Cytoskeleton-mediated Death Receptor and Ligand Concentration in Lipid Rafts Forms Apoptosis-promoting Clusters in Cancer Chemotherapy

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While investigating the mechanism of action of the novel antitumor drug Aplidin, we have discovered a potent and novel cell-killing mechanism that involves the formation of Fas/CD95-driven scaffolds in membrane raft clusters housing death receptors and apoptosis-related molecules. Fas, tumor necrosis factor-receptor 1, and tumor necrosis factor-related apoptosis-inducing ligand receptor 2/death receptor 5 were clustered into lipid rafts in leukemic Jurkat cells following Aplidin treatment, the presence of Fas being essential for apoptosis. Preformed membrane-bound Fas ligand (FasL) as well as downstream signaling molecules, including Fas-associated death domain-containing protein, procaspase-8, procaspase-10, c-Jun amino-terminal kinase, and Bid, were also translocated into lipid rafts, connecting death receptor extrinsic and mitochondrial intrinsic apoptotic pathways. Blocking Fas/FasL interaction partially inhibited Aplidin-induced apoptosis. Aplidin was rapidly incorporated into membrane rafts, and drug uptake was inhibited by lipid raft disruption. Actin-linking proteins ezrin, moesin, RhoA, and RhoGDI were conveyed into Fas-enriched rafts in drug-treated leukemic cells. Disruption of lipid rafts and interference with actin cytoskeleton prevented Fas clustering and apoptosis. Thus, Aplidin-induced apoptosis involves Fas activation in both a FasL-independent way and, following Fas/FasL interaction, an autocrine way through the concentration of Fas, membrane-bound FasL, and signaling molecules in membrane rafts. These data indicate a major role of actin cytoskeleton in the formation of Fas caps and highlight the crucial role of the clusters of apoptotic signaling molecule-enriched rafts in apoptosis, acting as concentrators of death receptors and downstream signaling molecules and as the linchpin from which a potent death signal is launched.

Drug-induced apoptosis in cancer chemotherapy has been postulated to be mediated by Fas ligand (FasL) expression followed by its subsequent interaction with death receptor Fas (a.k.a. CD95 or APO-1) (1). However, this hypothesis was questioned when FasL up-regulation, and even FasL, itself, was found nonessential for Fas activation (2, 3). Fas is a major member of the death receptor family, a subgroup of the tumor necrosis factor receptor superfamily characterized by a cytoplasmic death domain that is responsible for the transmission of apoptotic signaling through interaction with death domain-bearing adaptor molecules (4). Stimulation of Fas results in receptor oligomerization and recruitment of the adaptor molecule Fas-associated death domain-containing protein (FADD) through interaction between its own death domain and the clustered receptor death domains. In turn, FADD recruits procaspase-8 via a death effector domain interaction forming the so-called death-inducing signaling complex (5). The close proximity of procaspase-8 molecules in the complex drives its activation by self-cleavage, triggering downstream effector caspases and leading to apoptosis (4). We have recently shown the translocation of Fas and downstream signaling molecules into clusters of lipid rafts in the killing of tumor cells, independently of FasL, by the antitumor ether lipid 1-O-octadecyl-2-O-methyl-α-c-glycerol-3-phosphocholine (ET-18-OCH3; Edelfosine) (6, 7). Fas association with membrane rafts has also been reported following its engagement by the ligand (8, 9) and lipid rafts have been found to be essential for the initiation of Fas-mediated cell death signaling (6–10). These data have led to the notion that Fas-mediated apoptosis is triggered by its recruitment and clustering in membrane rafts. Lipid rafts are membrane microdomains highly enriched in cholesterol and sphingolipids with an estimated size between 50–70 nm. The proteins located in these microdomains are severely limited in their ability to freely diffuse over the plasma membrane (11). Thus, raft association tends to concentrate specific proteins within plasma membrane microdomains, and this could affect protein function (12).

Aplidin (Aplidine; dehydrodidiemnin B) is a naturally occurring cyclic depsipeptide isolated from the Mediterranean tunicate Aplidium albicans that shows strong in vitro and in vivo antitumor activity (13). Aplidin behaves as the most potent and rapid apoptosis inducer ever described and as a promising drug in the treatment of leukemia (14). Its apoptotic action is mediated by Fas/FasL, c-Jun amino-terminal kinase (JNK), and mitochondria (14, 15). Here, we have found a potent and novel mechanism of cell killing mediated not only by the clustering of Fas but also by the concentration of additional death receptors and membrane-bound FasL, together with downstream signaling molecules, into aggregated lipid rafts through a cytoskeleton-mediated process.

EXPERIMENTAL PROCEDURES

Drugs and Cell Culture—Aplidin was obtained from PharmaMar (Colmenar Viejo, Madrid, Spain) and prepared as a 1-mM stock solution
dissolved in Me2SO. Final solvent concentrations in cell culture were less than 0.1% (v/v), which had no effect in any of the parameters studied. [3H]Aplidin (87.2 mCi/mmol) was from PharmaMar. The human acute T-cell leukemia cell line Jurkat was grown in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 units/ml penicillin, and 24 μg/ml gentamicin. Fas-deficient Jurkat cells were generated following protracted cultures (over 10 months) of parental Jurkat cells. Mouse fibroblasts L929 and Fas-transfected L929 cells (7) were grown in Dulbecco’s modified Eagle’s medium–10% fetal calf serum.

**Immunofluorescence Flow Cytometry—**Cell surface expression of death receptors was analyzed by immunofluorescence flow cytometry (16) in a BD Biosciences FACSCalibur flow cytometer, using anti-Fas SM1/1 monoclonal antibody (mAb) (Bender MedSystems, Vienna, Austria) and specific antibodies against TNF receptor 1 (TNF-R1) and TRAIL-receptor 2/death receptor 5 (DR5) (Alexis Biochemicals, Lausen, Switzerland; Santa Cruz Biotechnology, Santa Cruz, CA). P3X63 myeloma culture supernatant, provided by F. Sánchez-Madrid (Hospital de La Princesa, Madrid, Spain), was used as a negative control. Measurement of FasL levels was achieved by flow cytometry in permeabilized cells as previously described (16).

**Aplidin Uptake—**Drug uptake was measured after incubating 1 × 10⁶ cells in fetal calf serum containing culture medium with 10–100 μg/ml Aplidin for the indicated times. For negative controls, cells were washed (five times) with 2% bovine serum albumin-phosphate-buffered saline (PBS) and placed in PBS supplemented with 10% fetal calf serum, 2 mM l-glutamine, and 100 μg/ml gentamicin. For drug uptake, 100% of the drug was available within 3 min of incubation in drug-free serum (data not shown).

**Results**

**Fas Involvement in Aplidin-induced Apoptosis—**We have previously found that L929 cells, lacking Fas, were resistant to Aplidin but became sensitive after being stably transfected with human Fas cDNA (14). Both L929 and Fas-transfected L929 cells possessed functional receptors for TRAIL and TNFα (90% and 74% apoptosis after incubation for 24 h with 50 ng/ml TRAIL and 100 units/ml TNFα, respectively, in the presence of 500 ng/ml actinomycin D). We then asked for the reverse approach, that is whether a Fas-positive cancer cell sensitive to Aplidin could become drug-resistant upon Fas loss. Toward this goal we generated a Fas-deficient subline from human T-lymphoid Jurkat cells, which are over 85% Fas-positive and very sensitive to Aplidin (Fig. 1). The cell surface expression of additional death receptors, such as TNF-R1 and TRAIL-receptor 2/DR5, remained at similar levels in both parental and Fas-deficient Jurkat cells (~40% and 63% positive cells for TNF-R1 and DR5, respectively). Both cell types were equally sensitive to apoptosis after incubation with rhTNFα and rhTRAIL (~42% apoptosis after 24 h of incubation), indicating that TNF-R1 and DR5 receptors were functional. However, unlike parental Jurkat cells, Fas-deficient Jurkat cells (<1% Fas-positive cells) were resistant to Aplidin as well as to the agonistic cytotoxic CH-11 anti-Fas monoclonal antibody or rhFasL (Fig. 1). The antitumor drug ET-18-OCH₃, which triggers Fas-mediated apoptosis (6, 7), also failed to induce cell death in Fas-deficient Jurkat cells (Fig. 1). These data indicate a major role of Fas in Aplidin-induced apoptosis that cannot be replaced by DR5 or TNF-R1.

**Aplidin Induces Recruitment of Death Receptors and Downstream Signaling Molecules into Clusters of Membrane Rafts—**We found Aplidin induced a rapid capping of lipid rafts (Fig. 2A) as assessed by the raft marker fluorescein isothiocyanate-labeled cholera toxin B subunit (FITC-CTx) that binds ganglioside G₄₄, mainly found in rafts (19). In addition, Fas cocapped with membrane rafts in Aplidin-treated Jurkat cells (Fig. 2B). Translocation of Fas into lipid rafts was further confirmed by isolation of membrane rafts in both untreated and Aplidin-treated Jurkat cells in sucrose gradients (Fig. 3A). Lipid rafts were isolated based on their insolubility in Triton X-100 lysates buffer at 4 °C and fractionated by discontinuous sucrose gradient centrifugation (7, 17). G₄₄-containing lipid rafts, at the upper part (fractions 3–5) of the sucrose gradient, showed no staining.

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**Apoptosis Assay—**Quantitation of apoptotic cells was calculated by flow cytometry as the percentage of cells with a DNA content less than G₁ (hypodiploidy) in cell cycle analysis (16). Death receptor-mediated apoptosis was induced by rhTNF-related apoptosis-inducing ligand (TRAIL) or rhFasL (Alexis), active in human and murine systems, and submitted to lipid raft isolation as above. Lipid raft-enriched fractions were run in parallel.

**RESULTS**

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Fig. 2. Co-clustering of membrane rafts and Fas in Aplidin-treated Jurkat cells. A, T-leukemic Jurkat cells were either untreated (Control) or treated with 10 nM Aplidin for 30 min, stained with FITC-CTx, and analyzed by confocal microscopy for raft visualization. Bar, 10 μm. B, colocalization of membrane rafts (Raft) and Fas in Aplidin-treated Jurkat cells. Cells were either untreated (Control) or treated with 10 nM Aplidin for 3 h and processed for confocal microscopy using FITC-CTx (green fluorescence for lipid rafts) and anti-Fas mAb, followed by CY3-conjugated anti-mouse antibody (red fluorescence for Fas). Areas of colocalization between membrane rafts and Fas in the merge panels are yellow. Bar, 10 μm. Images shown are representative of three independent experiments.

(Fig. 3A), were located using CTx conjugated to horseradish peroxidase (17). Interestingly, we also found that additional death receptors, DR5 and TNF-R1, were translocated into lipid rafts in Aplidin-treated cells (Fig. 3A). We found that Aplidin induced colocalization of DR5 and TNF-R1 with clusters of lipid rafts as assessed by confocal microscopy (data not shown). These data show for the first time the clustering of three major death receptors in lipid rafts and could explain the outstanding proapoptotic effect of Aplidin in cancer cells (14).

A number of additional apoptosis-related molecules were also translocated into membrane rafts, including FADD, procaspase-8, procaspase-10, JNK, and Bid (Fig. 3A). Caspase-10 was activated at a higher level than caspase-8, although only a minor proportion of caspase-10 and caspase-8 cleavage forms remained in lipid rafts (Fig. 3A). The recruitment in membrane rafts of JNK and Bid, the latter reported to act as a bridge between Fas signaling and mitochondria (20), may explain the dependence on both JNK and mitochondrial signaling of Aplidin-mediated apoptosis (14, 15).

On the other hand, we found that the protein tyrosine phosphatase CD45 was excluded from the membrane raft-containing fractions in both untreated and Aplidin-treated cells (Fig. 3A). This is in agreement with previous reports showing CD45 exclusion from membrane rafts under a wide array of experimental conditions (21–23). Taken together, our results indicate that Aplidin induces a reorganization of lipid raft protein content, translocating some proteins to these detergent-insoluble fractions, whereas others remain excluded from these fractions.

Membrane-bound FasL Is Concentrated in Membrane Rafts following Aplidin Treatment—Because Fas was required for an effective Aplidin-induced apoptotic response we investigated whether its cognate ligand FasL was affected following drug treatment. Aplidin did not induce FasL synthesis before triggering of the apoptotic response (data not shown) as assessed by flow cytometry analysis of FasL expression in permeabilized cells. In addition, Aplidin-induced apoptosis is independent of protein biosynthesis (14), further supporting that FasL synthesis is not required for the apoptotic response induced by Aplidin. However, we found translocation of a FasL-immunoreactive band into membrane rafts following Aplidin treatment (Fig. 3A). This band corresponded to ~40-kDa molecular mass, which is the expected size for membrane-bound FasL. We further confirmed the presence of membrane-bound FasL in membrane rafts isolated from Aplidin-treated Jurkat cells by biotinylation of cell surface proteins followed by treatment with Aplidin and subsequent immunoprecipitation of raft-enriched pooled fractions with a specific anti-FasL mAb (Fig. 3B). However, FasL was found to be virtually undetectable or in negligible amounts in membrane rafts isolated from biotinylated untreated control Jurkat cells (Fig. 3B). In addition, by confocal microscopy we found co-clustering of membrane-bound FasL and lipid rafts in Aplidin-treated Jurkat cells (Fig. 3C). Untreated cells showed a very faint and diffuse staining for membrane-bound FasL (data not shown). FasL translocation to lipid rafts following treatment with 10 nM Aplidin showed a kinetics similar to that of Fas receptor (data not shown), being optimum after 3 h treatment. Prevention of Fas/FasL interaction by the blocking anti-Fas SM1/23 antibody partially inhibited Aplidin-induced apoptosis (43.2% inhibition), confirming our previous findings (14) and suggesting that Aplidin induces cell death through both the Fas/FasL system and a FasL-independent process. This can be explained by our present data as Fas and membrane-bound FasL, located in membrane rafts may interact with each other through adjacent cells exposing the same complementary set of Fas and FasL proteins concentrated in lipid rafts. In addition, recruitment of Fas and the subsequent downstream signaling molecules into lipid rafts can facilitate triggering of the apoptotic signal with no participation of FasL (3, 7).

Integrity of Lipid Rafts Is Required for Aplidin-induced Fas Capping and Apoptosis—Disruption of rafts following pretreatment with methyl-β-cyclodextrin (MCD), which extracts cholesterol, prevented Aplidin-induced apoptosis (Fig. 4) and Fas capping (data not shown), indicating the critical role of lipid rafts in Aplidin-induced apoptosis.

Aplidin Is Incorporated into Lipid Rafts—We next asked whether Aplidin could promote the above rearrangements in lipid raft composition and clustering through its direct interaction with membrane rafts. We tested this directly by incubating Jurkat cells with [14C]Aplidin and subsequent isolation of membrane rafts using the well established method of Triton X-100 solubilization and sucrose gradient centrifugation (Fig. 5A). When cells were incubated with radiolabeled Aplidin for 10 min, most of the drug was found accumulated in raft fractions 3–5 (Fig. 5A). Radiolabeled Aplidin codistributed with the typical raft marker ganglioside GM1 (Fig. 5A). This association of Aplidin with lipid rafts preceded Fas translocation to these membrane microdomains (data not shown). Aplidin was rapidly incorporated by Jurkat cells, and disruption of rafts by treatment of cells with MCD resulted in the inhibition of Aplidin uptake (Fig. 5B), further demonstrating the association of Aplidin with membrane rafts.

Fas Capping Requires Actin Cytoskeleton—We next asked how Fas is concentrated in lipid rafts. Actin cytoskeleton is a dynamic intracellular structure that because of its continuous assembly/disassembly could be perfectly equipped to translo-
cate proteins and transmit signals. A link between raft-mediated signaling and the interaction of actin cytoskeleton with raft membrane domains has been suggested (24). Ezrin, a major protein of the so-called ERM proteins (ezrin, radixin, moesin) linking the actin cytoskeleton to the plasma membrane, has been reported to interact with Fas and mediate Fas cell membrane polarization during Fas-induced apoptosis in human T lymphocytes (25). We found that the actin-linking proteins ezrin, moesin, RhoA, and RhoGDI were accumulated in membrane rafts following treatment of leukemic cells with Aplidin (Fig. 6A). Pretreatment of Jurkat cells with the microfilament-disrupting agent cytochalasin B or with jasplakinolide, a cell-permeable inhibitor of microfilament-mediated events that induces microfilament polymerization and stabilization (26), inhibited Aplidin-induced apoptosis (Fig. 6B) as well as Fas clustering (Fig. 6C, and data not shown). When actin cytoskeleton was disorganized, the Aplidin-promoted Fas caps were no longer observed and Fas staining was weak and showed a rather scattered distribution (Fig. 6C). These data support the notion that actin cytoskeleton is involved in the generation of Fas-enriched lipid raft clusters.

FIG. 3. Recruitment of death receptors, membrane-bound FasL, and downstream signaling molecules into membrane rafts following Aplidin treatment. A, untreated Jurkat cells (Control) and Jurkat cells treated with 10 nM Aplidin for 3 h were lysed in 1% Triton X-100 and fractionated by centrifugation on a discontinuous sucrose density gradient. An equal volume of each collected fraction was subjected to SDS-PAGE before analysis of the indicated proteins using specific antibodies. Procaspses 8 and 10 as well as active p18 caspase-8 and p23 caspase-10 cleavage forms are indicated. Location of G_{m1}-containing rafts (fractions 3–5) was determined using CTx conjugated to horseradish peroxidase. Representative blots of three separate experiments are shown. B, the cell surface proteins of Jurkat cells were biotinylated, and membrane rafts were isolated as above from untreated control cells as well as from cells treated with 10 nM Aplidin for 3 h. Fractions 3–5 were pooled and used as the membrane raft fraction. Then, equal amounts of protein (45 μg) from the raft-enriched fraction were immunoprecipitated with X63 myeloma supernatant, used as a negative control, or anti-FasL mAb. The molecular masses (kDa) of protein markers are indicated on the left. C, Jurkat cells were treated with 10 nM Aplidin for 3 h and then analyzed for membrane raft and FasL localization using FITC-CTx (green fluorescence) and anti-FasL mAb, followed by CY3-conjugated anti-mouse antibody (red fluorescence), respectively. Areas of colocalization between membrane rafts and FasL in the merge panels are yellow. Images shown are representative of three independent experiments. Bar, 10 μm.

FIG. 4. Effect of MCD on Aplidin-induced apoptosis. Jurkat cells were pretreated with MCD at the indicated concentrations for 30 min at 37 °C in serum-free medium and washed three times; 10 nM Aplidin was then added for 3 h. The percentage of apoptotic cells was assessed by flow cytometry. Control untreated cells and cells treated only with MCD were run in parallel. Data shown are means of three independent experiments ± S.D.

DISCUSSION

The results reported here demonstrate the formation of clusters of lipid rafts that serve as platforms where death receptors, including Fas, DR5, and TNF-R1, membrane-bound FasL, and downstream signaling molecules, including FADD, pro-caspases 8 and 10, JNK, and Bid, are concentrated. Very recently, we have found that the antitumor ether lipid ET-18-OCH₃ is able to concentrate Fas as well as downstream signaling molecules into lipid rafts and this clustering process is required for its apoptotic activity (7). Our present results with the antitumor drug Aplidin further expand this finding.
showing that three major death receptors and membrane-bound FasL can be also translocated and concentrated into lipid rafts. To our knowledge, this is the first report showing the clustering of three major death receptors in lipid rafts and the concentration of both a death receptor and its corresponding ligand in specific areas of the cell surface that can act as potent apoptosis promoters. Taken together, these data lead us to define a new way by which apoptosis can be triggered through the concentration of death receptors, adaptors, and downstream signaling molecules in clusters of lipid rafts that trigger a potent cell death signal. The translocation of Bid to lipid rafts following drug treatment suggests an early link between death receptor (extrinsic)- and mitochondrial (intrinsic)-signaling pathways in apoptosis. In fact, we have found that overexpression of Bcl-2 in leukemic cells blocks mitochondrial-mediated apoptotic signaling and prevents cell death induced by Aplidin and by the anti-Fas CH-11 agonistic antibody (14). The clustering of apoptotic signaling molecule-enriched rafts described here seems to play a major role in the extrinsic death receptor signaling and in coupling of both extrinsic and intrinsic mitochondrial pathways of apoptosis.

We hypothesize that accumulation of Fas into aggregates of stabilized membrane lipid domains from a highly dispersed distribution may represent a general mode of regulating Fas activation and apoptosis. Membrane rafts could also serve to generate high local concentration of Fas as platforms for coupling adaptor and effector proteins required for Fas downstream signaling, facilitating and amplifying signaling processes by transient local assembly of various cross-interacting signaling molecules. This is of particular importance in Fas-mediated signal transduction because death receptors lack enzymatic activity and the initial triggering events depend largely on protein/protein interactions. Interestingly, there is an increasing number of agents that promote Fas clustering (3) as well as cocapping of Fas and membrane rafts, such as ET-18-OCH₃, resveratrol, and cisplatin (6, 7, 27, 28). This suggests that Fas capping is a general and efficient process in the triggering of apoptosis. However, efficiency in promoting the concentration of death receptors described here is largely stimulus-dependent. Aplidin is considered the most potent pro-apoptotic agent ever described (14); accordingly, the present data indicate the unprecedented concentration of such a high number of death receptors and apoptotic signaling molecules in membrane rafts that could explain the extraordinary ability of this antitumor drug in promoting apoptosis. In this regard, Aplidin is able to promote the translocation of three major death receptors, namely Fas, DR5, and TNF-R1, in clusters of lipid rafts.

Interestingly, the present data show for the first time the translocation of membrane-bound FasL into membrane rafts. This concentration of Fas and membrane-bound FasL into specific and small areas of the cell membrane may lead to an increase in cell-killing competence and suggests a regulatory mechanism by which cells concentrate receptors and ligands at specific regions of the cell surface, leading to a more effective cell response.

We have also found that Aplidin is rapidly incorporated into lipid rafts. This process is readily detected after only a few minutes of incubation with tumor cells and precedes Fas clustering. Thus, it is suggested that Aplidin could rearrange membrane rafts, promoting their clustering and redistributing the protein content. Our data indicate that membrane rafts are critical for both Aplidin uptake and concentration of death receptors, membrane-bound FasL, and downstream signaling molecules that eventually lead to apoptosis. In this regard, the antitumor ether lipid ET-18-OCH₃ has been also found to be incorporated into membrane rafts (7, 29) and to trigger the concentration of apoptotic proteins into lipid rafts (7), although to a lesser level than Aplidin. These results highlight the importance of membrane rafts in the regulation of apoptosis as well as in cancer chemotherapy; therefore, lipid rafts represent a potential target for therapeutic intervention.

Our findings on the concentration of both Fas and membrane-bound FasL in lipid rafts indicate that Aplidin can act through FasL-independent activation of Fas as well as through Fas/FasL interaction. This translocation of Fas and membrane-
bound FasL into clusters of lipid rafts gives an explanation for the long-standing dilemma on the involvement of the Fas/FasL system in cancer chemotherapy first postulated by Friesen et al. (1) in the mid 1990s. De novo FasL synthesis is not essential for the induction of apoptosis upon treatment with chemotherapeutic agents, contrary to previous suggestions (1), but a redistribution of pre-existing membrane-bound FasL into clusters of membrane rafts furnishes small areas of the cell surface as potent promoters of cell death. Fas could induce cell death independently of FasL, once clustered in membrane rafts together with downstream signaling molecules. Fas/FasL interactions may enhance this deadly response through binding of the concentrated Fas and membrane-bound FasL in lipid rafts in an autocrine way between adjacent cells. Interaction of the corresponding Fas/FasL pairs in both adjoining cells would lead to their apoptotic cell death. Our data show that raft association concentrates apoptotic molecules into membrane microdomains, allowing efficient death receptor and death ligand presentation and triggering a potent apoptotic response. The clusters of apoptotic-signaling molecule-enriched rafts described here act as scaffolds of Fas downstream signaling, providing a new framework in death receptor-mediated apoptosis and cancer chemotherapy. This can lead to a new way to elicit an efficient apoptotic response that could also sensitize cells to a variety of extracellular insults, including death receptor ligands.

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FIG. 6. Translocation of actin-linking proteins into membrane rafts and involvement of actin cytoskeleton on Fas capping and apoptosis in drug-treated Jurkat cells. A, untreated control cells and cells treated with 10 nM Aplidin for 3 h were lysed in 1% Triton X-100 and fractionated by centrifugation on a discontinuous sucrose density gradient. Individual fractions were subjected to SDS-PAGE and Western blotting to analyze the indicated proteins using specific antibodies and for the location of GM1-containing rafts (fractions 3–5). Representative blots of three separate experiments are shown. B, Jurkat cells were pretreated with 5 µg/ml cytochalasin B (CB) or 1 µm jasplakinolide (JASPL) for 30 min, incubated with 10 nM Aplidin for 3 h, and analyzed for apoptosis by flow cytometry. Data shown are means of three independent experiments ± S.D. C, Jurkat cells were pretreated with jasplakinolide and then incubated with 10 nM Aplidin for 3 h. Membrane rafts and Fas were analyzed by confocal microscopy using FITC-CTx (green fluorescence) and anti-Fas mAb followed by CY3-conjugated anti-mouse antibody (red fluorescence), respectively. Images shown are representative of three independent experiments. Bar, 10 µm.
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