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c-Jun NH_2-Terminal Kinase (JNK)-Dependent Nuclear Translocation of Apoptosis-Inducing Factor (AIF) Following Engagement of Membrane Immunoglobulin on WEHI-231 B Lymphoma Cells

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Abstract WEHI-231 B lymphoma cells have been employed for analysis of antigen-induced B cell unresponsiveness because these cells undergo cell cycle arrest in G1, accompanied by induction of apoptosis. In the present study, we examined the requirement for toxic small molecules apoptosis-inducing factor (AIF) and cytochrome *c*, and subsequent caspase activation in apoptotic cell death in WEHI-231 and CH31 B lymphoma cells following engagement of membrane immunoglobulin (mIg). Pan-caspase inhibitor BD-fmk blocked mIg-mediated increase in cells with sub-G1 DNA content, whereas it did not affect mIg-mediated loss of mitochondrial membrane potential and phosphatidylserine exposure on B cell membrane. Dominant-negative form of c-Jun NH_2-terminal kinase1 (JNK1) blocked the translocation of AIF into the nuclei and cytosol from the mitochondria in the WEHI-231 and CH31 cells following mIg engagement, whereas constitutively active form of JNK1 enhanced it. This AIF translocation was also blocked by Bcl-xL, but not by BD-fmk. Moreover, AIF-deficient clones via small interfering RNA (siRNA)-mediated method showed small increase in loss of mitochondrial membrane potential. After mIg engagement, the AIF-deficient clones displayed an enhanced sensitivity to mIg-mediated apoptosis, concomitant with translocation of a residual AIF into the nuclei, compared with control clone. Our findings are compatible with the notion that AIF has dual role, with a proapoptotic function in the nuclei and a distinct anti-apoptotic function in the mitochondria. These observations would be valuable for analysis of B cell unresponsiveness and hopefully for treatment of diseases involving B cell dysfunction. J. Cell. Biochem. 104: 1927–1936, 2008.

Key words: B lymphocytes; WEHI-231; apoptosis; caspases; c-Jun N-terminal kinase

Engagement of membrane immunoglobulin (mIg) plays a crucial role in elimination of high-affinity self-reactive B cells in several maturational stages, including immature and germinal center B cells [MacLennan, 1994; Nossal, 1994; Monroe, 2000; Rathmell and Thompson, 2002]. The mIg-mediated elimination of self-reactive B cells could be due to apoptotic cell death, resulting in induction of B cell unresponsiveness [Donjerkovic and Scott, 2000; Defrance et al., 2002; Deming and Rathmell, 2006]. However, the molecular mechanisms underlying mIg-mediated apoptosis remain incompletely understood. WEHI-231 and CH31 B lymphoma cells representing immature B cells served as a model for B cell unresponsiveness in this study because they undergo growth arrest and apoptosis following mIg engagement [Scott et al., 1986; DeFranco et al., 1987; Takada et al., 2001].

Apoptotic signaling pathways converge on mitochondrion in diverse cell types, including B cells [Reed, 1998; Susin et al., 1998; Gross et al., 1999]. Mitochondrial function is regulated by several components, including Bcl-2 family proteins, caspases, and mitogen-activated protein kinases (MAPKs) [Chao and Korsmeyer, 1998; Reed, 1998; Davis, 2000; Chang and Karin, 2001]. We and others have

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demonstrated that a prolonged activation of c-Jun NH_2-terminal kinase (JNK)1, a member of the MAPK family, occurs upon apoptotic stimulation and is involved in mitochondrial dysfunction in several cell types, including B cells [Xia et al., 1995; Graves et al., 1996; Takada et al., 2001, 2006]. Mitochondria contain several proapoptotic molecules in the intermembrane space, and some of the molecules, including apoptosis-inducing factor (AIF) and cytochrome *c*, are released from mitochondria to the cytosol and/or nuclei in response to apoptotic stimuli [Hong et al., 2004; Saelens et al., 2004]. Cytosolic cytochrome *c* and Apaf-1 in combination with ATP form apoptosomes, leading to activation of procaspase-9 through dimerization [Saelens et al., 2004]. Caspase-9 in turn activates effector caspases, such as caspase-3 and caspase-7, in some cell types. In contrast to these findings, there is some debate about whether mIg engagement induces activation of caspase-9 in combination with cytosolic cytochrome *c* [Bras et al., 1999; Ruiz-Vela et al., 1999; Herold et al., 2002]. Moreover, the dominant-negative form of caspase-9 prevented mIg-mediated cell death only modestly [Herold et al., 2002], suggesting that signaling molecule(s) other than those in the cytochrome *c*/caspase-9 pathways play some role in the mIg-mediated cell death. Although the pan-caspase inhibitor Z-VAD-fmk reduced some features of apoptosis, such as DNA fragmentation and chromatin condensation [Bras et al., 1999; Chen et al., 1998], this inhibition required higher concentrations of Z-VAD, exhibiting less specificity for caspases. These observations suggest that mIg-mediated cell death involves several distinct pathways in immature B cells. Indeed, ceramide generation and activation of cathepsin-B are involved in mIg-mediated apoptosis [Chen et al., 1998; Katz et al., 2001]. Recently, AIF has been demonstrated to induce caspase-independent cell death in some cell types [Joza et al., 2001; Hong et al., 2004; Porter and Urbano, 2006]. Upon apoptotic stimuli, AIF migrates from mitochondria to nuclei, where it might contribute to nuclear degradation [Daugas et al., 2000; Saelens et al., 2004]. In contrast, AIF within the mitochondria helps to generate energy through oxidative phosphorylation, contributing to cell survival [Cheung et al., 2006; Porter and Urbano, 2006].

In the present study, we examined the role for mitochondrial death-promoting factors AIF and cytochrome *c*, and the requirement for caspase activation in mIg-mediated apoptosis, and found that some form(s) of apoptotic phenotypes are caspase-dependent, while others are caspase-independent. The caspase-independent cell death might be due to the translocation of AIF into the nuclei, whereas AIF in the mitochondria functions as survival molecule, probably via oxidative phosphorylation. These findings suggest that AIF has both survival and proapoptotic functions in B lymphoma cells. Possible molecular mechanisms underlying JNK-mediated AIF-induced cell death will be discussed.

EXPERIMENTAL PROCEDURES

Cell Culture

WEHI-231 cells were maintained in RPMI-1640 medium consisting of 10% fetal bovine serum (FBS), 50 μM 2-mercaptoethanol, and 100 μg/ml kanamycin. CH31 cells were maintained in the RPMI-1640-based media for WEHI-231 cells by further adding 1 mM sodium pyruvate and 0.1 mM non-essential amino acid. Cells were cultured with anti-IgM (rat IgG, Bet-1 and Bet2; American Type Culture Collection, Manassas, VA) or actinomycin D for the indicated times. WEHI-231 and CH31 cells overexpressing dominant-negative JNK1 (dnJNK1) or a constitutively active JNK1 (MKK7-JNK1) constructs were employed, as previously reported [Takada et al., 2001, 2005]. In some experiments, WEHI-231 cells were pretreated with the pan-caspase inhibitor BD-fmk (dissolved in DMSO; Enzyme Systems Products, Aurora, OH) for 2 h, and then stimulated with anti-IgM, as described above.

Subcellular Fractionation

Cells stimulated with anti-IgM or cultured with the medium alone were fractionated as previously described [Takada et al., 2006]. Briefly, cells were lysed in a hypotonic buffer and homogenized. Samples were centrifuged at low speed to remove nuclei. The resulting pellet was used as the enriched nuclear fraction. The supernatants were centrifuged to obtain a rough mitochondrial fraction (pellet) and the resulting supernatant was centrifuged for the cytosol.

Western Blotting

Western blotting was carried out as previously described [Takada et al., 2006]. Briefly, cells were solubilized in lysis buffer (50 mM

Tris–HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotinin) on ice for 30 min. Samples were resolved on SDS–PAGE and transferred to PVDF membranes. Blots were incubated with a primary antibody (Ab): anti-AIF (Santa Cruz Biotechnology, Santa Cruz, CA), anti-PARP, anti-Cox (BD Biosciences, San Jose, CA), or anti-actin (Sigma-Aldrich, St. Louis, MO). After several washes, they were developed using a horseradish peroxidase-labeled second Ab (HRP-anti-rabbit IgG or HRP-anti-mouse IgG: Cappel, Aurora, OH), and enhanced chemiluminescence was assayed according to the manufacturer's recommendations (Amersham Biosciences, Piscataway, NJ). The density of each band was measured using NIH image software (Bethesda, MD).

Analysis of Flow Cytometry of Apoptotic Cells and Loss of Mitochondrial Membrane Potential

Induction of apoptosis and mitochondrial membrane potential was determined by propidium iodide (PI), annexin V-Cy5, and $DiOC_6$ staining methods, respectively, as previously described [Takada et al., 2005].

RNA Interference

For RNA interference from retroviral vectors, short hairpin small interference RNA (siRNA) constructs were designed to target AIF. AIF siRNA was derived from nt 1,150–1,168 in the sequence with accession no. nm_012019. The oligonucleotide sequences were the following: sense, 5′-GATCCGGATGGAAGGAAGGTAGAAATTCAAGAGATTTCTACCTTCCTTCCATCCTTTTTTG-3′; anti-sense, 5′-AATTCAAAAAAGGATGGAAGGAAGGTAGAAATCTCTTGAATTTCTACCTTCCTTCCATCCG-3′. The annealed oligonucleotides were subcloned into the pSIREN-RetroQ vector (BD Biosciences), resulting in pSIREN-RetroQ/AIF, as previously reported [Hata et al., 2007]. WEHI-231 cells were transfected with either pSIREN-RetroQ/AIF or the control pSIREN-RetroQ/Luc, which encodes luciferase siRNA. Resulting transformants were selected in the presence of puromycin (0.8 μg/ml), followed by a limiting dilution to obtain clones. Western blotting was used to determine the AIF levels in the clones.

Statistical Analysis

Data are expressed as the mean ± SD for each group. Statistical significance was determined by Student's t-test, and a difference of $P < 0.05$ was considered significant.

RESULTS

Pan-Caspase Inhibitor BD-fmk Abrogates Anti-IgM-Induced Increase in Cells With Sub-G1 DNA Content, But Not Loss of Mitochondrial Membrane Potential or Phosphatidylserine Exposure

It is not completely understood whether activation of caspases is involved in apoptotic cell death following mIg engagement of immature B cells. To address the requirement for caspases in mIg-mediated apoptotic processes in WEHI-231 cells representing immature B cells, cells were pretreated with the pan-caspase inhibitor BD-fmk (25, 50, or 100 μM) for 2 h and then stimulated with 10 μg/ml anti-IgM for a further 24 or 48 h. The cells were then assessed by PI staining method, which detects cells with sub-G1 DNA content. Pretreatment with BD-fmk had reduced increase in sub-G1 fraction induced by anti-IgM in a dose-dependent manner 48 h after stimulation (Fig. 1A). No sub-G1 fraction was detected 24 h after mIg engagement. The sub-G1 fraction induced by actinomycin D was also abrogated by BD-fmk. However, BD-fmk pretreatment did not affect mIg-mediated mitochondrial disruption (Fig. 1B) or phosphatidylserine exposure (Fig. 1C). These results suggest that mIg-induced apoptotic processes are regulated in both caspase-dependent and -independent fashions.

Anti-IgM Induces AIF Translocation Into the Nuclei and Cytosol From Mitochondria

AIF has been demonstrated to induce some features of apoptosis, including large-scale DNA fragmentation and phosphatidylserine exposure in a caspase-independent fashion [Susin et al., 1999; Daugas et al., 2000]. Upon apoptotic stimulation, mitochondrial AIF migrates into the nuclei in some cells, where it plays a crucial role in apoptosis induction [Cheung et al., 2006]. Therefore, we examined whether mIg engagement induces translocation of AIF in WEHI-231 cells. Cells were stimulated with anti-IgM for 24 and 48 h, followed by assay for AIF expression. In unstimulated cells, AIF was present mainly in the mitochondria, and small amount was found in the nuclei (Fig. 2A). AIF translocated into the nuclei and cytosol from the mitochondria 48 h

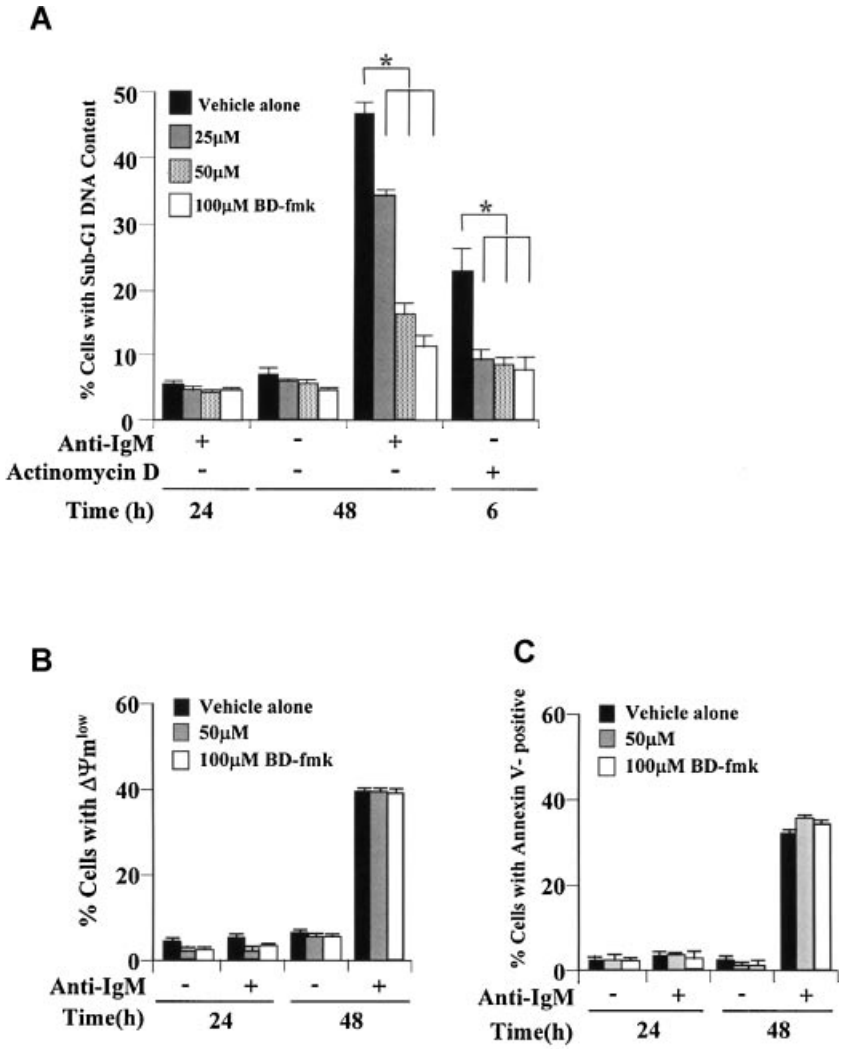

Fig. 1. Pan-caspase inhibitor BD-fmk reduces anti-IgM-induced increase in sub-G1 fraction, without apparent effect on mitochondrial membrane potential and phosphatidylserine exposure. WEHI-231 B lymphoma cells were pretreated with BD-fmk for 2 h, and then exposed to 10 μg/ml anti-IgM for 24 and 48 h, followed by assay for PI staining (**A**), ΔΨm (**B**), and annexin V staining (**C**). As a control, cells were also stimulated with actinomycin D. Results are shown as the mean ± SD of three independent experiments. *$P < 0.05$, vehicle control versus inhibitor.

after stimulation with anti-IgM. The mitochondrial translocation of AIF was also detected 24 h after anti-IgM stimulation in another B lymphoma cell line CH31, which is more sensitive to anti-IgM-induced apoptosis than WEHI-231 (Fig. 2B). The mitochondrial fractions were not contaminated with the nuclei and/or cytosolic fractions, based on the findings that PARP, COX, and actin were specifically found in nuclei, mitochondria, and cytosol, respectively.

Unlike AIF, cytochrome *c* release into the cytosol from the mitochondria was confirmed to be undetected up to 72 h after anti-IgM (Fig. 2C), as reported by Ruiz-Vela et al. [1999]. As expected, actinomycin D induced the relocation of cytochrome *c* into the cytosol. These findings suggest that mIg engagement induces translocation of AIF, but not cytochrome *c*, into the nuclei and cytosol from the mitochondria.

Anti-IgM Induces JNK1-Mediated Translocation of AIF Into the Nuclei and Cytosol

We recently demonstrated that sustained mIg-mediated activation of JNK1 is involved

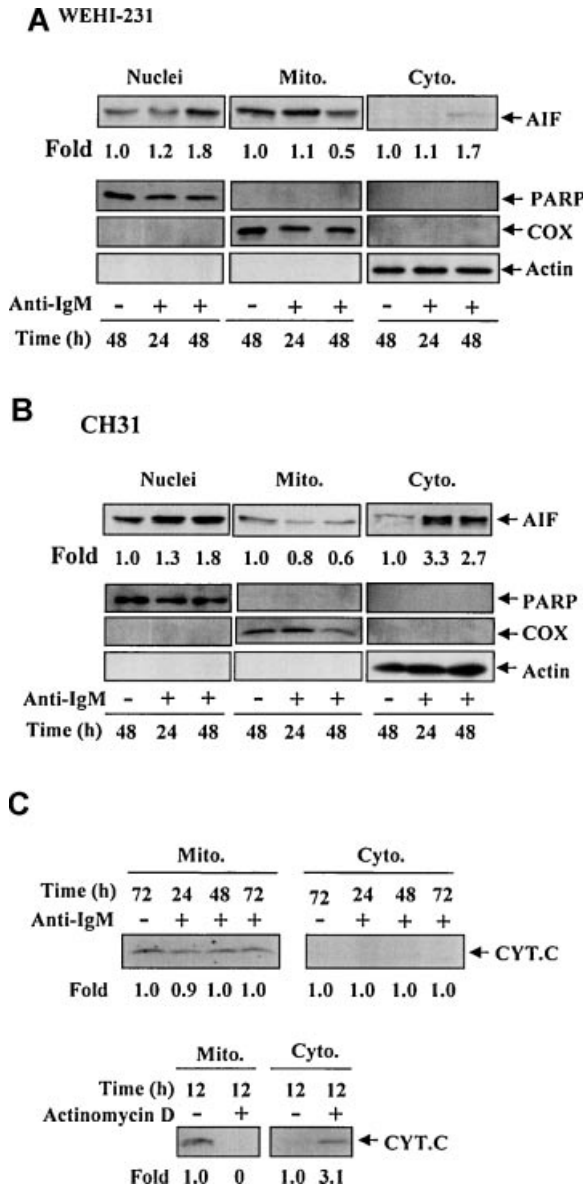

Fig. 2. Anti-IgM induces AIF translocation into the nuclei and cytosol from the mitochondria. WEHI-231 (**A**,**C**) and CH31 (**B**) cells were cultured with 10 μg/ml anti-IgM (A,B) or 0.2 μg/ml actinomycin D (C) for the indicated periods, and cell lysates were fractionated into the cytosol, mitochondria, and nuclei. Levels of AIF and cytochrome *c* were determined by Western blotting and expressed as a fold of the AIF or cytochrome *c* level from unstimulated cells. As controls to confirm the fidelity of the fractionation procedure, the expression of PARP, COX, and actin was also determined. Experiments were done three times with essentially similar results.

in mitochondrial disruption and apoptotic cell death in both WEHI-231 and CH31 B lymphoma cells [Takada et al., 2001, 2006]. To address whether JNK activation is implicated in the AIF translocation, WEHI-231 and CH31 cells overexpressing dnJNK1, MKK7-JNK1, or the control vector alone were employed [Takada et al., 2001, 2005] (Fig. 3A). The dnJNK1-overexpressing WEHI-231 or control cells were stimulated with anti-IgM for the indicated periods, and AIF expression levels in the mitochondria, nuclei, and cytosol were determined. The mIg-mediated translocation of

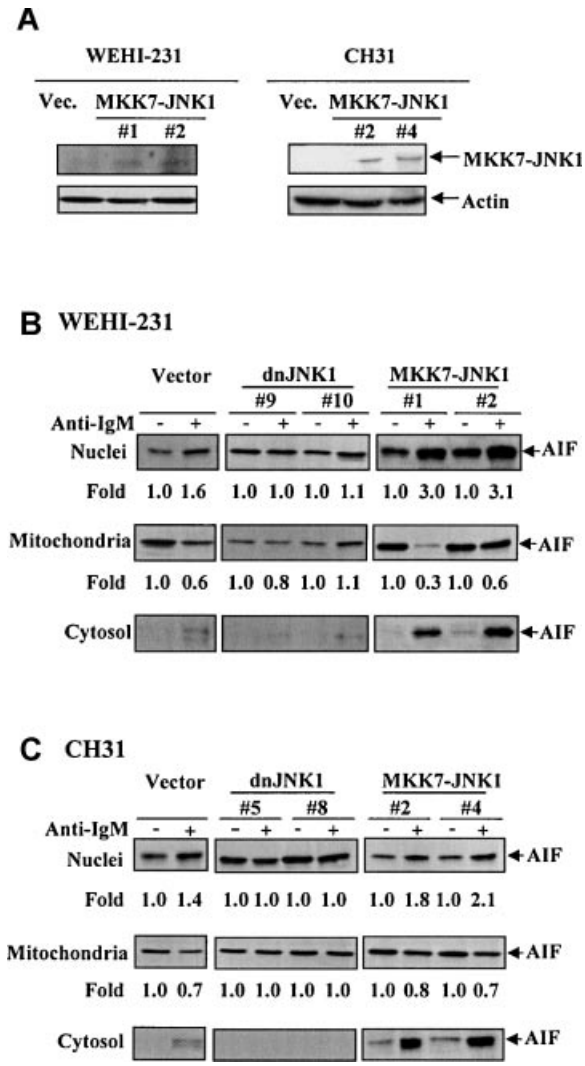

Fig. 3. Reduced translocation into the nuclei and cytosol from the mitochondria is found in the cells expressing dnJNK1, whereas increased translocation is found in those expressing MKK7-JNK1, compared with vector control. Levels of MKK7-JNK1 protein in the WEHI-231 and CH31 clones expressing MKK7-JNK1 were assessed by Western blotting (**A**). WEHI-231 (**B**) and CH31 (**C**) clones overexpressing dnJNK1, MKK7-JNK1, or vector alone were stimulated with anti-IgM or medium alone for 48 h, followed by assay for AIF expression of nuclei, mitochondria, and cytosolic fractions. Levels of AIF were expressed, as described in Figure 2. Experiments were done three times with essentially similar results.

AIF to both the nuclei and cytosol in the dnJNK1 clones (#9 and #10) was substantially abrogated in comparison with the vector control (Fig. 3B), suggesting that JNK1 activation is implicated in the mIg-mediated AIF translocation. Consistent with this observation, a constitutively active form of JNK1, MKK7-JNK1 (#1 and #2), considerably enhanced the anti-IgM-induced AIF translocation into the nuclei and cytosol compared with control. A similar profile of the AIF translocation was found in CH31 clones overexpressing dnJNK1 or MKK7-JNK1 (Fig. 3C). Together, these results indicate that the anti-IgM-induced AIF translocation into the nuclei and cytosol from the mitochondria involves JNK activation in B lymphoma cells.

Bcl-xL, But Not Pan-Caspase Inhibitor BD-fmk, Prevents the anti-IgM-Induced Translocation of AIF

mIg engagement of WEHI-231 cells has been shown to initiate caspase-7 activation independent of cytochrome *c* release into the cytosol from mitochondria [Ruiz-Vela et al., 1999]. To address whether the mIg-mediated AIF translocation requires caspase activation, cells pretreated with 50 μM BD-fmk for 2 h were stimulated with 10 μg/ml anti-IgM for a further 48 h, followed by assay for AIF expression. The mIg-mediated AIF translocation was unaffected in the presence of BD-fmk (Fig. 4), suggesting that caspases are not necessary for mIg-mediated AIF translocation.

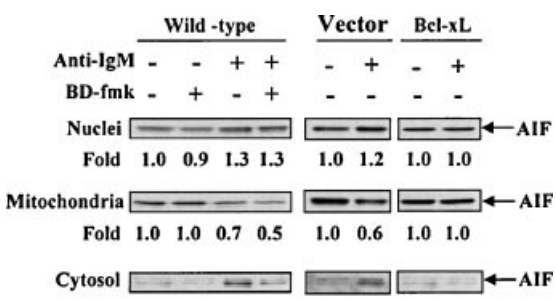

Fig. 4. Anti-IgM-induced translocation of AIF is prevented by Bcl-xL but not pan-caspase inhibitor BD-fmk. WEHI-231 cells preincubated with 50 μM BD-fmk for 2 h were stimulated with 10 μg/ml anti-IgM for a further 48 h, followed by assay for AIF expression in the nuclei, cytosol, and mitochondria, respectively. WEHI-231 cells expressing Bcl-xL or control vector alone were also stimulated with anti-IgM, followed by assay for AIF expression. Levels of AIF were expressed, as described in Figure 2. Experiments were done three times with essentially similar results.

The Bcl-2 family member Bcl-xL reduced mIg-mediated induction of apoptosis in WEHI-231 cells [Choi et al., 1995]. To address whether Bcl-xL is implicated in the mIg-mediated AIF translocation, WEHI-231 cells expressing exogenous Bcl-xL (levels equivalent to endogenous Bcl-xL) were stimulated with 10 μg/ml anti-IgM for 48 h. The mIg-mediated translocation was substantially prevented in the Bcl-xL-overexpressing cells compared with the control (Fig. 4). Thus, mIg-mediated JNK-induced AIF translocation is upstream or independent of caspase activation in B cells, which is prevented by Bcl-xL.

AIF Deficient Clones Display Increased Sensitivity to Anti-IgM-Induced Apoptotic Cell Death

To examine whether mIg-mediated AIF relocation is associated with apoptotic cell death, we generated stable AIF-deficient clones via siRNA-mediated knockdown approach in WEHI-231 cells. The two independent clones (#1, #3) showed approximately 75–90% reduction in AIF protein levels, compared with control clone with irrelevant siRNA (Fig. 5A). After anti-IgM stimulation, these AIF-deficient clones displayed translocation of residual AIF into the nuclei, concomitant decrease in mitochondrial AIF levels lower than control clone (Fig. 5B). The AIF-deficient clones showed an enhanced sensitivity to anti-IgM, as assessed by loss of mitochondrial membrane potential (Fig. 5C), annexin-positive cells (Fig. 5D), and sub-G1 fraction (Fig. 5E). Interestingly, the AIF-deficient clones showed a small increase in loss of $\Delta\Psi m$ compared with control, suggesting that AIF within the mitochondria serves as survival molecule. Together, AIF might serve as anti-apoptotic as well as proapoptotic molecule in B lymphoma cells.

DISCUSSION

Engagement of mIg of WEHI-231 cells, a model for analysis of antigen-induced unresponsiveness in immature B cells, results in apoptotic cell death, accompanied by loss of mitochondrial membrane potential [Donjerkovic and Scott, 2000; Defrance et al., 2002; Deming and Rathmell, 2006]. Whether mIg engagement of WEHI-231 cells induces mitochondrial cytochrome *c* release into the cytosol remains to be established [Ruiz-Vela et al., 1999; Herold et al., 2002], although caspase-7

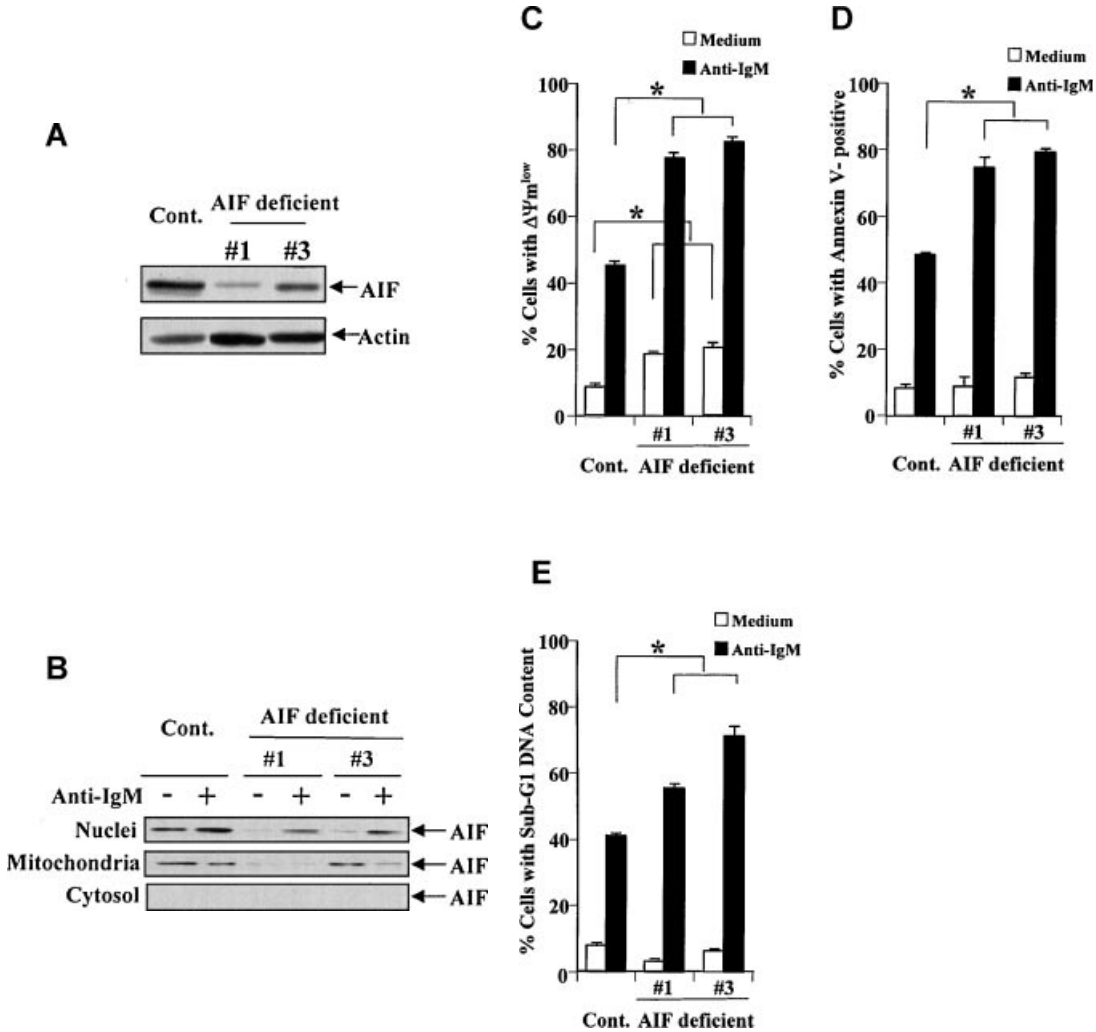

Fig. 5. AIF-deficient clones via RNA interference display increased sensitivity to anti-IgM-induced apoptosis. Two AIF-deficient and one control clones were assayed for AIF expression (**A**). After stimulation with anti-IgM for the indicated times, cell lysates from these clones were fractionated into the mitochondria, cytosol, and nuclei, followed by assay for AIF expression (**B**). These clones were also assayed for mitochondrial membrane potential (**C**), annexin V staining (**D**), and sub-G1 DNA content (**E**). Experiments were done three times with essentially similar results. $^*P < 0.05$, control versus AIF-deficient clones.

activation takes place upon mIg ligation, independent of cytochrome *c* release [Bras et al., 1999]. However, dominant negative caspase-9 did not completely abrogate the mIg-mediated cell death of WEHI-231 cells [Herold et al., 2002], suggesting that mIg-mediated apoptotic cell death occurs in at least a caspase-independent manner. In the present study, we examined molecular mechanism underlying mIg-mediated caspase-independent apoptotic processes probably involving AIF in WEHI-231 and CH31 cells.

A variety of apoptotic signaling pathways converge on the mitochondrion, which is thought to play a central role in the decision for cell death [Reed, 1998; Susin et al., 1998; Gross et al., 1999]. Upon apoptotic stimulation, mitochondrial permeabilization occurs, accompanied by mitochondrial cytochrome *c* release into the cytosol in some cell types. In combination with Apaf-1, ATP, and caspase-9, cytochrome *c* provokes assembly of apoptosomes, leading to activation of effector caspases such as caspase-3 [Susin et al., 1998]. Caspase-3 activation was induced in germinal center B cells after mIg engagement [van Eijk and de Groot, 1999]. However, release of cytochrome *c* into the cytosol was not detected in WEHI-231 cells, representing immature B cells, upon

mIg engagement [Ruiz-Vela et al., 1999] (Fig. 2C). These results suggest that cytochrome *c*/apoptosome-mediated caspase activation is differentially employed depending on the maturational stage of B cells.

The role of activation of caspases in mIg-mediated apoptosis in WEHI-231 cells remains to be elucidated, although caspases are activated upon mIg engagement [Bras et al., 1999; Ruiz-Vela et al., 1999]. For example, mIg engagement caused caspase-7 activation at least through activation of calpain, probably independent of cytochrome *c*-mediated apoptosome assembly [Ruiz-Vela et al., 1999]. The pan-caspase inhibitor BD-fmk abrogated anti-IgM-induced increase in sub-G1 fraction [Bras et al., 1999] (Fig. 1A), but not mitochondrial depolarization (Fig. 1B) and phosphatidylserine exposure (Fig. 1C). These results suggest that some feature(s) of apoptotic cell death require caspase activation, but other form(s) of cell death are caspase-independent, probably involving AIF. Indeed, anti-IgM induced translocation of AIF into the nuclei and cytosol (Fig. 2A,B). Consistent with our findings, toxic small molecule AIF within the mitochondria has been reported to translocate into the nuclei and/or cytosol upon apoptotic stimuli in some cell types [Saelens et al., 2004] where AIF induces large-scale DNA fragmentation (more than 50 kb) in a caspase-independent manner [Susin et al., 1999; Daugas et al., 2000].

Several activation pathways result in AIF translocation into the nuclei and/or cytosol [Saelens et al., 2004]. We recently demonstrated that sustained mIg-mediated activation of JNK1 initiates migration of Bax from the cytosol to the mitochondria [Takada et al., 2006], leading to mitochondrial permeabilization, probably through formation of a heterodimer of Bax with BimL. Bax-mediated mitochondrial permeabilization might participate in the release of AIF into the cytosol or nuclei. Indeed, AIF mediated Bax-dependent cell death in neuronal cells [Cheung et al., 2005]. This JNK-mediated pro-apoptotic action of Bax is antagonized by anti-apoptotic molecules such as Bcl-xL and Bcl-2. Consistent with this notion, mIg-mediated AIF translocation was abrogated by dnJNK1 (Fig. 3) and BCl-xL (Fig. 4) and promoted by the active form of JNK MKK7-JNK1 (Fig. 3). Thus, mIg-mediated JNK activation regulates AIF translocation into the nuclei and/or cytosol.

Whether the mIg-mediated mitochondrial AIF release involves caspase activation remains to be established [Daugas et al., 2000]. Pretreatment with pan-caspase inhibitor BD-fmk did not affect mIg-mediated AIF translocation from mitochondria (Fig. 4). In contrast, another study showed that AIF failed to translocate into the nuclei or cytosol from mitochondria when caspase activity was suppressed, suggesting that caspase is required for AIF translocation [Arnoult et al., 2003]. Further studies are required to resolve these apparently contradictory observations.

Some controversy arises on the role of AIF in cell death upon diverse stimuli in several cell types [Lipton and Bossy-Wetzel, 2002; Porter and Urbano, 2006; Srivastava et al., 2007]. Thus, gene silencing of AIF with siRNA protected some cells from apoptosis [Cheung et al., 2005; Seth et al., 2005], suggesting that AIF contribute to cell death via nuclear translocation in response to some stresses. Conversely, AIF-deficient cells displayed a failure to grow in soft agar [Urbano et al., 2005] and an enhanced sensitivity to other insults [Urbano et al., 2005; Srivastava et al., 2007], suggesting that AIF plays a crucial role in survival probably via oxidative phosphorylation and energy generation. Indeed, our AIF-deficient clones showed a slower growth than control clone (Takada et al., unpublished work) and an increased sensitivity to anti-IgM-induced apoptosis (Fig. 5C–E). These findings suggest that AIF functions as both survival and proapoptotic component, dependent on the cell lineage and insults.

Understanding the molecular pathways leading to antigen-induced apoptosis is essential for analysis of mIg-mediated tolerance in B cells, and hopefully for elucidation of the molecular mechanisms of autoimmune diseases. In the present study, we clearly demonstrated that mIg engagement induces AIF relocation into the nuclei and cytosol from the mitochondria, which is dependent on JNK activation. Our present data support the notion that AIF within the mitochondria contributes to survival, while AIF relocation into the nuclei contributes to apoptosis.

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