CD47 and the 19 kDa Interacting Protein-3 (BNIP3) in T Cell Apoptosis*

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CD47 is a surface receptor that induces either coactivation or apoptosis in lymphocytes, depending on the ligand(s) bound. Interestingly, the apoptotic pathway is independent of caspase activation and cytochrome c release and is accompanied by early mitochondrial dysfunction with suppression of mitochondrial membrane potential (ΔΨm). Using CD47 as a bait in a yeast two-hybrid system, we identified the Bcl-2 homology 3 (BH3)-only protein 19 kDa interacting protein-3 (BNIP3), a pro-apoptotic member of the Bcl-2 family, as a novel partner. Interaction between CD47 and the BH3-only protein was confirmed by immunoprecipitation analysis, and CD47-induced apoptosis was inhibited by attenuating BNIP3 expression with antisense oligonucleotides. Finally, we showed that the C-terminal domain of thrombospondin-1 (TSP-1), but not signal-regulatory protein (SIRPa1), is the ligand for CD47 involved in inducing cell death. Immunofluorescence analysis of CD47 and BNIP3 revealed a partial colocalization of both molecules under basal conditions. After T cell stimulation via CD47, BNIP3 translocates to the mitochondria to induce apoptosis. These results show that the BH3-dependent apoptotic pathways, previously shown to be activated by intracellular pro-apoptotic events, can also be turned on by surface receptors. This new pathway results in a fast induction of cell death resembling necrosis, which is likely to play an important role in lymphocyte regulation at inflammatory sites and/or in the vicinity of thrombosis.

Multicellular organisms eliminate excess, damaged or infected cells by stereotypic programs of cell death (PCD).1 In its classic form, apoptosis is characterized by well defined ultrastructural changes including cell shrinkage, exposure of phosphatidylserine at the outer leaflet of the cytoplasmic membrane, changes in mitochondrial permeability, membrane blebbing, caspases activation, and DNA degradation. Lymphocyte PCD plays an important role in controlling immune responses and occurs both in central and peripheral lymphoid organs. Disturbed PCD may contribute to multiple immune disorders such as cancer and autoimmune and degenerative diseases. Upon signaling, pathways that influence T cell proliferation and survival, CD95/CD95L and tumor necrosis factor receptor pathways have been extensively studied over the past few years (1). However, a number of others T cell surface receptors such as major histocompatibility complex class I and II (2), CD2 (3), CD4 (4), CD45 (5, 6), or CD99 (7) can also trigger these pathways, they have all been described to act independently of any of the known caspases (8), and the molecular mechanisms and the physiological and/or the pathological relevance of these death pathways remains to be established. Among these molecules, recent reports implicate CD47 in the triggering of atypical cell death (9, 10).

CD47 (also known as IAP for integrin-associated protein), expressed on all mammalian cells (11), displays an extracellular Ig-like domain with five transmembrane (TM) segments and a short C-terminal cytoplasmic tail. CD47 is associated with β2 integrins on several cell types. However, on other cell types such as lymphocytes, no association with integrins has been documented. More generally, CD47 has been shown to activate integrins, either through direct interaction or at a distance (11–13). The two natural ligands currently known for CD47 are thrombospondin-1 (TSP-1), a protein found in extracellular matrix and released in large amounts by platelets upon activation, and the signal-regulatory protein (SIRPa1), expressed on the surface of macrophages and endothelial and dendritic cells. Moreover, we and others have shown that CD47 can trigger T cell activation and proliferation (14–16) or induce T cell spreading (17). Therefore an important task has been to determine under which condition(s) CD47 induces T cell death and/or survival. Indeed, in addition to the cytoplasmic proteins Gi (18, 19) and proteins linking IAP with cytoskeleton (PLC) (20), we show in the present study that CD47 associates with the pro-apoptotic molecule 19 kDa interacting protein-3 (BNIP3).

BNIP3 belongs to the Bcl-2 homology 3 (BH3)-only family, a Bcl-2-related family possessing an atypical Bcl-2 homology 3 (BH3) domain, which regulates PCD from mitochondrial sites by selective Bcl-2/Bcl-XL interactions (21, 22). BNIP3 family members contain a C-terminal transmembrane domain that is required for their mitochondrial localization, homodimerization, as well as regulation of their pro-apoptotic activities (23). BNIP3-mediated apoptosis has been reported to be independ-
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EXPERIMENTAL PROCEDURES

Reagents—The CD47 mAb Ad22 was kindly provided by Dr. Rolf D. Pettersen (Department of Pediatric Research and Pediatrics, The National Hospital, Oslo, Norway) and has been described elsewhere (9). CD47 mAb B6H12 was from the American Type Culture Collection (ATCC, Rockville, MD). Rabbit polyclonal anti-BNIP3 and mouse monoclonal anti-BNIP3 antibodies were described elsewhere (21). MAb against Fas CH11 was from Immunotech (BD Biosciences), mAb against Bcl-2 was from Santa Cruz Biotechnology, and mAb against V5 was from Invitrogen. SIRPα-Fc fusion proteins have previously been described (25). The peptides 4N1K (KRFFYVMMWKK) and 4NGO (KRFFYGMMWKK) were from Genosys Biotechnologies (The Woodlands, TX). Plasmids encoding green fluorescent protein (GFP)-tagged cytochrome c and Bcl-2 (pCMV-Bcl-2) were kindly provided by Dr. A. Galmiche (INSERM U462, Nice, France).

Cells—The Jurkat T cell line (J6.1) was obtained from ATCC and cultured in RPMI 1640 (Invitrogen) supplemented with 5% fetal bovine serum (Duchet, Brumath, France), 50 units/ml penicillin, 50 μg/ml streptomycin, 2 μg/ml fungizone, and 1 μg/ml pyruvate (Merck, Darmstadt, Germany). The CD47-deficient Jurkat T cell line (JIN) for Jurkat IAP-negative cells has been previously described (26).

Assay for Apoptosis—Phosphatidylserine exposure and decrease in mitochondrial membrane potential (ΔΨm) were measured by flow cytometry using a FACScan (BD Biosciences). For phosphatidylserine exposure, cells were double-stained with annexin V-phalocyanin (VP) and propidium iodide as described by the manufacturer (Roche Applied Science). The decrease in ΔΨm was assessed by incubating Jurkat cells with 40 nM 3,3-dihexylxocarboxylic acid (DiOC6) (Molecular Probes, Eugene, OR) for 30 min at 37°C.

Confocal Microscopy—Jurkat cells, treated or not with the pro-apoptotic 4N1K peptide for 2 h, were plated on polylysine glass slides (Menzel-Glaser, Freiburg, Germany). For mitochondrial staining, Texas Red MitoTracker (Molecular Probes) was added to the medium to a final concentration of 300 nM for 45 min. Cells were then fixed with 3.7% formaldehyde, permeabilized with 0.2% Triton X-100, and blocked for 20 min with phosphate-buffered saline containing bovine serum albumin (0.5% bovine albumin (0.05%). Cells were stained for CD47 using SIRPα-Fc and FITC-conjugated mouse anti-human antibody (Dako) at 4°C before fixation, and for BNIP3 using the mouse monoclonal anti-BNIP3 antibody (21) followed by a Cyan V-conjugated rabbit anti-mouse antibody. Samples were mounted in Mowiol (Calbiochem) and observed with a confocal microscope (Leica TCS-SP, Heidelberg, Germany).

Co-immunoprecipitation and Western Blot Analysis—Cells were washed twice in cold phosphate-buffered saline and lysed in 2% Triton X-100 isotonic buffer with freshly added protease inhibitors (50 μM HEPES, pH 7.6, 150 mM NaCl, 20 mM EDTA) with freshly added protease inhibitors. Cellular extracts were then incubated in a 96-well plate with 10 mM diethiothreitol and 200 μM of Ac-DEVD-pNA as substrate for various times at 37°C. Caspase activity was measured by the release of pNA at 410 nm in either the presence or absence of 1 μM Ac-DEVD-CHO, an irreversible inhibitor of caspase-3. Caspase-3 activity represents the Ac-DEVD-CHO inhibitable activity and is expressed as nanomoles of substrate hydrolyzed per min and mg of protein.

BNIP3 Antisense Oligodeoxynucleotides—Two μM of BNIP3 antisense (sequence number 2.04252) or scrambled phosphorothioate oligodeoxynucleotides (Biosearch, Inc., Corte Madera, CA) was added to cells at the start of culture and every time the culture medium was renewed during 72 h.

Yeast Two-hybrid System—The C-terminal 146 amino acids of type 2 CD47 corresponding to the multispan and cytoplasmic portion of the molecule were fused to the LexA DNA-binding domain in pLex-9 (pLex-CD47 multimeric membrane spanning (MMS)) and used as a bait for screening a human cDNA library (29, 30). Standard techniques were used for nucleic acid manipulations and preparation of DNA constructs. Primers used to generate the DNA fragments were 5′-GAATTCCTGGT-ATTTAACACCTTAAATAGTCCGGT-3′ (sense oligonucleotide, EcoRI site underlined), and 5′-COCGATCCCTGAGTTATCTCCTAGAGG-GTTTG-3′ (antisense oligonucleotides, BamHI site underlined, mutation introduced in bold). A second round of screening was done using a LexA-lamin plasmid as a negative control. CD47 MMS domain and BNIP3 interactions were assayed for growth on the basis of histidine prototrophy on DOBA Ura -Trp -Leu -His plates. Subsequent testing for β-galactosidase activity was performed. Deletion mutants for BNIP3 gene were previously described (21). BNIP3-H1 (Δ106–164), BNIP3-H2 (Δ104–119), BNIP3-H3 (Δ110–164), and BNIP3-H4 (Δ184–194). cDNAs were cloned into the EcoRI/BamHI sites of the two yeast-hybrid expression vector pActII.

RESULTS

CD47 Induces a Rapid Mitochondrial Dysfunction and Apoptosis—As previously described (9, 10), CD47 antibody Ad22 in a soluble form induces a fast cell death with an early phosphatidylserine exposure (Fig. 1A) and mitochondrial abnormalities. Indeed, using DiOC6 to measure ΔΨm following cell stimulation via CD47 (Fig. 1B), we observed ΔΨm drop within 1 h, indicating that the mitochondrial dysfunction occurred as early as cell death. CD47 stimulation was nearly as efficient as CD95 at suppressing ΔΨm, as measured after optimal times.
Despite early mitochondrial dysfunction following CD47 stimulation, we observed only a late and low release of cytochrome c which was undetectable before 3 h, in marked contrast with the effects of CD95 stimulation (Fig. 1C). Therefore, cytochrome c release appears to be a secondary event. To further ensure that cytochrome c release occurs after cell death,
we transfected Jurkat cells with GFP-tagged cytochrome c and observed its mitochondrial release after CD47 stimulation while simultaneously measuring Annexin binding (Fig. 1D). Quite clearly, mitochondrial release was a later event. No role for caspases could be demonstrated by attempts to inhibit PCD with the broad-spectrum caspase inhibitor z-VAD-fmk (result not shown). Moreover, no activation of caspase 3 could be detected while PCD occurred in the same experiment, in marked contrast with controls measuring the effect of a CD95 antibody (Fig. 1E).

**CD47 Interacts with the Pro-apoptotic Molecule BNIP3**—To determine the apoptotic pathway triggered by CD47 stimulation, we searched for CD47 interacting molecule(s) in the yeast two-hybrid system. We used the CD47 MMS domain fused to Gal4 as a bait for screening a human lymphocyte cDNA library. We ascertained the requirement for the MMS domain of CD47 to induce apoptosis by using a chimeric form of CD47, composed of the extracellular domain of CD47 and the membrane and cytoplasmic domains of CD7 (CD47ec-CD7) (15). This chimera was unable to transmit an apoptotic signal when transfected in the CD47-deficient T-cell line which we previously generated (JIN cells) (26), (Fig. 2A). CD47ec-CD7 mutants and wild-type CD47 transfected cells showed comparable levels of Fas-induced apoptosis (result not shown). Note that the use of MMS domain as bait to perform the two-hybrid screen has already been successively performed (31–36). In the present screen, five positive clones were selected of an estimated 5 × 10⁶ colonies, for their ability to reconstitute a functional Gal4 transcription complex. Among them, we found a clone encoding the pro-apoptotic BH3-only family protein BNIP3 (Fig. 2B). To determine the domain of the BNIP3 molecule involved in binding to CD47, we tested a series of BNIP3 deletion mutants (21) by using the two-hybrid system. Whereas full-length BNIP3 showed a strong interaction with CD47, BNIP3ΔTM2 was unable to interact with CD47 (Fig. 2B) indicating that the transmembrane domain of BNIP3 is required for interaction with CD47. Mutants lacking the NH₂ terminus, the BH3 domain, or the COOH terminus did not bind strongly to CD47 (Fig. 2B), suggesting that other regions in the full-length structure of BNIP3 are necessary for the tight interaction with CD47.

To confirm the association between CD47 and BNIP3 in vivo, we performed co-immunoprecipitation assays (Fig. 2C). BNIP3 was specifically co-immunoprecipitated with CD47 from Jurkat T cells. Nevertheless, as expected, no co-immunoprecipitation was observed from JIN cells or cells expressing the CD47ec-CD7 mutant. Thus, BNIP3 interacts with the MMS domain of CD47 both in yeast and mammalian cells.

Next, we investigated whether, by blocking BNIP3 synthesis with antisense oligodeoxynucleotides, the CD47-generated apoptotic signal could be interrupted. Jurkat cells were incubated with phosphorothioate-derivatized antisense oligodeoxynucleotides, and apoptosis in response to soluble CD47 antibody Ad22 or anti-Fas antibody CH11 was measured (Fig. 3A). Antisense oligodeoxynucleotides corresponding to BNIP3 blocked CD47-induced cell death, showing a 2-fold reduction in cell death, whereas a scrambled sequence of the same oligodeoxynucleotide had no effect. Moreover, oligodeoxynucleotides had no effect on either survival of control cells or on apoptosis induced by the anti-Fas antibody. Immunoblot analysis confirmed a loss of BNIP3 expression from cells cultured with the BNIP3 antisense oligodeoxynucleotides (Fig. 3A). Thus, reduction of BNIP3 expression specifically leads to a reduction of cell death induced via CD47. Finally, because it has been previously shown that BNIP3 binds Bcl-2 at the mitochondrial membrane, an event likely to play a role in BNIP3-dependent PCD (24), we induced overexpression of Bcl-2 in Jurkat cells. Overexpressing cells were less susceptible to PCD induced either via CD47 than their normal counterparts (Fig. 3B). Thus, reduction of BNIP3 expression or overexpression of Bcl-2 leads to a reduction of cell death induced via CD47, demonstrating a
functional interaction between CD47 and BNIP3.

**TSP-1 Promotes CD47-induced Apoptosis and BNIP3 Translocation to Mitochondria**—CD47 acts as a receptor both for the C-terminal domain of TSP-1 and for SIRPα1 (37, 38). TSP-1 is a protein expressed on endothelial cells, found in large amounts in extracellular matrix and released by activated platelets, whereas SIRPα1 is expressed on the surface of macrophages and endothelial and dendritic cells. To determine which natural ligand induces cell death, we treated Jurkat cells or JIN cells with the CD47-binding agonist peptide from TSP-1 named 4N1K (37) or with a recombinant SIRPα1-Fc fusion protein (25) (Fig. 4A). At high concentrations (400 μM), soluble 4N1K induced a rapid death of Jurkat T cells, but had no nonspecific cytotoxic effect on CD47-deficient cells. Control peptide 4NGG failed to induce cell death in the two cell types. By contrast, the soluble SIRPα1-Fc fusion protein, which efficiently bound CD47 (results not shown), had no apoptotic effect, regardless of the amount of protein or the incubation time we used. It is worth noting that only high concentrations of 4N1K (400 μM) are able to induce cell death, whereas lower concentrations have been reported to sustain clonal expansion of inflammatory T cells (39) or to induce anergy of naive T cells (40, 41). This apoptotic effect of 4N1K on T cells is not due to a nonspecific cytotoxicity, because CD47-deficient cells are resistant to cell death induced by 4N1K.

BNIP3 has been described to localize at and to act on the mitochondria when overexpressed (21, 23, 42). The finding of a potential association between the cell surface CD47 molecule and BNIP3 prompted us to examine the subcellular localization of native BNIP3. We detected BNIP3 at the inner leaflet of the plasma membrane in addition to mitochondrial localization (results not shown). Because it was shown that BNIP3 is active when integrated in the outer membrane of the mitochondria, we have investigated whether the plasma membrane-associate-

**FIG. 3. CD47 is functionally associated with BNIP3.** A. Jurkat cells were incubated with 2 μM BNIP3 antisense or scrambled phosphorothioate oligodeoxynucleotides for 72 h prior stimulation with soluble CD47 Ad22 (1 μg/ml, 1 h) or soluble CD95 CH11 (0.5 μg/ml, 3 h) antibodies. Cell death was measured as in Fig. 1A. The effect of oligodeoxynucleotides on BNIP3 protein expression was evaluated by Western blot analysis 72 h after addition of BNIP3 antisense oligodeoxynucleotides. Equal concentrations (50 μg/well) of cell lysates were loaded into each well. Actin expression served as a loading control. B. Jurkat cells were transfected with a plasmid allowing overexpression of Bcl-2. 48 h after transfection, cells were incubated in medium alone or stimulated via CD47. Cell death was measured as above. The level of Bcl-2 expression was assessed by Western blotting with an anti-Bcl-2 antibody. Actin served as a loading and transfer control. Results are representative of three independent experiments performed in triplicate.

**FIG. 4. TSP-1 induces apoptosis via CD47 and BNIP3 relocalization to mitochondria.** A. Jurkat T cells or JIN cells were stimulated for 3 h either with the soluble CD47 antibody Ad22 (1 μg/ml), the soluble 4N1K peptide (400 μM), the soluble 4NGG peptide (400 μM), or the soluble SIRPα1-Fc fusion protein (10 μg/ml). Apoptosis was monitored as in Fig. 1A. B. Jurkat cells were stimulated 1 h with the soluble Ad22 antibody (1 μg/ml) or incubated with medium alone. Cells lysates were subfractionated by ultracentrifugation and each fraction was blotted with appropriate antibodies. Mitochondrial and microsomal fractions were monitored by immunoblotting for the presence of translocase (TOM40) and placental alkaline phosphatase (PLAP), respectively. Note that dilution factors of the mitochondrial and microsomal fractions cannot be adjusted and are quite different, the mitochondrial fraction being the most concentrated. C. Jurkat cells were stimulated with the soluble 4N1K peptide (400 μM) or incubated with medium alone. CD47 was labeled with SIRPα1-Fc using FITC-conjugated second antibody, BNIP3 with anti-BNIP3, and Cyan V-conjugated second antibody, and mitochondria were stained with the Texas Red MitoTracker. The three images were merged (BNIP3 + CD47 + mitochondria). Scale bar represents 10 μM. This image is representative of a larger field of view, and data are from a representative experiment performed three times.
confirm that BNIP3 translocates from the plasma membrane to the mitochondria upon CD47 stimulation, we performed double labeling experiments in which T cells were stimulated or not with the pro-apoptotic 4N1K peptide and then double labeled for either CD47 or BNIP3, and mitochondria were revealed with the MitoTracker (Fig. 4C). When T cells were pretreated with the pro-apoptotic 4N1K peptide, BNIP3 was seen to localized to mitochondria, whereas substantial amounts of BNIP3 remained localized at the cell membrane in basal conditions (Fig. 4C).

**DISCUSSION**

Our data demonstrate that a BH3-only protein can be pivotal in transducing an external signal via a surface receptor. So far BH3-only proteins have been mainly regarded as sensors of intracellular or stress-induced damages, and BNIP3 was shown to mediate hypoxia-induced apoptosis (43). However, the latter process appears to be slow, likely due to a requirement for de novo synthesis of BNIP3, because the molecule is quickly degraded by the proteasome (44, 45). It has been previously observed (21, 42) that, when overexpressed, BNIP3 localizes to mitochondria. Although we made the same observation, we also collected several lines of evidence demonstrating that under basal conditions a significant amount of BNIP3 is localized at the plasma membrane where it might associate with CD47. In a yeast two-hybrid system using the membrane-spanning domain of CD47 as bait, we identified BNIP3. Moreover, immunoprecipitation of CD47 was accompanied by coprecipitation of BNIP3. Finally, double immunofluorescence visualization of CD47 and BNIP3 and subcellular fractionation revealed that a significant proportion of both molecules colocalize. The use of deleted forms of BNIP3 allowed us to identify the transmembrane domain of BNIP3 as the region necessary for interaction with the TM domains of CD47.

A second series of experiments showed that BNIP3 is required for the pro-apoptotic effect of CD47. First, we observed a substantial reduction in CD47 pro-apoptotic effect when BNIP3 expression was markedly reduced, using an antisense oligodeoxynucleotide of BNIP3, or when the anti-apoptotic molecule Bcl-2, another BNIP3 interactor, was overexpressed. Because it has been shown that BNIP3 exerts its pro-apoptotic effect at the mitochondria membrane (22, 23, 44), this suggests a simple model based on the translocation of BNIP3 from the inner surface of the cell membrane to the mitochondria. Consistent with this model, we showed that, after a pro-apoptotic signal transmitted via CD47 upon binding of a mAb or a peptide mimicking TSP-1, BNIP3 translocates from the plasma membrane to the mitochondria as assessed both by subcellular fractionation and immunofluorescence studies. This model accounts for a fast apoptosis and is consistent with the “rheostatic” effect exerted by the Bcl-2 family proteins (46–49). It is worth noting that several other BH3-only proteins were reported to also be subjected to fast post-translational modification and translocation based effect (50). It must be emphasized, however, that BNIP3, together with Nix, forms a peculiar and quite conserved subfamily (45, 51) within the BH3-only proteins. Their BH3 domain was found to be non-functional in terms of association with the anti-apoptotic proteins Bcl-2 and Bcl-XL. Rather it was demonstrated that their transmembrane C-terminal segment as well as N-terminal residues are necessary for these associations. Although a series of observations have shown that “classical” BH3-only protein associates with BAX and BAK to induce cell death (46, 52), the mitochondrial and post-mitochondrial mechanisms leading to cell death with the BNIP3 subfamily remain to be established. Moreover, whether other BH3-only proteins would mediate apoptotic signals transduced via distinct transmembrane receptors remains to be determined.

T cell PCD via CD47 can be triggered by TSP-1 and not by its other natural ligand SIRPα1. The fact that only high amounts of TSP-1 can trigger apoptosis also fits with the above model. By contrast, lower amounts of TSP-1 and 4N1K can sustain clonal expansion of T cells (14–16). It can therefore be assumed that this pro-apoptotic pathway could quickly limit the inflammatory response, which tends to develop after thrombosis. We have connected in vitro evidence, in an inflammation model, for such a role of CD47. 2Consistent with this view, it must be kept in mind that the CD47 pro-apoptotic pathway acts only on activated, but not resting, normal T cells (9). Interestingly, an overexpression of TSP-1 has recently been observed after myocardial infarction, forming a belt around injured tissues (55). Moreover, cell types other than T cells could be subjected to an apoptotic regulation via the TSP-1/CD47 pathway, as both TSP-1 and CD47 have been reported to be strongly up-regulated on vascular endothelial cells upon disturbance of blood laminar flow and can induce their apoptosis (53, 54).

**REFERENCES**

1. Siegel, R. M., Chan, F. K., Chun, H. J., and Lenardo, M. J. (2000) Nat. Immunol. 1, 469–474
2. Skov, S., Klausen, P., and Claesson, M. H. (1997) J. Cell Biol. 139, 1523–1531
3. Deas, O., Dumont, C., MacFarlane, M., Boureau, M., Hebib, C., Harper, F., Hirsch, F., Charpentier, B., Cohen, G. M., and Senik, A. (1996) J. Immunol. 161, 3375–3383
4. Berndt, C., Mops, B., Angermuller, S., Gierschik, P., and Kramer, P. H. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12556–12561
5. Lesage, S., Steff, A. M., Philippot, F., Page, T., Trop, S., Mateo, V., and Hugo, P. (1997) J. Immunol. 159, 4762–4771
6. Klaus, S. J., Sidenranks, S. P., and Clarke, E. A. (1996) J. Immunol. 156, 2743–2753
7. Bernard, G., Brettmattia, J. P., de Matteis, M., Tramppont, P., Hofman, P., Senik, A., and Bernard, A. (1997) J. Immunol. 158, 2543–2550
8. Scaffidi, C., Kirchhoff, S., Krammer, P. H., and Peter, M. E. (1999) Curr. Opin. Immunol. 11, 277–285
9. Pettersen, R. D., Hestdal, K., Olafsen, M. K., Lie, S. O., and Lindberg, F. P. (1999) J. Immunol. 163, 4900–4909
10. Mateo, V., Lagrouex, L., Bron, D., Biron, G., Arman, M., Delesgesse, G., and Sarfatti, M. (1999) Nat. Med. 5, 1277–1284
11. Brown, E., Hooper, L., Ho, T., and Gresham, H. (1990) J. Cell Biol. 111, 2783–2794
12. Lindberg, F. P., Gresham, H. D., Schwarz, E., and Brown, E. J. (1993) J. Cell Biol. 123, 485–496
13. Brittain, J. E., Milnar, K. J., Anderson, C. S., Orringer, E. P., and Parise, L. V. (2001) J. Clin. Invest. 107, 1555–1562
14. Tichioni, M., Deckert, M., Mary, F., Bernard, G., Brown, E. J., and Bernard, A. (1997) J. Immunol. 158, 677–684
15. Reinhold, M. I., Lindberg, F. P., Kersh, G. J., Allen, P. M., and Brown, E. J. (1999) J. Exp. Med. 189, 201–11
16. Wachter, C., Majdz, O., Stullng, T., Berger, M., Baumrucker, T., Knapp, W., and Pichl, W. F. (1997) J. Immunol. 159, 5345–5354
17. Reinhold, M. I., Green, J. M., Lindberg, F. P., Tichioni, M., and Brown, E. J. (1999) Int. Immunol. 11, 707–718
18. Fraizer, W. A., Gao, A. G., Dimitry, J., Chung, J., Brown, E. J., Lindberg, F. P., and Linder, M. E. (1999) J. Biol. Chem. 274, 8545–8560
19. Gao, A. G., Lindberg, F. P., Dimitry, J. M., Brown, E. J., and Fraizer, W. A. (1996) J. Cell Biol. 135, 533–544
20. Wu, L. L., Wang, J., Zheleznyak, A., and Brown, E. J. (1999) Mol. Cell. 4, 619–625
21. Ray, R., Chen, G., Vande Velde, C., Cizeau, J., Park, J. H., Reed, J. C., Gietz, R. D., and Greenberg, A. H. (2000) J. Biol. Chem. 275, 1439–1448
22. Yasuda, M., Theodorakis, P., Subramanian, T., and Chinnadrani, G. (1998) J. Biol. Chem. 273, 12415–12421
23. Chen, G., Ray, R., Dubik, D., Shi, L., Cizeau, J., Bleackley, R. C., Saxena, S., Gietz, R. D., and Greenberg, A. H. (1997) J. Exp. Med. 186, 1795–1803
24. Van de Velde, C., Cizeau, J., Dubik, D., Alimonti, J., Brown, T., Israels, S., Hirokawa, H., and Greenberg, A. H. (2000) Mol. Cell. 20, 5454–5469
25. Rebers, R. A., Green, J. M., Reinhold, M. I., Tichioni, M., and Brown, E. J. (2001) J. Biol. Chem. 276, 7672–7680
26. Tichioni, M., Raimondi, V., Lamy, L., Wijdenes, J., Lindberg, F. P., Brown, L. Lam, M., Tichioni, A. Foussat, E. J. Brown, and A. Bernard, manuscript in preparation.
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E. J., and Bernard, A. (2001) FASEB J. 15, 341–350
27. Storrie, B., and Madden, E. A. (1990) Methods Enzymol. 183, 203–225
28. Ricci, J. E., Maulon, L., Battaglione-Hofman, V., Bertolotto, C., Luciano, F., Mari, B., Hofman, P., and Auberger, P. (2001) Eur. Cytokine Netw. 12, 126–134
29. Deckert, M., Tartare-Deckert, S., Couture, C., Mustelin, T., and Altman, A. (1996) Immunity 5, 591–604
30. Foucault, I., Liu, Y. C., Bernard, A., and Deckert, M. (2002) J. Biol. Chem. 24, 24
31. Xu, X., Shi, Y., Wu, X., Gambetti, P., Sui, D., and Cui, M. Z. (1999) J. Biol. Chem. 274, 32543–32546
32. Alberici, A., Moratto, D., Benussi, L., Gasparini, L., Ghidoni, R., Gatta, L. B., Finazzi, D., Frisoni, G. B., Trabucchi, M., Grooten, J. H., Nitsch, R. M., and Auberger, P. (2001) Eur. Cytokine Netw. 12, 126–134
33. Passer, B. J., Pellegrini, L., Vito, P., Ganjei, J. K., and D'Adamio, L. (1999) J. Biol. Chem. 274, 24007–24013
34. Imafuku, I., Masaki, T., Waragai, M., Takeuchi, S., Kawabata, M., Hirai, S., Obno, S., Nee, L. K., Lipps, C. F., Kanazawa, I., Imagawa, M., and Okazawa, H. (1999) J. Cell Biol. 147, 121–134
35. Xu, X., Shi, Y. C., Gao, W., Mao, G., Zhao, G., Agrawal, S., Chisolin, G. M., Sui, D., and Cui, M. Z. (2002) J. Biol. Chem. 277, 48913–48922
36. Hebert, S. S., Godin, C., Tomiyama, T., Mori, H., and Levesque, G. (2003) Biochim. Biophys. Res. Commun. 301, 119–126
37. Gao, A. G., Lindberg, F. P., Finn, M. B., Blystone, S. D., Brown, E. J., and Frazier, W. A. (1996) J. Biol. Chem. 271, 21–24
38. Jiang, P., Lagenaur, C. F., and Narayanan, V. (1999) J. Biol. Chem. 274, 559–562
39. Vallejo, A. N., Mugge, L. O., Klumpp, P. A., Weyand, C. M., and Goronzy, J. J. (2000) J. Immunol. 164, 2947–2954
40. Avice, M. N., Rubio, M., Sergerie, M., Delespesse, G., and Sarfati, M. (2000) J. Immunol. 165, 4624–4631
41. Li, Z., He, L., Wilson, K., and Roberts, D. (2001) J. Immunol. 166, 2427–2436
42. Boyd, J. M., Malstrom, S., Subramanian, T., Venkatesh, L. K., Schaeper, U., Elangovan, B., D'Sa-Epper, C., and Chinnadurai, G. (1994) Cell 79, 341–351
43. Bruck, R. K. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9082–9087
44. Chen, G., Cizeau, J., Vande Velde, C., Park, J. H., Bosek, G., Bolo, J., Shi, L., Dubik, D., and Greenberg, A. (1999) J. Biol. Chem. 274, 7–10
45. Cizeau, J., Ray, R., Chen, G., Gietz, R. D., and Greenberg, A. H. (2000) Oncogene 19, 5453–5463
46. Letai, A., Bassik, M., Walensky, L., Sorcinelli, M., Weiler, S., and Korsmeyer, S. (2002) Cancer Cell 2, 183
47. Cheng, E. H., Wei, M. C., Weiler, S., Flavell, R. A., Mak, T. W., Lindsten, T., and Korsmeyer, S. J. (2001) Mol. Cell 8, 705–711
48. Adams, J. M., and Cory, S. (1998) Science 281, 1322–1326
49. Zimmermann, R. C., Bonzon, C., and Green, D. R. (2001) Pharmacol. Ther. 92, 57–70
50. Huang, D. C., and Strasser, A. (2000) Cell 103, 839–842
51. Yasuda, M., D'Sa-Epper, C., Gong, X. L., and Chinnadurai, G. (1998) Oncogene 17, 2525–2530
52. Martinou, J. C., and Green, D. R. (2001) Nat. Rev. Mol. Cell. Biol. 2, 63–67
53. Freyberg, M. A., Kaiser, D., Graf, R., Vacher, P., and Friedl, P. (2000) Biochem. Biophys. Res. Commun. 271, 584–588
54. Freyberg, M. A., Kaiser, D., Graf, R., Buttenbender, J., and Friedl, P. (2001) Biochem. Biophys. Res. Commun. 286, 141–149
55. Ren, G., Mendoza, L. H., Jackson, P., Michael, L. H., Smith, W. C., Entman, M. L., and Frangogiannis, N. G. (2002) FASEB J. 16, A541. (Abstr.)