 2) DNA damage in turn activated a p53-dependent pathway, and 3) both the p53-independent and p53-dependent apoptotic pathways are located in the mitochondria, and their activation resulted in amplification of the apoptotic process. This conclusion is supported by the results of previous studies demonstrating that splenic B cells from p53-null mice were not more resistant to apoptosis than normal mice (31), and that BCR ligation activated a p53-independent pathway of c-Myc-induced apoptosis in murine WEHI-231 cells (32). To our knowledge, however, no report has described the relationship between p53-dependent and p53-independent pathways or the relationship between CD27/CD40 signaling and p53 activation in BCR-mediated apoptosis in humans. This issue is important in the consideration of the potential biological role of p53 in human B cell fate and responses. It should be noted that murine B cells showed only a marginal expression of CD27 (33–35) and that a normal B cell response to antigen challenge was observed in CD27-deficient mice (36). CD27 and CD40 signals primarily inhibited p53-independent pathway in human BCR-mediated apoptosis at the mitochondrial level (Fig. 7).

It is possible that the findings obtained in the present study are specific for Ramos cells. To rule out this possibility, we performed the same series of experiments using another human B cell line, P32/ISH. P32/ISH cells express IgM, CD40, and CD27, and are susceptible to BCR-mediated apoptosis (37). Our results in P32/ISH cells were similar to those described in Ramos cells (data not shown), thus indicating that the aforementioned findings were not specific to Ramos cells but rather were common to B cells.

In conclusion, we have demonstrated in the present study that at the mitochondrial level CD27 and CD40 co-stimulatory
signals suppressed the p53-mediated apoptotic pathway in human B cells by inhibiting p53-independent apoptotic pathway induced by BCR ligation. In humans, CD40 signals induce proliferation of B cells and generation of memory B cells, and CD27 signals induce marginal proliferation of B cells and generation of plasma cells, resulting in Ig production (3, 7, 18, 21, 22, 38). Therefore, in humans CD27 signals might rescue the differentiation of germinal center B cells, especially memory B cells (35, 39, 40), into plasma cells from apoptosis in the secondary immune response, whereas CD40 signals might rescue the clonal expansion and differentiation of germinal center B cells into memory B cells.

Acknowledgments—We thank Drs. Mitsufumi Mayumi and Yoshinobu Matsuo for helpful discussion and Yoshie Nitta for excellent secretarial assistance.

REFERENCES
1. Craxton, A., Otipoby, K., Jiang, A., and Clark, E. A. (1999) Adv. Immunol. 73, 79–152
2. Goodnow, C. C., Cyster, J. G., Hartley, S. B., Bell, S. E., Coole, M. P., Hearly, J. I., Akkaraju, S., Rathmell, J. C., Pogue, S. L., and Shukat, K. P. (1995)

![Fig. 7. Effects of CD27 and CD40 ligation on BCR-mediated apoptosis of Ramos cells that express dominant-negative p53 protein.](http://www.jbc.org/)

Stable transfected Ramos cells (1 x 10^6/well) with p53DN gene or vector alone were co-cultured with mitomycin C-treated transfected cells (2 x 10^5/well) in the presence of control Ab or anti-IgM Ab (10 μg/ml). Twenty-four h after the treatment, annexin V-positive cells were determined by fluorescence-activated cell sorter analysis (A), or whole cell lysates (B) or cytoplasmic fraction (D) were prepared for Western blotting to detect the indicated proteins. Their expression levels were presented in the histograms shown in C and E, respectively. Data shown are representative of three different experiments, and are presented as mean ± S.D.; *, p < 0.05; **, p < 0.01.
17. Ghia, P., Boussiotis, V., Schulzke, J., Cardoso, A., Dorfman, D. H., Gribben, J., Freeman, A., and Nadler, L. (1998) Blood 91, 244–251
18. Lee, H. H., Dadgostar, H., Cheng, Q., Shu, J., and Cheng, G. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9136–9141
19. Choi, M. S., Boise, L. H., Gottschalk, A. R., Quintans, J., Thompson, C. B., and Klaus, G. G. (1995) Eur. J. Immunol. 25, 1352–1357
20. Wang, Z., Karras, J. G., Howard, R. G., and Rothstein, T. L. (1995) J. Immunol. 155, 3722–3725
21. Kobata, T., Jacquot, S., Kozlowski, S., Agematsu, K., Schlossman, S. F., and Morimoto, C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11249–11253
22. Jacquot, S., Kobata, T., Iwata, S., Morimoto, C., and Schlossman, S. F. (1997) J. Immunol. 159, 2652–2657
23. Akiba, H., Nakano, H., Nishinaka, S., Shindo, M., Kohata, T., Atsuta, M., Morimoto, C., Ware, C. F., Malinin, N. L., Wallach, D., Yagita, H., and Okumura, K. (1998) J. Biol. Chem. 273, 13533–13538
24. Kruse, N., Pette, M., Toyka, K., and Rieckmann, P. (1997) J. Immunol. Methods. 210, 195–203
25. Scheffner, M., Takahashi, T., Huibregtse, J. M., Minna, J. D., and Howley, P. M. (1992) J. Virol. 66, 5100–5105
26. Karasuyama, H., Kudo, A., and Melchers, F. (1990) J. Exp. Med. 172, 969–972
27. Giaccia, A. J., and Rastan, M. B. (1996) Genes Dev. 10, 2073–2083
28. Zhang, Y., and Xiong, Y. (2001) Science 292, 1910–1915
29. Prasad, K. V. S., Ao, Z., Yoon, Y., Wu, M. X., Rizk, M., Jacquot, S., and Schlossman, S. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6346–6351
30. Siegel, R. M., K-M Chan, F., Chun, H. J., and Lenardo, M. J. (2000) Nat. Immunol. 1, 469–474
31. Shiek, L., Carman, J. H., Choi, J. K., Somasundaram, K., Burrell, M., Hill, D. E., Zeng, Y. X., Wang, K. G., Sathany, K., Kadesch, T. R., Monroe, J. G., Donehower, L. A., and El-Deiry, W. S. (1997) Cell Growth Differ. 8, 121–131
32. Hagiwara, H., Adachi, T., Yoshida, T., Nomura, T., Miyasaka, N., Honjo, T., and T. Tsuchiya. (1999) Oncogene 18, 4091–4098
33. Gravestein, L. A., Nieland, J. D., Rieckmann, P. M., and Borst, J. (1995) Int. Immunol. 7, 551–557
34. Oshima, H., Nakano, H., Nobara, C., Kobata, T., Nakajima, A., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., Muto, T., Yagita, H., and Okumura, K. (1998) Int. Immunol. 10, 517–526
35. Kleiman, U., Raje, R. K., and Kupper, P. (1998) J. Exp. Med. 188, 1679–1689
36. Hendriks, J., Gravestein, L. A., Tessler, L. K., van Lier, R. A. W., Schumacher, T. N. M., and Borst, J. (2000) Nat. Immunol. 1, 433–440
37. Suzuki, T., Kiyokawa, N., Taguchi, T., Sekino, T., Katagiri, Y. U., and Fujimoto, J. (2001) J. Immunol. 166, 5567–5577
38. Morimoto, S., Ranno, Y., Tanaka, Y., Tokano, Y., Hashimoto, H., Jacquot, S., Morimoto, C., Schlossman, S. F., Yagita, H., Okumura, K., and Kobata, T. (2000) J. Immunol. 164, 4097–4104
39. Tangye, S. G., Liu, Y. J., Aversa, G., Phillips, J. H., and de Vries, J. E. (1998) J. Exp. Med. 188, 1691–1703
40. Agematsu, K., Hohkibara, S., Nagumo, H., and Komiyama, A. (2000) Immunol. Today 21, 204–206
CD27 and CD40 Inhibit p53-independent Mitochondrial Pathways in Apoptosis of B Cells Induced by B Cell Receptor Ligation
Hidenori Hase, Yumiko Kanno, Hidefumi Kojima, Chikao Morimoto, Ko Okumura and Tetsuji Kobata

J. Biol. Chem. 2002, 277:46950-46958.
doi: 10.1074/jbc.M209050200 originally published online September 24, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M209050200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 23 of which can be accessed free at http://www.jbc.org/content/277/49/46950.full.html#ref-list-1