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Sensitization of AIDS-Kaposi’s Sarcoma Cells to Apo-2 Ligand-Induced Apoptosis by Actinomycin D

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Kaposi’s sarcoma (KS) is the most frequent malignancy associated with HIV infection (AIDS-KS), a complication that leads to high mortality and morbidity. AIDS-KS cells are resistant to killing by chemotherapeutic drugs/NK cells and Fas-induced apoptosis, suggesting that the acquisition of antiapoptotic characteristics by AIDS-KS cells may contribute to their prolonged survival. Apo-2 ligand (Apo-2L)/TNF-related apoptosis-inducing ligand, a new member of the TNF family, has been identified as an apoptosis-inducing molecule. In this study we examined the sensitivity of 10 different AIDS-KS isolates to Apo-2L-mediated cytotoxicity. AIDS-KS cells were relatively resistant to Apo-2L; however, Apo-2L and actinomycin D (Act D) used in combination synergistically potentiated the induction of cell death in nine of the 10 isolates. Apo-2L induced apoptosis in >80% of AIDS-KS cells pretreated with Act D. The caspase inhibitors, zIETD-fmk and zDEVD-fmk, inhibited apoptosis in AIDS-KS by sApo-2L, suggesting that caspase 3-like and caspase 8 or 10 activities are essential for Apo-2L-mediated apoptosis. Act D treatment of AIDS-KS cells markedly and selectively down-regulated Bcl-xL expression, while the expressions of decay receptors 1 and 2, Bax, cellular FLICE (Fas-associated death domain protein-like IL-1-converting enzyme) inhibitory protein, FADD (Fas-associated death domain protein), procaspase 8, and p53 were not affected. These findings suggest the possible involvement of Bcl-xL in Act D-induced sensitization of AIDS-KS cells to Apo-2L-mediated apoptosis. Furthermore, Act D did not sensitize PBMC or fibroblast cells to Apo-2L. Thus, Apo-2L and Act D used in combination may be of therapeutic value in the treatment of AIDS-KS. The Journal of Immunology, 1999, 162: 5616–5623.

Two death domain-containing receptors, DR4 and DR5, have been identified as Apo-2L/TRAIL receptors (4–12). Unlike the Fas ligand, the expression of Apo-2L/TRAIL and its receptors is observed in various normal human tissues, suggesting that Apo-2L/TRAIL must not be cytotoxic to most normal tissues in vivo (13). It has also been reported that Apo-2L/TRAIL can bind a decoy receptor 1 (Dr61; also called TRID, TRAIL-R3, or LIT) (5, 6, 11, 12, 14, 15) and DcR2 (also called TRAIL-R4 or TRUNDD) (16–18); however, these receptors cannot transduce the apoptotic signal into the cells because of their lack of functional death domains. Normal cells express high levels of Dr61 and DcR2, while malignant cells express only minimum amounts of these receptors (13). Therefore, it is hypothesized that resistance of normal cells to Apo-2L/TRAIL may be due to the expression of Dr61 and DcR2.

Kaposi’s sarcoma (KS) is the most common malignancy arising in persons with HIV infection (AIDS-KS). The clinical course of AIDS-KS is highly variable, ranging from a minimal disease presenting as an incidental finding, to a rapidly progressive or extensive disease resulting in significant morbidity and mortality (19). Extracutaneous spread is common, involving most frequently the oral cavity, the gastrointestinal tract, the lung, and the lymph nodes. AIDS-KS is a highly vascular tumor consisting of proliferating spindle-shaped cells (KS cells), microvascular endothelial cells, infiltrating mononuclear cells, and edema (19). Experimental data using cultured KS cells indicate that KS cells contribute to the development and progression of KS lesions by producing growth-promoting, inflammatory, and angiogenic cytokines (20–24).

Although a number of modalities have been used for 15 yr, cure or long term complete remission from KS is unlikely with the currently available therapeutic modalities (25). We have reported that AIDS-KS cells are resistant to Fas-mediated apoptosis (26). In addition, AIDS-KS cells are resistant to chemotherapeutic drugs (25). Several lines of evidence suggest that KS cells may acquire...
resistance to several apoptotic stimuli through the expression of antiapoptotic molecules such as Bcl-2 (27) and Bcl-xl (28). The resistance of KS cells to apoptosis may hamper the development of therapeutic agents for the treatment of AIDS-KS. In this study we present evidence that actinomyclin D (Act D) sensitizes AIDS-KS cells to sApo-2L-mediated apoptosis. In addition, we address the possible roles of apoptosis-related molecules in Act D-induced sensitization of AIDS-KS cells to sApo-2L.

Materials and Methods

Cells and reagent

AIDS-KS cells were developed from the pleural effusion of lung KS (KS-6, KS-7, KS-9, KS-11, KS-21, KS-22, KS-23), lung KS (KS-3, KS-8), and oral mucosa KS (KS-10B) of HIV-infected patients. KS-21, KS-22, and KS-23 cells were derived from the Institute of Molecular Medicine, Huntington Memorial Hospital (Pasadena, CA), by using previously described methods (29). KS-3, KS-6, KS-7, KS-9, KS-10B, and KS-11 were developed in the Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health (Bethesda, MD). AIDS-KS cells were maintained in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS and conditioned medium from human oncostatin M (OM) expressing Chinese hamster ovary cells to a final OM concentration of 15 ng/ml (30). During the study KS-6, KS-8, and KS-11 cells died, and these cells were not available for all tests performed in the other KS cell cultures. Human foreskin fibroblast cells were maintained in EMEM supplemented with 10% FBS (29). Human PBMC were purified using Ficoll-Hypaque and cultured in RPMI 1640 supplemented with 10% FBS. Act D was purchased from Sigma (St. Louis, MO). The sApo-2L was prepared as previously described (6). Caspase inhibitors, zVAD-fmk and zDEVD-fmk, were purchased from Calbiochem-Novabiochem (San Diego, CA). The zETD-fmk was obtained from MBL (Nagoya, Japan). The agonistic anti-Fas mAb (CH-11) was purchased from Upstate Biotechnology (Lake Placid, NY).

Cytotoxicity assay

AIDS-KS cells or human foreskin fibroblast cells were seeded in 24-well culture plates and cultured for 18–24 h at 37°C. Each well was washed twice with medium and incubated in the presence or the absence of Act D, with or without sApo-2L or CH-11. Eighteen hours later, the number of adherent cells was counted. The medium was removed, and the plates were washed with Hank’s solution (Life Technologies). After detaching the cells with trypsin/EDTA (Life Technologies), the number of cells was counted using a particle counter (Coulter, Hialeah, FL). Cell viability was also evaluated using a cell proliferation kit-XTT assay (Boehringer Mannheim, Indianapolis, IN). The cell viability determined by counting the adherent cells correlated with that determined by the XTT assay. The data are represented as the mean ± SD (n = 3). Cytotoxicity for PBMC was determined using a cell proliferation kit-XTT assay according to the manufacturer’s instructions. Cell viability (%) = 100 × (ODtreated/ODuntreated) obtained from sApo-2L-treated cultures/ODsApo-2L untreated cultures.

Morphology

KS-10B cells (2 × 10^6 cells) were cultured for 18 h in RPMI 1640 supplemented with 10% FBS in a 24-well culture plate. The sApo-2L (500 ng/ml) and Act D (10 ng/ml) were added to the cultures and then incubated for 18 h. The cells were observed under a phase-contrast microscope (Olympus Optical, Tokyo, Japan).

DNA fragmentation assays

KS-10B cells were cultured in RPMI 1640 supplemented with 10% FBS in 75-cm² culture flask. The sApo-2L (250 ng/ml) and Act D (10 ng/ml) were added to cultures, then incubated for 18 h. Detached and attached cells (~1 million cells) were collected, washed three times with PBS, and then suspended in 100 μl of ice-cold 70% ethanol. After incubation for 20 min at room temperature, the cells were washed, suspended in staining buffer (PBS/10 mg/ml propidium iodide/50 μg/ml RNase A), incubated for 30 min at room temperature, and subjected to flow cytometric analysis using an EPICS XL flow cytometer (Coulter).

RT-PCR

Confluent AIDS-KS cells were cultured for 18 h in the presence or the absence of Act D (10 ng/ml). Total RNA was prepared using TRIZol (Life Technologies). First-strand cDNA was synthesized in 60 μl of reaction mixture containing 6 μg of total RNA, using a cDNA Preamplification Kit (Life Technologies). The cDNA synthesis reaction mixtures were subjected to PCR amplification (100 μl) under the following conditions for DrG1, DrG2, cellular FLICE (Fas-associated death domain protein-like-1,-1-converting enzyme) inhibitory protein (cFLIP), and β-actin: 30 cycles at 94°C for 1 min, at 55°C for 1 min, and at 72°C for 1 min. PCRs were performed using the following upstream and downstream primers. DrG1 (upstream), 5'-CAG TGT AAA GAA GGC ACC TTC CGG-3'; DrG1 (downstream), 5'-GCA GGA GTC CTT GGG CGT GTG-3'; DrG2 (upstream), 5'-CAC TCT ATT ATC ATA GTC GTT TT-3'; DrG2 (downstream), 5'-GAA GGA CAT GAA CGC CGC AAG AG-3'; cFLIP (upstream), 5'-AGG CAT TTC GAT CCC TAG TC-3'; cFLIP (downstream), 5'-GAG CGT GAG GGT CTC CAC AC-3'; and β-actin (upstream), 5'-ATC TGCCGAC CAC TCC TTC AAC GAT CG-3'; β-actin (downstream), 5'-CGT ACT CAT CCT GTC TGA TCA TCA TCT GC-3'. Ten microliters of PCR reaction were subjected to agarose gel electrophoresis. Densitometric analysis was performed using Scan Analy- sis (Biosoft, London, U.K.). The intensity of the PCR products generated from 5 μl of Act D-unreントed cell-derived template was set as 1.0.

Western blot analysis

Confluent AIDS-KS cells were incubated for 18 h in the presence or the absence of Act D (10 ng/ml). The cells were lysed at 4°C in RIPA buffer (50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSE, 1 μM of aprotinin, 1 μM leupeptin, 1 μM of pepstatin, 1 mM NaN3, and 1 mM NaF). The cell lysates (5–30 μg) were electrophoresed on 10–20% or 12% SDS-PAGE (Novex, San Diego, CA) and subjected to Western blot analysis. The transfer of proteins from gels onto Hybond nitrocellulose membranes (Amer- sham, Arlington Heights, IL) was electrothermally conducted in a transblotting cell (Bio-Rad, Hercules, CA). The membranes were blocked by immersing for 1 h at room temperature in 5% nonfat skim milk/PBS and then incubated with the respective Ab for 18 h at room temperature. Mouses anti-Bcl-x and FADD (Fas-associated death domain protein) mAbs were purchased from Transduction Laboratory (Lexington, KY). Mouse anti-Bax mAb and rabbit anti-p53 polyclonal Ab were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-procaspase 8 mAb was obtained from PharMingen (San Diego, CA). Rabbit anti-cFLIP polyclonal Ab was obtained from Upstate Biotechnology. After washing in PBS/0.1% Tween-20, the membranes were incubated for 2 h with horseradish peroxi- idase-conjugated anti-mouse or anti-rabbit IgG Ab (New England Biolabs). Western blot detection was performed with a Photoprobe Western blot detection kit (New England Biolabs).

Results

Sensitization of AIDS-KS cells to sApo-2L-induced cell death

We determined the cytotoxic effect of sApo-2L on 10 kinds of AIDS-KS cells obtained from tissues of different patients with HIV infection. AIDS-KS cells were incubated with 500 ng/ml of sApo-2L. As shown in Table 1, KS-7, KS-11, and KS-22 were moderately sensitive to 500 ng/ml of sApo-2L, and the other KS cells were resistant. Protein synthesis inhibitors or chemotherapeutic drugs are known to markedly augment Fas- or TNF-α-mediated cytoxicity for some types of cells (31). We have also reported that the combination of an agonistic anti-Fas mAb, CH-11, and a subtoxic concentration of Act D (10 ng/ml) induced significant levels of cell death in AIDS-KS cell populations (Fig. 1A) (26). Therefore, we examined the combined effect of sApo-2L and Act D treatment on AIDS-KS cell viability. Combination treatment of sApo-2L with Act D efficiently induced cell death in KS isolates, except in KS-8 (Table 1). We further analyzed the effects of sApo-2L and Act D (10 ng/ml) on KS-10B and KS-11. AIDS-KS cells were incubated with various concentrations of sApo-2L in the presence or the absence of Act D (10 ng/ml) (Fig. 1B). In the presence of Act D, sApo-2L induced cell death in KS-10B and KS-11 in a concentration-dependent manner.

To determine whether the cytotoxic effect of Act D and sApo-2L on KS cells was the result of a synergistic or an additive effect, KS-9 cells were treated with various concentrations of sApo-2L or Act D (Fig. 1C). Based on the methods described by Berenbaum.
additive effects of treatment with Act D alone and sApo-2L alone.

To characterize the Apo-2L/Act D-induced AIDS-KS cell death occurs via apoptosis

To examine whether the combination treatment with Act D and sApo-2L to AIDS-KS cells, we examined the morphological changes following sApo-2L treatment of these cells (Fig. 3A). When KS-10B cells were treated for 18 h with sApo-2L (500 ng/ml) plus Act D (10 ng/ml), most sApo-2L-treated cells displayed cell blebbing within 2 h, and almost all cells were detached within 6 h (Fig. 1D). These data indicate that Act D sensitizes AIDS-KS cells to sApo-2L. The effect of Act D and sApo-2L on KS-9 was less pronounced compared with those on the other KS isolates (Fig. 2A). However, efficient cell death induction was obtained by increasing the concentration of Act D (Fig. 2B) or pretreatment of KS-3 with Act D (Fig. 2C).

The sApo-2L/Act D-induced AIDS-KS cell death occurs via apoptosis.

To examine whether the combination treatment with Act D and sApo-2L to AIDS-KS cells, we examined the morphological changes following sApo-2L treatment of these cells (Fig. 3A). When KS-10B cells were treated for 18 h with sApo-2L (500 ng/ml) plus Act D (10 ng/ml), most cells were detached from the culture dishes and formed extensive blebbing on the cell surface, a characteristic of apoptosis. In contrast, the effect of either agent used alone was extremely weak. We evaluated apoptosis by the propidium iodide staining method. As shown in Fig. 3B, flow cytometric analysis revealed that the combined treatment of sApo-2L (250 ng/ml) and Act D (10 ng/ml) induced DNA fragmentation in 55.1% of the KS cell population, while only small numbers of sApo-2L-treated or Act D-treated KS cells underwent apoptosis. The number of apoptotic cells shown in Fig. 3B correlated well with the viability assessed by counting the number of adherent cells shown in Fig. 1B. These findings demonstrate that the combination treatment with sApo-2L and Act D induces KS cell death by apoptosis.

Act D failed to sensitize fibroblast cells and PBMC to sApo-2L.

To examine whether the combination treatment with Act D and sApo-2L also kills normal cells, cytotoxic assays were performed with foreskin fibroblast cells and PBMC. As shown in Fig. 4, foreskin fibroblast cells and PBMC were resistant to sApo-2L. Act D inhibited the growth of fibroblast cells and reduced the viability of PBMC; however, the combination of Act D and sApo-2L failed to induce synergistic cell death in both cell types. These data show that Act D cannot sensitize fibroblast cells and PBMC to sApo-2L.
The sApo-2L-induced apoptosis in AIDS-KS cells is caspase-dependent

Caspases play essential roles in many types of apoptosis (33). Caspase inhibitors block Apo-2L/TRAIL-induced apoptosis (34, 35). We tested the effects of caspase inhibitors, zVAD-fmk, zDEVD-fmk, and zIETD-fmk, on sApo-2L-induced apoptosis of AIDS-KS cells (Fig. 5). In vitro, zVAD-fmk is a general caspase inhibitor, and zDEVD-fmk and zIETD-fmk inhibit caspase 3-like and caspase 8 and 10 activities, respectively. AIDS-KS cells were pretreated for 18 h with or without Act D (10 ng/ml) and incubated for 6 h with sApo-2L. The data represent the mean ± SD (n = 3). A and C, Open bars, without Act D; solid bars, with Act D plus sApo-2L (250 ng/ml). B, Open bars, without sApo-2L; solid bars, with sApo-2L (250 ng/ml).

FIGURE 2. Synergistic effect of Act D and sApo-2L on the viability of KS-3 as a function of increasing concentrations of sApo-2L in the presence of Act D (10 ng/ml; A), increasing the concentration of Act D with sApo-2L (250 ng/ml; B), or pretreatment with Act D (C). KS-3 cells were pretreated for 18 h with or without Act D (10 ng/ml) and incubated for 6 h with sApo-2L. The data represent the mean ± SD (n = 3). A and C, Open bars, without Act D; solid bars, with Act D plus sApo-2L (250 ng/ml). B, Open bars, without sApo-2L; solid bars, with sApo-2L (250 ng/ml).

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Molecular mechanism of Act D-induced sensitization of AIDS-KS cells to sApo-2L

Act D is an inhibitor of transcription. To investigate the mechanism of sensitization of KS cells to sApo-2L by Act D, we examined the effect of Act D on the expression of both the decoy receptors for sApo-2L, DcR1 and DcR2 and cFLIP (36, 37), a cellular homologue of viral FLIP (38), using RT-PCR (Fig. 6). We performed PCR from 5 and 0.5 μl of RT reaction mixture. As shown in Fig. 6, no significant down-regulation of these molecules was observed in Act D-treated KS cells. Further, the levels of cFLIP protein in AIDS-KS cells were not changed following Act D treatment (Fig. 7).

Members of the Bcl-2 family regulate apoptosis induced by various stimuli (39–41). Bcl-2 and Bcl-xL function as antiapoptotic molecules, while Bax and Bcl-xS function as proapoptotic molecules. The p53 regulates the expression of these Bcl-2 family molecules (39–41). As shown in Fig. 7, high levels of p53 proteins were expressed in Act D-untreated cells, and Act D treatment did not affect the expression of p53. Bax proteins are abundantly expressed, and Act D treatment did not affect the expression of Bax proteins. Bcl-xL and Bcl-xS are generated from a single gene by alternative splicing (42). Anti-Bcl-x mAb recognized only a molecule with a Mr of 30 kDa, indicating that AIDS-KS cells preferentially expressed the Bcl-xL protein. Act D treatment markedly
reduced the level of the Bcl-xL protein. The expressions of bcl-2 mRNA and Bcl-2 protein in AIDS-KS cells were low compared with their expression in the Daudi B lymphoma (26, 28).

More recently, several reports have indicated that FADD is essential for Apo-2L/TRAIL-induced apoptosis (7, 9, 12). Following stimulation of Fas, the death-inducing signaling complex, Fas/FADD/pro-caspase 8 complex, is formed, leading to the activation of a caspase cascade and the induction of apoptosis (13, 43). Since we have shown their involvement of the caspase 8-like activity in sApo-2L-induced apoptosis in AIDS-KS cells (Fig. 5), we examined the expression of FADD and procaspase 8 (Fig. 7). The levels of FADD and procaspase 8 proteins were unaffected by Act D treatment. Together, these findings indicate that Act D preferentially down-regulated the expression of anti-apoptotic Bcl-xL protein.

Discussion

Currently, various chemotherapeutic treatments are implemented for patients with rapidly progressive, extensive, or symptomatic KS; however, it has been difficult to control symptoms caused by KS (25). Most patients with AIDS-KS present with severe immune dysregulation caused by HIV infection, and therefore, there is a limitation to the use of high dosages of chemotherapeutic drugs (25). In this study we have obtained evidence in vitro that the Apo-2L–Act D combination treatment can reduce the minimum effective dosage of Act D. Act D and sApo-2L efficiently induced cell death in nine of 10 AIDS-KS isolates. Further, following pretreatment with Act D, sApo-2L killed AIDS-KS cells more efficiently. Transcripts of Apo-2L/TRAIL and its receptors, DR4 and DR5, were detected in many types of normal tissues, suggesting that these tissues may be resistant to Apo-2L/TRAIL (13). In this study we found that Act D cannot sensitize PBMC and fibroblast cells to sApo-2L. Consequently, sApo-2L and subtoxic Act D may prove to be beneficial biological therapeutic agents in the absence of undesirable drug-mediated side effects on AIDS-KS patients.
Several lines of evidence show that the AIDS-KS lesion is, at least in its early stages, a reversible hyperplasia initiated by a complex cascade of cytokines and growth factors (20, 21, 44). Experimental data corroborate that KS cells, central elements in KS lesion, proliferate rapidly in response to various external stimuli, such as OM (30, 45), IL-6/soluble IL-6R complexes (46), IL-1β, and TNF-α (20, 47). Recently, we have reported that endogenous basic fibroblast growth factor is essential for the proliferation of AIDS-KS cells induced by external cytokines (23). We have also reported that dexamethasone synergistically enhances OM- or IL-6/soluble IL-6R-mediated proliferation of AIDS-KS cells (47), a finding that may explain the clinical observations that corticosteroid therapy is associated with the development of new KS (48) and the deterioration of preexisting KS (49). KS lesions are rapidly aggravated in response to environmental factors such as opportunistic infection and glucocorticoid use for immune suppression therapy (21, 44). Large amounts of basic fibroblast growth factor and vascular endothelial growth factor produced from KS cells (24) may induce severe angiogenesis with bleeding and edema, resulting in increasing mortality. Therefore, agents that induce rapid death of AIDS-KS cells may be more effective compounds for the treatment of AIDS-KS. Herein, we show that the combination treatment of sApo-2L with Act D induced a more rapid cytotoxic response to AIDS-KS cells than Act D used alone. For instance, sApo-2L-induced cell blebbing became detectable within 2 h in almost 100% of KS cells pretreated with Act D. These findings suggest that the combined therapy of sApo-2L and Act D is anticipated to improve the efficiency of KS treatment. In addition to Act D, we have also tested the effect of chemotherapeutic drugs, such as adriamycin, cisplatinum, and etoposide. Combination treatment of any of these drugs with sApo-2L synergistically induced cell death, although the sensitization effects were much less pronounced than those achieved with Act D (data not shown).

It has been proposed that induction of apoptosis by Apo-2L/TRAIL requires caspase activation. Crm A, a viral caspase inhibitor, and YYAD-CHO, a tetrapeptide ICE inhibitor, inhibit Apo-2L/TRAIL-induced apoptosis (34, 35). In the present study we show that zVAD-fmk, zDEVD-fmk, and zIETD-fmk markedly inhibited sApo-2L-induced cell death in KS. The caspase 8 inhibitor, but not the caspase 3 inhibitor, blocked sApo-2L-induced apoptosis in melanoma (50). However, in our studies, there was significant inhibitory effect by both caspase inhibitors. These data indicate that caspase 3-like and caspase 8 or 10 activities are essential for induction of apoptosis in AIDS-KS cells by sApo-2L. The caspase cascade in apoptosis may be activated by both a mitochondria-dependent and a mitochondria-independent pathway. In the mitochondrial-dependent pathway, the caspase cascade is activated by death-inducing signaling complex, which consists of death receptors, apoptotic adaptor molecules, and initiator types of caspases, such as caspase 2 and 8. TRADD (TNFR-associated death domain protein), FADD, RIP (receptor interacting protein), and RAIDD (RIP-associated ICH-1/Ced-3 homologous death domain protein) are known as apoptotic adaptor molecules (13, 43, 51, 52). In the mitochondria-dependent pathway, the initiator caspase, caspase 9, is activated by Apaf-1 and cytochrome c, which is released from mitochondria following apoptotic stimuli (39, 40). It is as yet unknown which pathway is involved in the Apo-2L-induced apoptosis. Ectopic expression of dominant negative FADD did not inhibit apoptosis by Apo-2L, indicating that a FADD-independent pathway is linked to activation of a caspase cascade (4–6, 10, 34). Yeh et al. (53) showed that DR4-induced apoptosis is not inhibited in fibroblast cells derived from FADD-deficient mice, while TNF receptor type 1-, Fas-, and DR3-induced apoptosis was inhibited. However, other laboratories demonstrated that the dominant negative FADD inhibits DR4- or DR-5-induced apoptosis (7, 9, 12, 54). Pan et al. (5) claimed no association of DR4 or DR5 with FADD, TRADD, and caspase 8, while others presented the association of DR4 or DR5 with TRADD, FADD, TRAF (TNFR-associated factor), and RIP (9, 12). Further, Pan et al. (5) showed that DR4 and DR5 are associated with FLICE2 (caspase 10b). Our findings showing inhibition of sApo-2L-induced apoptosis in AIDS-KS cells by zIETD-fmk may suggest the involvement of caspase 10.

Several lines of evidence show that Bcl-2 and Bcl-xL function as antiapoptotic molecules mainly at the level of the mitochondria (39–41). It was reported that Bcl-xL binds Apaf-1 and inhibits activation of caspase 9 (55). Overexpression of Bcl-2 and Bcl-xL inhibits apoptosis or necrosis induced by a variety of stimuli (56–58). Further, Bcl-2 is strongly stained in spindle cells of advanced AIDS-KS (27). Foreman et al. (28) reported that high levels of Bcl-x are detected in AIDS-KS lesions, and cultured AIDS-KS cells preferentially express Bcl-xL. These studies suggest that high levels of Bcl-2 and Bcl-xL may lead to prolonged survival of AIDS-KS cells. In this study we show that AIDS-KS spindle cells preferentially express Bcl-xL, which is markedly reduced by Act D treatment. Previously, we have reported that the level of bcl-2...
mRNA in AIDS-KS cells was very low (26). Similar findings were reported by Foreman (28). In contrast, the expressions of cFLIP mRNA and Bax, p53, FADD, and procaspase 8 proteins were not affected by Act D treatment. Marked down-regulation of Drcl and Dcr2 mRNA was not observed in AIDS-KS cells by Act D. Griffith et al. (50) reported that there was no correlation between the resistance of cells (>60 different tumors) to TRAIL and the expression of Drcl and Dcr2. Thus, it seems unlikely that Drcl and Dcr2 expression are responsible for resistance of AIDS-KS cells to sApo-2L. Saffidi et al. (58) demonstrated that Fas signals are transduced exclusively through the mitochondria-dependent pathway in Jurkat and CEK cells, although most Fas-sensitive cells are killed through the mitochondria-independent pathway. Thus, it is possible that Apo-2L-induced signal may pass exclusively through the mitochondria in AIDS-KS cells. Down-regulation of Bcl-xL may be associated with sensitization of AIDS-KS cells by Act D. 

Recently, it has been reported that the sensitivity of several melanoma cells to Apo-2L/TRAIL is increased by cyclohexamide or Act D treatment (50, 59). However, the underlying mechanism of sensitization remains unknown. Chemotherapeutic drugs up-regulated the level of DR5 mRNA in a p53-dependent manner (8). Up-regulation of DR5 was also reported by TNF-α in p53-independent manner (60). Up-regulation of DR5 may lead to an increase in sensitivity to sApo-2L.

In summary, our data show that Act D sensitizes AIDS-KS cells to sApo-2L-mediated apoptosis. Sensitization was achieved with subtoxic concentrations of Act D. Furthermore, we show that normal fibroblasts and PBMC are not sensitized by Act D to sApo-2L killing. Therefore, our findings in vitro showing synergistic cytotoxic activity of sApo-2L in combination with Act D support their use in vivo in the therapy of drug-resistant AIDS-KS.

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