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A Fas-associated protein factor, FAF1, potentiates Fas-mediated apoptosis

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ABSTRACT **Fas, a member of the tumor necrosis factor receptor family, can induce apoptosis when activated by Fas ligand binding or anti-Fas antibody crosslinking. Genetic studies have shown that a defect in Fas-mediated apoptosis resulted in abnormal development and function of the immune system in mice. A point mutation in the cytoplasmic domain of Fas (a single base change from T to A at base 786), replacing isoleucine with asparagine, abolishes the signal transducing property of Fas. Mice homozygous for this mutant allele (lpr^{cg}/lpr^{cg} mice) develop lymphadenopathy and a lupus-like autoimmune disease. Little is known about the mechanism of signal transduction in Fas-mediated apoptosis. In this study, we used the two-hybrid screen in yeast to isolate a Fas-associated protein factor, FAF1, which specifically interacts with the cytoplasmic domain of wild-type Fas but not the lpr^{cg}-mutated Fas protein. This interaction occurs not only in yeast but also in mammalian cells. When transiently expressed in L cells, FAF1 potentiated Fas-induced apoptosis. A search of available DNA and protein sequence data banks did not reveal significant homology between FAF1 and known proteins. Therefore, FAF1 is an unusual protein that binds to the wild type but not the inactive point mutant of Fas. FAF1 potentiates Fas-induced cell killing and is a candidate signal transducing molecule in the regulation of apoptosis.**

Little is known about the molecular mechanism of signal transduction by molecules that regulate apoptosis. Several members of the tumor necrosis factor (TNF) receptor family are known to trigger cell death when activated by the respective ligand. Fas, a member of this receptor family, mediates apoptosis when triggered by its ligand or antibody crosslinking (1).

Apoptosis induced by Fas plays an important role in the development and function of the immune system (1, 2). Mutations or deletions of the Fas gene and Fas ligand in mice result in lymphadenopathy and systemic lupus erythematosus-like autoimmune disease, which naturally occurs in *lpr/lpr* (Fas mutant), lpr^{cg}/lpr^{cg}, and *gld/gld* mice (Fas ligand mutant) (3). In lpr^{cg} mice, a single base change at position 786 (from T to A), replacing isoleucine with asparagine in the cytoplasmic domain of Fas, completely abolished the signal transducing property of Fas (4). This suggests that Fas-mediated apoptosis is involved in deletion of the autoreactive lymphocytes. Gene knockout studies have also shown that the Fas pathway is one of the two mechanisms utilized by cytotoxic T cells and natural killer cells in cell killing to eliminate cancer cells and cells infected by viruses (2).

To understand the mechanism of Fas-mediated apoptotic signal transduction, we have studied the downstream events after Fas ligand binding by identifying a protein factor associated with the cytoplasmic domain of activated Fas. First, using chimeric molecules, we showed that the cytoplasmic domain of Fas is sufficient to mediate apoptosis. Using the cytoplasmic domain of Fas as a target for the two-hybrid screening system in yeast, we were able to clone a protein (FAF1; Fas-associated factor 1) specifically interacting with the wild-type cytoplasmic domain of Fas but not with the lpr^{cg} mutant.‡ When expressed transiently in L cells, FAF1 potentiated the apoptotic signal generated through Fas.

MATERIALS AND METHODS

Antibodies. GK1.5, used as a primary crosslinking antibody, is a monoclonal antibody specific to murine CD4 (L3T4) (Caltag, South San Francisco, CA). PE-L3T4 (Caltag) is GK1.5 conjugated with phycoerythrin and was used for surface staining of CD4/fas expression by fluorescence-activated cell sorting (FACS) analysis (FACS IV; Becton Dickinson). A rabbit anti-rat IgG was used as secondary crosslinking antibody (Zymed). 12CA5 is a monoclonal antibody specific for the hemagglutinin (HA) epitope of the influenza virus (5). A rabbit serum specific for murine CD4 (a gift from D. R. Littman, New York University) was used for detection of CD4/fas by Western blot.

DNA Constructs. A chimeric molecule of CD4 and fas was subcloned into vector PSM (6), which has a simian virus 40 (SV40) replication origin and a SV40 early promoter. PSMCD4/fas contains the chimera with a wild-type cytoplasmic domain of Fas. PSMCD4/fas786A has a T to A point mutation at base 786 in the cytoplasmic domain of fas. Fusion molecules of the λ repressor dimerization domain and Fas cytoplasmic domain were inserted in-frame with the GAL4 DNA-binding domain and HA epitope in the vector PAS-CHY (7). FAF1 tagged with a HA epitope at the N terminus was subcloned into the PCGN vector (with a cytomegalovirus promoter) to make PCGN8.1.

Cells, Transfections, and Immunoprecipitation. For coimmunoprecipitation experiments, COS cells were transiently transfected with PCGN8.1 alone or PCGN8.1 plus PSMCD4/fas or plus PSMCD4/fas786A by the DEAE-dextran method (8). Transfectants were lysed by lysis buffer (20 mM Tris·HCl, pH 7.5/137 mM NaCl/1% Triton X-100) 2 days later. The expression level of FAF1 was quantitated by Western blot analysis with 12CA5 antibody. Cell lysates were used for immunoprecipitation of CD4 by GK1.5. Immune complexes were analyzed by SDS/8% PAGE and transferred to nitrocellulose paper. The paper was then incubated with 12CA5 antibody and developed by an alkaline phosphatase method (Boehringer Mannheim). To quantitate the amount of CD4/fas or CD4/fas786A immunoprecipitated, the blot was stripped off, reprobed with anti-CD4 antiserum, and then developed by the enhanced chemiluminescence (ECL) method (Amersham).

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Abbreviations: TNF, tumor necrosis factor; FACS, fluorescence-activated cell sorting; HA, hemagglutinin.

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‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. U39643).

L cells were transfected with PSMCD4/fas or PSMCD4/fas786A plus a thymidine kinase promoter-driven *neo* gene expression vector (Stratagene) by calcium precipitation. The cells were then selected in medium with G418 (400 μg/ml) for 10 days (9). Individual clones were analyzed for CD4 surface expression by FACS. Multiple clones expressing either wild type or the mutant chimera were generated.

For FAF1 functional studies, PCGN8.1 or PCGN vector plus PSV-β-galactosidase (5:1 ratio) was transiently transfected into both CD4/fas- and CD4/fas786A-expressing cells by either DEAE-dextran or Lipofectin (BRL). Transfected cells were identified by their expression of β-galactosidase [stained blue cells with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal)] (10).

Antibody Crosslinking and Assays for Apoptosis. CD4/fas- or CD4/fas786A-expressing cells were incubated with appropriate amounts of GK1.5 at 4°C for 20–30 min and rinsed with prewarmed 37°C medium (0% serum). Actinomycin D (final concentration, 0.5 μg/ml) (8) and secondary antibody (5 μg/ml) were added to the cells. The cells were then incubated at 37°C for different times.

To detect DNA fragmentation, DNA was extracted from cells 2 hr after crosslinking, electrophoresed in 2% agarose gel, and visualized with ethidium bromide.

For FAF1 functional assays, the cells were crosslinked 48–72 hr after transfection. Then the cells were visualized under the microscope and photographs were taken at different times to detect changes in cellular morphology. To quantitate the percentage of apoptotic cell death at a given time, the crosslinked cells were fixed, stained with X-Gal, and counted for the number of apoptotic cells (membrane blebbing) in 100 blue cells.

RESULTS

The Cytoplasmic Domain of Fas Is Sufficient to Initiate an Apoptotic Signal. A chimeric cDNA (CD4/fas) including the cytoplasmic domain of murine Fas linked to the extracellular and transmembrane domains of murine CD4 (CD4/fas) was constructed. As control, mutant Fas with the point mutation of *lpr*^{cg} mice (T786A) was also expressed as an analogous chimeric molecule (CD4/fas786A). The chimeras were transfected into L cells and stable clones expressing equivalent levels of wild-type (CD4/fas) or mutant (CD4/fas786A) chimeric molecules were chosen for analysis (Fig. 1). L cells expressing CD4/fas (CD4/fas-16) underwent apoptotic cell death when crosslinked by monoclonal antibody against CD4 (L3T4; Caltag) in the presence of actinomycin D (9). DNA fragmentation, characteristic of apoptosis, was observed 2 hr after antibody crosslinking (Fig. 2*A*). Cells were shrunk and detached from the bottom of the culture dish at 10 hr (Fig. 2*B*). However, L cells expressing the mutant chimera (CD4/fas786A-23), under the same treatment, did not undergo apoptotic cell death (Fig. 2*C*). Multiple clones of each type were analyzed and gave the same results. We concluded that the cytoplasmic domain of Fas can initiate an apoptotic signal.

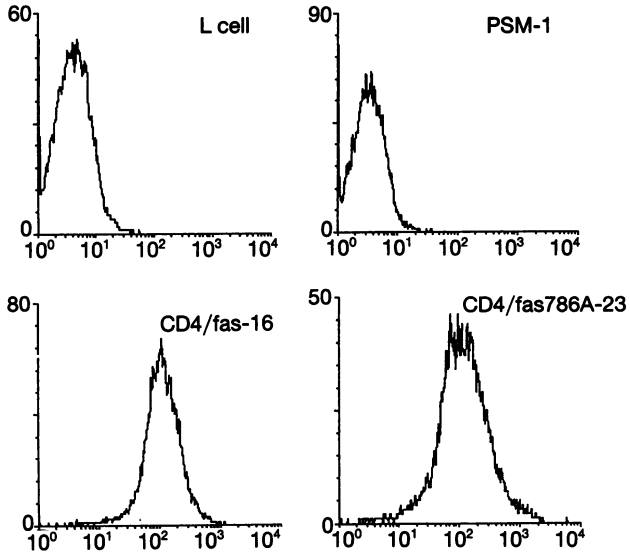

FIG. 1. Surface expression of CD4/fas and CD4/fas786A detected by FACS analysis. Single clones of untransfected L cells (L cells), L cells transfected with PSM vector alone (PSM-1), and L cells transfected with PSMCD4/fas (CD4/fas-16) or with PSMCD4/fas786A (CD4/fas786A-23) were analyzed by FACS (FACS IV; Becton Dickinson) as described in *Materials and Methods*.

Our results also show that dimerization or oligomerization of the Fas cytoplasmic domain is sufficient to generate an apoptotic signal. The antibody L3T4 (GK1.5), which we used to induce apoptosis through the Fas cytoplasmic domain, is a bivalent rat IgG-2b. As shown in Fig. 2*A*, there was no significant change upon the addition of a secondary anti-rat IgG antibody, suggesting that dimerization was sufficient for Fas activation.

Two-Hybrid Screening in Yeast Using the Cytoplasmic Domain of Fas. To simulate activated dimeric Fas, we constructed a fusion molecule of the λ repressor dimerization domain and the Fas cytoplasmic domain. The fusion molecule was then linked to the DNA binding domain of GAL4 for two-hybrid screening (7). As a control, we made a similar construction with the T786A mutation (*lpr*^{cg} mutation) in the Fas cytoplasmic domain.

More than 1.1×10^6 clones of a cDNA library from a murine T-cell line (a generous gift from S. Elledge, Baylor University, Houston) were screened for their ability to interact with the Fas fusion molecule in the two-hybrid system. Four independent clones interacted specifically with the wild-type Fas constructs but not the mutant Fas constructs (data not shown). Two clones had 2.2-kb inserts and two others had 2-kb inserts. Sequence analysis showed they were derived from the same gene and fused to the activation domain of GAL4 in the same reading frame. The inserts of the shorter clones were missing ≈150 bp at the 5′ end and ≈50 bp at the 3′ end of the sequence of the longer clones. A murine thymus cDNA library (a gift from M. Davis, Stanford University) was screened with a DNA probe including 0.7 kb of the 5′ end of the longer clone isolated by two-hybrid screening, and two independent cDNA clones of ≈2.6 kb were obtained.

Sequence analysis indicated that these were full-length cDNAs and contained an open reading frame encoding a protein of 649 amino acids (Fig. 3). The deduced molecular mass is 74 kDa and the pI is 4.6. The translation start site contains a perfect Kozak consensus sequence (11). There are two regions, amino acids 280–310 and 490–590, that are highly negatively charged and have a predicted α-helical secondary structure. There are three potential myristoylation sites located at amino acids 50, 306, and 310. There are also three N-glycosylation sites at amino acids 163, 209, and 423. No significant sequence homology of our clones was found with any protein sequence in available sequence data banks. This protein was named Fas-associated factor 1 (FAF1).

FAF1 Interacts Specifically with the Cytoplasmic Domain of Wild-Type Fas in COS Cells. To show that the association between FAF1 and Fas can occur in mammalian cells, FAF1 tagged with an influenza virus HA epitope was transiently coexpressed with CD4/fas or CD4/fas786A in COS cells. The level of FAF1 was approximately equivalent in the two types of cells (Fig. 4*A*). A much greater quantity of FAF1 was coimmunoprecipitated with CD4/fas (lane 3) than with CD4/fas786A (lane 4) (Fig. 4*B*), although more CD4/786A was immunoprecipitated than CD4/fas (Fig. 4*C*). As shown in Fig. 4, the molecular mass of FAF1 detected on SDS/polyacryl-

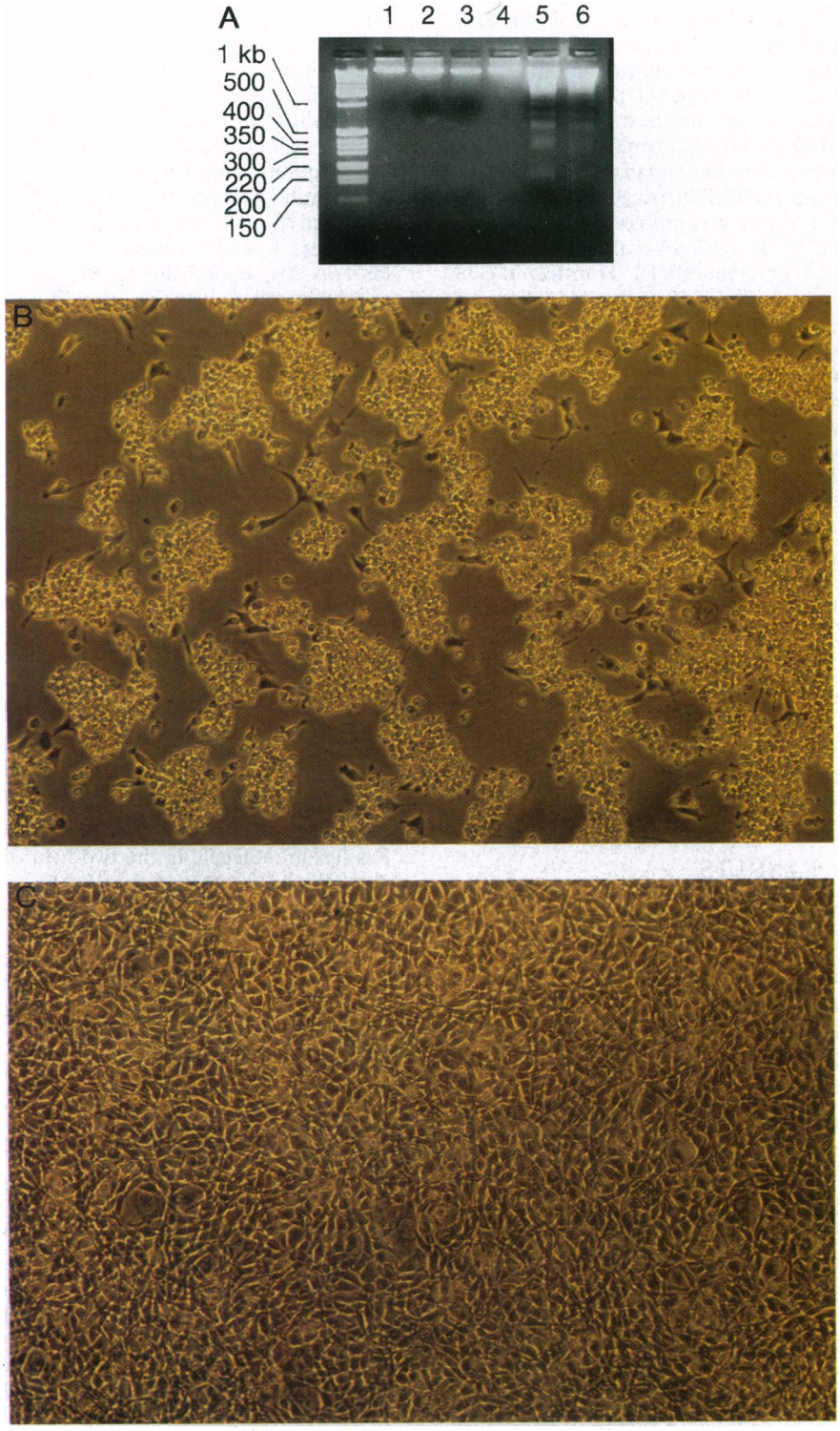

FIG. 2. (*A*) Fragmentation of DNA from CD4/fas786A-23 cells (lanes 1–3) and from CD4/fas-16 cells (lanes 4–6) after anti-CD4 crosslinking. Lanes: 1 and 4, DNAs from control cells incubated with actinomycin D only; 2 and 5, DNAs from cells crosslinked with L3T4 alone; 3 and 6, DNAs from cells incubated with both L3T4 and anti-rat IgG. (*B* and *C*) Morphology of cells after anti-CD4 crosslinking. Cells were treated as described in *Materials and Methods* and photographs were taken 10 hr later. (*B*) CD4/fas-16 cells. (*C*) CD4/fas786A-23 cells.

amide gel is 75–80 kDa, which is higher than the predicted 74 kDa. This could be accounted for by posttranslational modifications such as glycosylations. Thus, FAF1 specifically associated with the cytoplasmic domain of wild-type Fas in COS cells.

The observation that FAF1 binds to the Fas cytoplasmic domain is probably not an artifact caused by overexpression of FAF1 in COS cells because the level of FAF1 expression was relatively low (≈1/10th) compared to other proteins that we have expressed with the same expression vector (data not shown).

FAF1 Potentiates Apoptosis Mediated by Fas. To determine the significance of the association between FAF1 and Fas, FAF1 was transiently expressed in CD4/fas-16 and CD4/fas786A-23 cells. Different amounts of L3T4 antibody were incubated with the cells in the presence of actinomycin D as

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1                                                    20
MetAlaSer AsnMetAspLeu ProMetIle LeuAlaAsp PheGlnAlaCys ThrGlyIle
21                                                   40
GluAsnIle AspGluAlaIle ThrLeuLeu GluGlnAsn AsnTrpAspLeu ValAlaAla
41                                                   60
Ile Asn Gly ValIleProGln GluAsnGly IleLeu Gln SerAspPhe Gly GlyGluThr
61                                                   80
MetPro Gly ProThrPheAsp ProAlaSer HisProAla ProAla Ser ThrPro Ser Ser
81                                                   100
Ser Ala  PheArgProValMet Pro SerArg GlnIleVal GluArgGlnProArgMetLeu
101                                                  120
AspPheArgValGluTyrArg AspArgAsn ValAspValValLeuGluAspSerCysThr
121                                                  140
Val GlyGlu Ile LysGln Ile Leu GluAsn Glu Leu Gl IleProValPro LysMetLeu
141                                                  160
LeuLysGly TrpLysThrGly AspValGlu AspSerThr ValLeuLysSer LeuHisLeu
161                                                  180
ProLysAsn AsnSerLeuTyr ValLeuThr ProAspLeu ProProPro Ser Ser Ser Ser
181                                                  200
HisAlaGly AlaLeuGlnGlu SerLeuAsn GlnAsnPhe MetLeu Ile Ile Thr His Arg
201                                                  220
GluValGln ArgGluTyrAsn LeuAsnPhe SerGlySer SerThrValGln GluValLys
221                                                  240
ArgAsnVal TyrAspLeuThr SerIlePro ValArgHis GlnLeuTrpGlu Gly Trp Pro
241                                                  260
AlaSerAla ThrAspAspSer MetCysLeu AlaGluSer GlyLeuSerTyr ProCysHis
261                                                  280
ArgLeuThr ValGlyArgArg ThrSerPro ValGlnThr ArgGluGlnSer GluGluGln
281                                                  300
SerThrAsp ValHisMetVal SerAspSer AspGlyAsp AspPheGluAsp AlaSerGlu
301                                                  320
PheGlyVal AspAspGlyGlu ValPheGly MetAlaSer SerThrLeuArg LysSerPro
321                                                  340
MetMetPro GluAsnAlaGlu AsnGluGly AspAlaLeu LeuGlnPheThrAlaGluPhe
341                                                  360
SerSerArg TyrSerAspCys HisProVal PheTyrIle Gly Ser Leu Glu Ala Ala Phe
361                                                  380
GlnGluAla PheTyrValLys AlaArgAsp ArgLysLeu LeuAlaIleTyr Leu His His
381                                                  400
AspGluSer ValLeuThrAsn ValPheCys SerGlnMet LeuCysAlaGlu Ser IleVal
401                                                  420
SerTyrLeu SerGlnAsnPhe IleThrTrp AlaTrpAsp LeuThrLysAsp ThrAsnArg
421                                                  440
AlaArgPhe LeuThrMetCys AsnArgHis PheGlySer ValIleAlaGln Thr Ile Arg
441                                                  460
ThrGlnLys ThrAspGlnPhe ProLeuPhe LeuIleIle MetGlyLysArg Ser Ser Asn
461                                                  480
GluValLeu AsnValIleGln GlyAsnThr ThrValAsp GluLeuMetMet ArgLeuMet
481                                                  500
AlaAlaMet GluIlePheSer AlaGlnGln GlnGluAsp IleLysAspGlu AspGluArg
501                                                  520
GluAlaArg GluAsnValLys ArgGluGln AspGluAlaTyrArgLeuSer LeuGluAla
521                                                  540
AspArgAla LysArgGluAla HisGluArg GluMetAlaGluGlnPheArgLeuGluGln
541                                                  560
IleArgLys GluGlnGluGlu GluArgGlu AlaIleArg Leu Ser Leu Glu Gln AlaLeu
561                                                  580
ProProGlu ProLysGluGlu AsnAlaGlu ProValSer LysLeuArg Ile Arg Thr Pro
581                                                  600
SerGlyGlu PheLeuGluArg ArgPheLeu AlaSerAsn LysLeuGlnIle ValPheAsp
601                                                  620
PheValAla SerLysGlyPhe ProTrpAsp GluPheLys LeuLeuSerThr PheProArg
621                                                  640
ArgAspVal ThrGlnLeuAsp ProAsnLys SerLeuLeu GluValAsnLeu PheProGln
641                649
GluThrLeu PheLeuGlnAla LysGlu***>
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FIG. 3. Amino acid sequence deduced from nucleotide sequence of FAF1 cDNAs. Underlined sequences are the two α-helix domains.

described in *Materials and Methods.* In CD4/fas-16 cells, transient expression of FAF1 resulted in more rapid and extensive apoptosis than in mock-transfected cells (Fig. 5). One hour after addition of L3T4 crosslinking antibody (200 ng/ml) ≈60% of CD4/Fas-16 cells expressing FAF1 had undergone apoptotic cell death compared with 30% in the cells without FAF1 overexpression (Fig. 5). Increasing the L3T4 concentration to 1 μg/ml increased the apoptotic cell death to ≈70% from 40% with FAF1 overexpression (Fig. 5). There was no obvious apoptosis observed in CD4/fas786A-23 cells treated similarly (Fig. 5). Apoptosis induced through Fas was thus increased from 30–40% in the controls to 60–70% when FAF1 was expressed. Similar results were obtained in the human T-cell leukemia line Jurkat, where transient expression of FAF1 potentiated apoptosis induced by anti-human Fas antibody (data not shown). These data suggest that FAF1 can potentiate apoptosis mediated by Fas and acts downstream of Fas.

DISCUSSION

The TNF receptor family includes the following receptors: TNF receptors (P55 and P75), nerve growth factor receptor

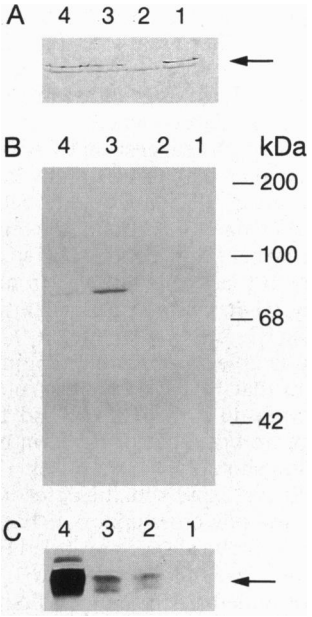

FIG. 4. Coimmunoprecipitation of CD4/fas and FAF1 in COS cells. (*A*) Expression of FAF1 in COS cells detected in whole-cell lysates by anti-HA epitope antibody (12CA5). Arrow indicates HA-tagged FAF1. (*B*) Western blot with 12CA5 after immunoprecipitation of CD4. (*C*) Immunoprecipitated chimeric molecules of CD4/fas or CD4/fas786A detected by Western blot. Blot from *B* was reprobed with anti-CD4 antiserum (gift from D. R. Littman). The COS cells were transfected with PSM plus PCGN8.1 (lane 1), PSMCD4/fas plus PCGN (lane 2), PSMCD4/fas plus PCGN8.1 (lane 3), or PSMCD4/fas786A plus PCGN8.1 (lane 4) as described. FAF1 expression level was determined by Western blot analysis with 12CA5 (*A*). Immunoprecipitation of CD4/Fas was done with anti-CD4 antibody and the Western blot was first probed with 12CA5 and developed by an alkaline phosphatase method to detect FAF1 (*B*). The antibody was then stripped off, and the blot was incubated with anti-CD4 antibody and developed by ECL to detect CD4/fas (*C*).

(low affinity), CD40, OX40, CD27, CD30, 4-1BB, and Fas (12). Receptors of this family can mediate very divergent biological responses, including cell proliferation, differentiation, and apoptosis (12–14). Fas is no exception and can not

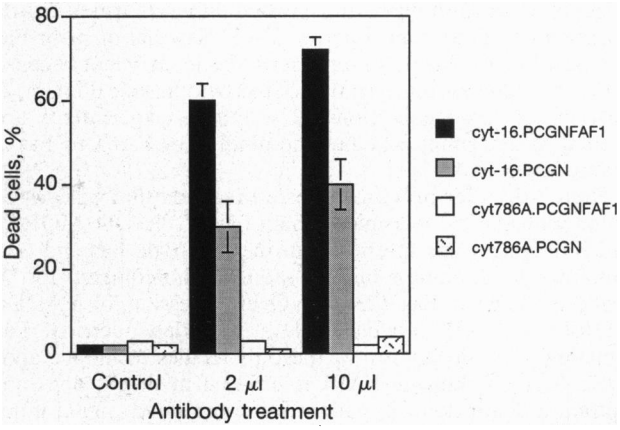

FIG. 5. Percentage of cells undergoing apoptosis 1 hr after L3T4 treatment. CD4/fas-16 and CD4/fas786A-23 cells were cotransfected with PSV-β-galactosidase and PCGN8.1 or PCGN as described. Transfectants were crosslinked by 10 μl (1 μg/ml) or 2 μl (200 ng/ml) of L3T4 or a control rat IgG as described in *Materials and Methods*. Cells were fixed 1 hr after antibody crosslinking and assayed for β-galactosidase expression. Blue cells were counted by light microscopy. Percentage of apoptotic cells is the number of cells with cell membrane blebbing among 100 blue cells counted. Bars indicate means ± SD for four independent experiments.

only induce apoptosis in many cell types but in certain cell types mediate cell proliferation (15, 16). Thus, Fas signal transduction provides a model system for studies on the molecular regulation of signals generated from a single receptor leading to different consequences.

In this study, our results suggest that FAF1 is an apoptotic signaling molecule that acts downstream in the Fas signal transduction pathway. FAF1 directly interacts with the wild-type cytoplasmic domain of Fas but not the mutant type of Fas that is deficient in signal transduction. This specific interaction occurred not only in yeast cells but also in mammalian cells. More importantly, when expressed transiently in both mouse fibroblast cell line (L cells) and human T-cell tumor line (Jurkat), FAF1 was able to potentiate apoptosis induced by Fas. This indicates that FAF1 is involved in the Fas-induced apoptotic signal transduction pathway and functions downstream of Fas. Future knockout of FAF1 in mice by homologous recombination should give an answer.

When FAF1 was overexpressed, the percentage of apoptotic cells induced by antibody crosslinking of Fas increased from 30% to 60% (Fig. 5). In the absence of added FAF1, Fas killing of cells is mediated by endogenous FAF1. Therefore, the apparent effect of added FAF1 may be limited because the amount of FAF1 is not the main rate-limiting factor in Fas-induced apoptosis of the cells tested. Overexpression of FAF1 in cells where FAF1 is a limiting factor should result in a greater increase in Fas-induced apoptosis.

It is not known yet how FAF1 functions in signal transduction during Fas-mediated apoptosis. The TNF receptor is thought to induce apoptosis by activating two types of sphingomyelinase—neutral and acidic (14, 17, 18). The neutral sphingomyelinase generates ceramide, which acts as secondary messenger to activate protein kinases/phosphatases and phospholipase A_2 (18). The acidic sphingomyelinase directs the activation of NF-κB (18). The cytoplasmic domains of Fas and P55 TNF receptor share a conserved "death domain" sequence (19), and mutagenesis studies have shown that conserved amino acids in this region of P55 TNF receptor and Fas are essential for their function (19, 20). This structural similarity between Fas and TNF receptor suggests that Fas and perhaps FAF1 might also activate a sphingomyelin pathway (21).

In the two-hybrid screening, we used the Fas cytoplasmic domain fused to the λ repressor dimerization domain as the target in order to simulate an activated dimeric form of Fas. It is difficult to determine whether FAF1 association with Fas requires dimeric Fas or a monomeric Fas is sufficient because of the spontaneous dimerization of Fas cytoplasmic domain, at least under some conditions (22). Future experiments are needed to determine whether the binding of FAF1 to Fas is ligand dependent.

There are other proteins reported recently that were associated with the cytoplasmic domain of Fas. FADD/MORT1 and RIP specifically interact with the wild-type Fas and also contain a death domain in their sequence homologous to the death domain of Fas (23–25). Overexpression of FADD/MORT1 and RIP induced cell death independent of Fas signaling. The mechanism of these molecules to induce apoptosis is still unknown. FAF1 is unusual in that it does not contain a death domain, cannot initiate a death signal independently, and can augment a Fas-induced apoptotic signal. FAF1 may be a signaling molecule downstream of the death domain-containing proteins during apoptotic signal transduction. Further studies on the function of FAF1 and the interaction of Fas, FAF1, FADD/MORT1, and RIP should provide insight into the mechanism of how an apoptotic signal(s) is transduced from the plasma membrane into the cytoplasm.

In summary, our data suggest that FAF1 is a downstream target of activated Fas and, as such, is a candidate for Fas signaling effectors. Understanding the function of this downstream molecule should lead to a better understanding of the mechanisms by which divergent biological responses are mediated by different members of the TNF/nerve growth factor receptor family.

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