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Granzyme B-mediated Degradation of T-Cell Receptor ζ Chain[1]

Eva Wieckowski, Gui-Qiang Wang, Brian R. Gastman, Leslie A. Goldstein, and Hannah Rabinowich[2]

Departments of Pathology [E. W., G-Q. W., L. A. G., H. R.], and Otolaryngology [B. R. G.], The University of Pittsburgh School of Medicine, and The University of Pittsburgh Cancer Institute [H. R.], Pittsburgh, Pennsylvania 15213

Abstract

We recently reported that the T-cell receptor (TCR)-ζ chain is cleaved by caspase-3 and -7 in apoptotic T lymphocytes or in a cell-free system. We report here that the ζ chain is also a direct substrate for granzyme B (GrB) proteolytic activity. Loss in expression of TCR-ζ was observed in Jurkat T leukemic cells treated by a combination of GrB and a replication-deficient adenovirus. Although the apoptosis initiated in these cells by GrB was significantly reduced by the pancaspase inhibitor Z-VAD-FMK, TCR-ζ degradation was not prevented. These findings suggest that the GrB-mediated degradation of TCR-ζ chain can proceed despite the efficient inhibition of caspase activity. An *in vitro* translated TCR-ζ product was efficiently cleaved by GrB, which suggests that the TCR-ζ protein is a direct substrate for GrB. As assessed by site-directed mutagenesis, the activity of GrB was directed toward aspartic acid residues that were different from those of recombinant caspase-3. Whereas caspase-3 cleavage products appear to accumulate, the GrB-generated products seem to undergo further degradation, which suggests the presence of multiple GrB-specific cleavage sites within the TCR-ζ protein. These findings suggest that the TCR-ζ protein in target T lymphocytes serves as a substrate for the proteolytic activities that are featured by the two major mechanisms of cytotoxicity: death receptor pathways mediated by caspases and granule exocytosis mediated by direct GrB activity or GrB-activated caspases. TCR-ζ protein degradation may be of significance in cytotoxic mechanisms directed against T cells infected with viruses, such as HIV-1, in which the TCR-ζ protein is used for viral pathogenesis.

Introduction

CTLs and NK[3] cells play an essential role in the clearance of virally infected cells and tumor cells (1). Two major mechanisms of cytotoxicity used by these cells include the stimulation of the Fas death receptor pathway and granule exocytosis (2, 3). Apoptotic signaling through Fas is mediated by the activation of caspase-8 (4) and can proceed via mitochondria-dependent as well as independent pathways (5). In granule exocytosis, CTL granules stream toward the contact site between the cytotoxic lymphocyte and its target, in which granule contents are exposed to the target cell membrane (6). Studies in gene-knockout mice have indicated that the two major components of the granules, perforin and GrB, are essential for the granule exocytosis mechanism of cell death (7–11). Although perforin by itself cannot induce apoptosis, it allows granzymes, cosecreted serine proteases, to access their substrates. Perforin is required for the disruption of granzyme-containing target cell endosomes, a function that can be substituted for by endosomolytic agents such as adenovirus (12), listeriolysin, and pneumococcal pneumolysin (13).

GrB, the prototypic member of the granzyme family of serine proteases, shares substrate specificity with caspases for cleavage after aspartate residues (14). GrB has been reported to cleave caspases, including caspase-3, -6, -7, -8, and -10 *in vitro* (15–18). It has been assumed that it can initiate apoptosis at multiple points along the caspase-dependent apoptotic cascade (19, 20). However, recent evidence suggests that GrB may not rely solely on caspase activation to inflict target cell death. It has recently been reported that GrB can initiate apoptosis by cleavage of Bid, which subsequently induces the mitochondrial apoptotic cascade (21–24), or by cleaving DFF45/ICAD to liberate active DFF40/CAD, which induces nuclear apoptosis (25, 26).

The TCR-ζ chain is an essential component of the signal transduction mechanism of T lymphocytes (27). Experiments using ζ-knockout mice showed that the ζ protein is required for surface expression of TCRs on almost all T cells and for normal T-cell development (28). Numerous studies have documented the loss of the TCR-ζ protein in tumor-infiltrating lymphocytes in various types of cancer (29–33). Loss of TCR-ζ protein has also been detected in T and NK lymphocytes from HIV-infected individuals (34, 35). This loss has been associated with dysfunction of T cells in either cancer patients or HIV-infected donors (34, 36, 37). Although several mechanisms have been considered for the observed loss in TCR-ζ, including hydrogen peroxide or other soluble factors secreted by the tumor or tumor macrophages (38–41), the biochemical mechanism(s) responsible for TCR-ζ loss in cancer patients has not yet been resolved. We have recently reported that the TCR-ζ chain is a direct substrate for caspase-3 and -7 (42). Caspase-mediated degradation of ζ chain may serve as a partial explanation for the observed reduced expression of this protein in tumor-associated lymphocytes (43, 44). Degradation of TCR-ζ has also been observed in leukemic or virally infected cells undergoing apoptosis induced by cytotoxic T or NK cells. Such degradation may result from activation of caspase cascades by either death receptors or GrB/perforin mechanisms of cytotoxicity. In the present study, we provide evidence that the TCR-ζ protein is a direct substrate for GrB, a prominent component of the granule exocytosis mechanism of cytotoxicity.

Materials and Methods

Reagents. The COOH terminus-specific anti-ζ mAb (clone 8D3) used for immunoblotting was purchased from PharMingen (San Diego, CA) and an additional mAb (clone TIA-2) used for flow cytometry was from Coulter. Anti-β-actin mAb was purchased from Sigma (St. Louis, MO). Anti-caspase-3 Ab, anti-PARP mAb, and recombinant caspase-3 were from PharMingen; Z-VAD-FMK and purified GrB were from Enzyme Systems (Livermore, CA); GrB inhibitor Z-AAD-CH_2Cl was from Oncogene (Boston, MA); FITC-annexin V was from Clontech (Palo Alto, CA); Amplify, [^{35}S]methionine and [^{3}H]leucine were from Amersham-Pharmacia (Piscataway, NJ).

Cells, Cell Lysates, and Cell Extracts. The Jurkat T leukemic cell line was obtained from American Type Culture Collection (Rockville, MD). Cell lysates were prepared with 1% NP40, 20 mM Tris-base, 137 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. To prepare cell extracts for GrB or caspase-3 reactions, cultured Jurkat cells were washed twice with PBS and then resuspended in

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[2] To whom requests for reprints should be addressed, at University of Pittsburgh Cancer Institute, W952 Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, Pennsylvania 15213. Phone: (412) 624-0289; Fax: (412) 624-7737; E-mail: rabinow@pitt.edu.

[3] The abbreviations used are: NK, natural killer; TCR, T-cell receptor; GrB, granzyme B; Ab, antibody; mAb, monoclonal Ab; PARP, poly(ADP-ribose) polymerase; Ad, adenovirus type V; pfu, plaque-forming unit(s); PVDF, polyvinylidene difluoride.

ice-cold buffer [20 mM HEPES (pH 7.0), 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM sodium EDTA, 1 mM sodium EGTA, 1 mM DTT, 250 mM sucrose, and protease inhibitors]. After incubation on ice for 20 min, cells (2.5×10^6/0.5 ml) were disrupted by Dounce homogenization (20 strokes). Nuclei were removed by centrifugation at $650 \times g$ for 10 min at 4°C. Cellular extracts were obtained as the supernatants resulting from centrifugation at $14{,}000 \times g$ at 4°C for 30 min.

Induction of Apoptosis. CTL-free apoptosis was induced by incubation of target cells with GrB (33–200 nM) and replication-deficient Ad (10 pfu/ml) for 2–24 h as described previously (45). The cells were then washed to remove the excess of exogenous GrB. To avoid enzymatic activity of GrB during the lysis procedure, GrB inhibitor, Z-AAD-CH_2Cl (100 μM) was added to the lysis buffer at a dose demonstrated to inhibit GrB activity (data not shown).

Assessment of Apoptosis. Cytofluorometric analyses of apoptosis were performed as described previously (46) by staining with FITC-annexin V conjugate (Clontech), which served to assess aberrant phosphatidylserine exposure. Assessment of TCR-ζ protein expression by flow cytometry was performed on Saponin-permeabilized cells as described previously (44).

Site-directed Mutagenesis. Site-directed mutagenesis was performed using the QuickChange site-directed mutagenesis kit from Stratagene (La Jolla, CA), according to the manufacturer's procedure. Human TCR-ζ cDNA (wild type) cloned into *Hin*dIII- *Xho*I sites of pCDM8 vector was a generous gift from Dr. Lewis Lanier (DNAX, Palo Alto, CA). Thus, site-directed mutagenesis of TCR-ζ was performed with the wild-type recombinant plasmid. Complementary primers for the mutants were as follows: for D28A, 5′-GCTTTGGCCTGCTGGCTC-CCAAACTCTGCTA-3′ and 5′-GCAGAGTTTGGGAGCCAGCAGGCCAAA-GCTCT-3′; for D36A, 5′-CTGCTACCTGCTGGCTGGAATCCTCTTCA-TCTA-3′ and 5′-GATGAAGAGGATTCCAGCCAGCAGGTAGCAGAG-3′; for D84A, 5′-GACGAAGAGAGGAGTACGCTGTTTTGGACAAGAGAC-3′ and 5′-GTCTCTTGTCCAAAACAGCGTACTCCTCTCTTCGTC-3′; and for D87A, 5′-GTACGATGTTTTGGCCAAGAGACGTGGCCGGGA-3′ and 5′-CGGCCACGTCTCTTGGCCAAAACATCGTACAGGA-3′. Aliquots of transformed MC1061/P3 cells were plated on LB agar containing 25 μg/ml ampicillin and 10 μg/ml tetracycline. Three colonies were picked for each mutant. Purified plasmid DNA (one for each mutant) was subjected to DNA sequence analysis to confirm the presence of the site-directed mutation.

***In Vitro* Transcription-Translation of ζ Protein.** Wild-type and mutant TCR-ζ cDNAs were expressed in the TNT T7 transcription-translation reticulocyte lysate system or wheat germ extract system (Promega). Each coupled transcription-translation reaction contained 1 μg of plasmid DNA in a final volume of 50 μl in a methionine-free amino acid mixture supplemented with [^{35}S]methionine, or in a leucine-free amino acid mixture supplemented with [^{3}H]leucine, according to the manufacturer's instructions. After incubation at 30°C for 90 min, the reaction products were immediately used or stored at −70°C.

***In Vitro* Cleavage Reaction with Caspase-3 or GrB.** *In vitro* cleavage reactions were performed in a total volume of 20 μl. The reaction buffer consisted of 20 mM HEPES (pH 7.4), 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 20% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. Each reaction also contained 5 μl of ^{3}H-labeled ζ, and reticulocyte lysate or wheat germ extract in the presence or the absence of recombinant caspase-3 (1 μM) or GrB (66–200 nM) for 1–3 h at 37°C. Reactions were terminated by the addition of SDS loading buffer and boiling for 5 min. Products of the cleavage reactions were separated by 18% SDS/PAGE. Gels were fixed, impregnated with Amplify, vacuum-dried, and subjected to autoradiography. Alternatively, the reaction products were detected by Western blotting with anti-ζ mAb.

Western Blot Analysis. Proteins were separated by SDS/PAGE and were subsequently transferred to PVDF membranes, as described previously (45). After probing with a specific primary Ab and horseradish peroxidase-conjugated secondary Ab, the protein bands were detected by enhanced chemiluminescence (Pierce, Rockford, IL).

Results and Discussion

Loss of TCR-ζ Protein Expression during GrB-mediated Apoptosis. Our previous studies demonstrated that the loss of TCR-ζ protein is associated with apoptosis that is initiated via death receptor or mitochondrial pathways and is mediated by caspase-3 and -7 (42). Because GrB can impact the caspase cascade by a direct cleavage of procaspases and the generation of active caspase enzymes, we investigated a potential role for GrB in the degradation of the TCR-ζ protein. To initiate a GrB-mediated apoptotic cascade, we have used a CTL-free system in which purified GrB was added with Ad to the Jurkat target cell suspension. As reported previously (45), such treatment induced the membranous exposure of phosphatidylserine in a significant proportion of the treated cells (Fig. 1*A*). In the presence of the pancaspase inhibitor Z-VAD-FMK, annexin V binding to the exposed phosphatidylserine was significantly reduced, suggesting that the apoptosis initiated by GrB was mediated mainly by the activation of caspases. In addition to caspase activation, GrB initiates a mitochondrial apoptotic cascade, which is caspase independent in its initial phase. Thus, the presence of Z-VAD-FMK did not block, but rather slowed down, the apoptotic cascade. As assessed by the trypan blue exclusion dye, 24–30 h of exposure of Jurkat cells to Ad/GrB resulted in the loss of 90% of the cells, whereas Ad/GrB treatment in the presence of Z-VAD-FMK yielded such a low level of survival only after 72 h. GrB-induced apoptosis was also associated with a loss in

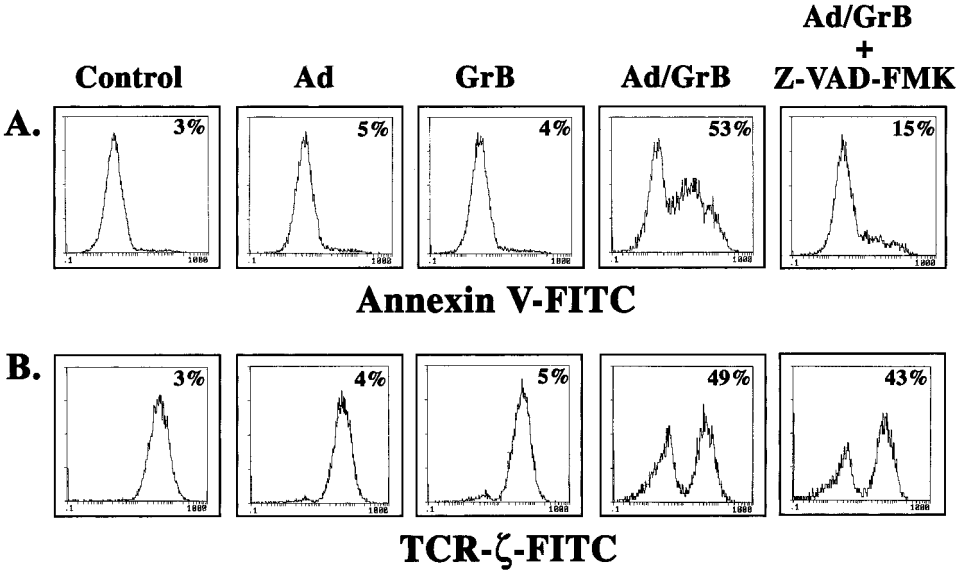

Fig. 1. Loss in TCR-ζ protein expression mediated by CTL-free GrB cytotoxicity is not inhibited by Z-VAD-FMK. *A*, induction of apoptosis in Ad/GrB-treated Jurkat cells. Jurkat cells were treated with Ad (10 pfu/ml), GrB (33 nM), or a combination of the two for 16 h. Z-VAD-FMK (100 μM) or control DMSO were added 2 h before GrB. The treated cells were assessed for phosphatidylserine exposure by binding of annexin V-FITC. Percent annexin V-positive cells is indicated. *B*, loss of TCR-ζ protein expression in Ad/GrB-treated Jurkat cells. The cells were permeabilized by Saponin and assessed for TCR-ζ protein expression using ζ-specific TAI-2 mAb. The percentage of cells negative for TCR-ζ protein is indicated. Treatment with DMSO as vehicle control for Z-VAD-FMK did not have an effect on annexin V binding nor on TCR-ζ expression.

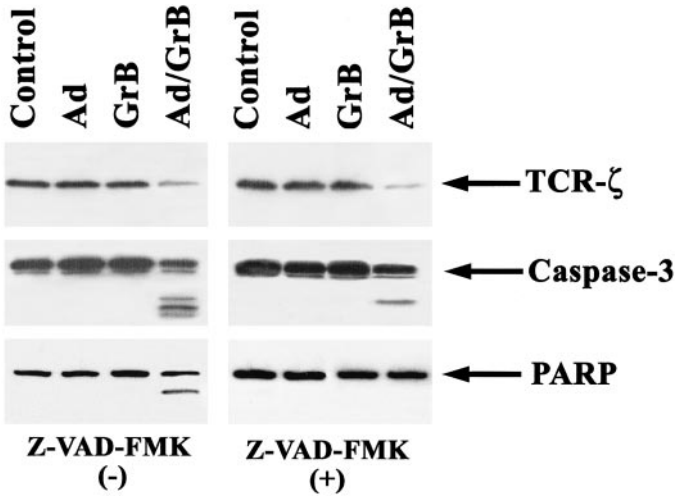

Fig. 2. GrB-mediated loss of the TCR-ζ protein can proceed despite the inhibition of caspase-3. Jurkat cells were treated with Ad (10 pfu/ml), GrB (33 nM), or a combination of the two for 2 h in the presence or absence of Z-VAD-FMK (100 μM). The cells were washed to remove excess GrB and lysed in the presence of the GrB inhibitor Z-AAD-CH_2Cl (100 μM) to inhibit GrB activity during generation of cell lysates. Proteins were resolved by SDS/PAGE and assessed by immunoblotting with anti-ζ Ab, which detects full-length, but not cleavage, products of ζ protein (*top panel*). The membranes were stripped and sequentially reprobed with anti-caspase-3 Ab (*middle panel*) and anti-PARP mAb (*bottom panel*).

TCR-ζ protein expression as assessed by flow cytometry (Fig. 1*B*). In contrast to the exposure of phosphatidylserine, which was significantly inhibited by the presence of Z-VAD-FMK during 16 h of exposure to Ad/GrB, the loss in TCR-ζ protein expression was not significantly affected by the presence of this inhibitor. These observations were also confirmed by immunoblot analyses of the GrB-treated cell lysates for TCR-ζ, caspase-3 and PARP. For these analyses, a GrB inhibitor, Z-AAD-CH_2Cl, was added to the lysis buffer to block any activity of GrB during the lysate preparation. Loss of TCR-ζ was observed in cells treated with Ad/GrB for 2 h in the presence or absence of Z-VAD-FMK (Fig. 2, *top*). In these cells, p20 caspase-3, previously reported to be generated by GrB cleavage of procaspase-3 at D175 (47), was detected in the presence or absence of the caspase inhibitor (Fig. 2, *middle*). However, in the presence of Z-VAD-FMK, activity of caspase-3 p20 was inhibited, because no auto-processing of the p20 protein or PARP cleavage were observed (Fig. 2, *middle* and *bottom*). Because Z-VAD-FMK has no direct effect on GrB proteolytic activity, these findings suggest that GrB may directly cleave the TCR-ζ protein, or mediate its activity via a Z-VAD-FMK-insensitive protease. Furthermore, the similar proportion of loss in TCR-ζ protein expression in the presence or absence of the inhibitor, which was documented by flow cytometry and immunoblotting, suggests that when caspase activity is inhibited, the proteolytic activity responsible for the TCR-ζ degradation is as efficient as that of caspases.

Degradation of Endogenous TCR-ζ Protein by GrB. To better characterize the mechanism used by GrB to induce the observed loss in expression of the TCR-ζ protein, extracts of Jurkat cells were treated with recombinant caspase-3 or purified GrB. In either case, a significant loss in TCR-ζ protein was detected (Fig. 3, *A* and *B*). The presence of Z-VAD-FMK in the GrB-treated extract had no effect on the observed loss in TCR-ζ protein. However, it blocked the ability of GrB-formed caspase-3 p20 to auto-process itself into active subunits, as seen in the absence of Z-VAD-FMK (Fig. 3*B*). These results further demonstrate that, although GrB cleaves and activates caspase-3, the observed loss in TCR-ζ protein can be attained independently of caspase-3 activity.

Cleavage of *in Vitro* Translated TCR-ζ Protein by GrB. To determine whether GrB has a direct proteolytic effect on the TCR-ζ protein, we used *in vitro* coupled transcription-translation systems of either reticulocyte lysates or wheat germ extracts to obtain *in vitro* ζ translation products. Exposure of such *in vitro* translated ζ products to either recombinant caspase-3 or GrB resulted in significantly reduced detection of the 18-kDa-ζ product and the appearance of radiolabeled cleavage products of molecular mass of approximately 7 kDa (Fig. 4*A*). The newly generated radiolabeled signal detected after treatment with recombinant caspase-3 appears to be composed of the two cleavage products of 7- and 10-kDA detected by us previously. Whereas the cleavage products generated by caspase-3 appeared to accumulate during the reaction, the low level of detection of cleavage products after treatment with GrB suggest that they may be susceptible to further degradation by GrB (Fig. 4*B*). The detection of only one cleavage product after treatment with GrB at various doses or incubation periods of time (1–3 h; data not shown) suggests that GrB targets several sites within the TCR-ζ protein, and that the small-size cleavage products run off the gel. The degradation of *in vitro* translated ζ protein by either recombinant caspase-3 or GrB was also confirmed by immunoblotting (Fig. 4, *bottom panels*) using ζ-COOH terminus-specific Ab (capable of detecting full-length ζ protein).

GrB and Caspase-3 Target Different Cleavage Sites within TCR-ζ Protein. We have previously reported that at least two cleavage products of TCR-ζ protein are generated by the exposure of either Jurkat cell extract or *in vitro* translated ζ products to recombinant

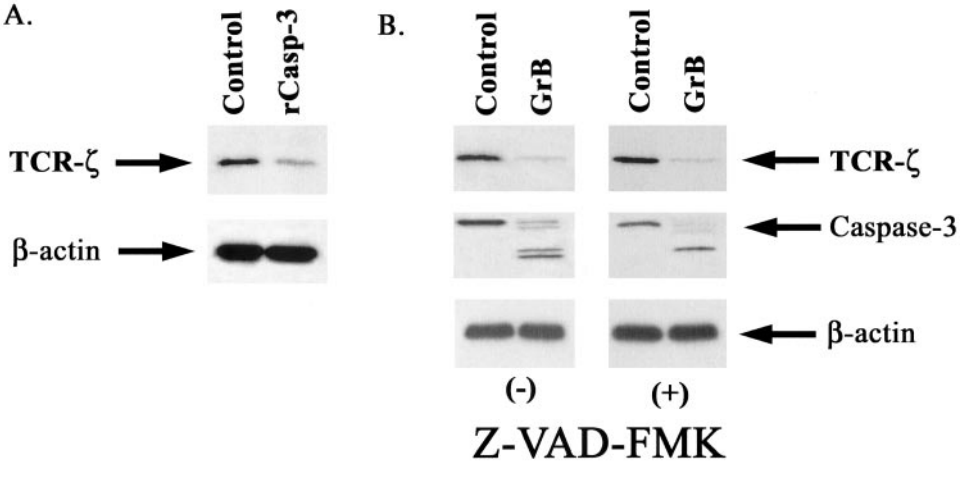

Fig. 3. GrB-mediated degradation of the TCR-ζ protein in Jurkat cell extract. Extracts of Jurkat cells were treated with recombinant caspase-3 (1 μM; *A*) or GrB (66 nM; *B*) for 1 h at 37°C. Z-VAD-FMK (100 μM) was added 20 min before the addition of GrB. Proteins were resolved by SDS/PAGE and transferred to PVDF membranes, which were probed with an anti-ζ mAb capable of detecting the full-length ζ protein and with anti-caspase-3 Ab. β-actin served to demonstrate equal loading.

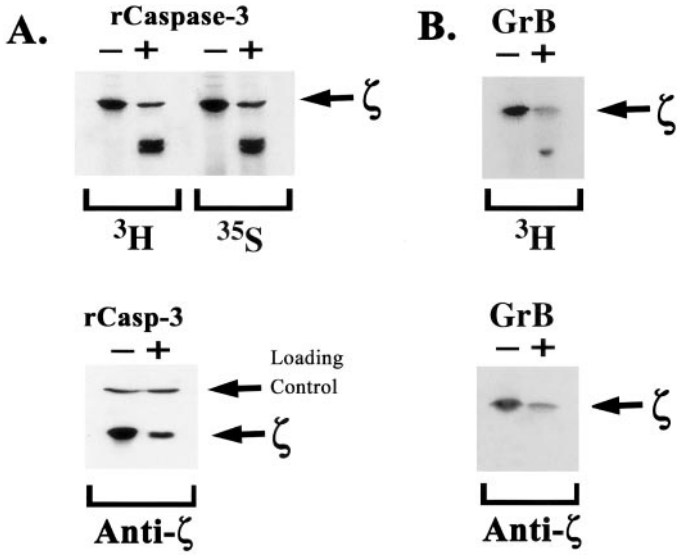

Fig. 4. Cleavage of *in vitro* translated ζ protein by recombinant caspase-3 (*A*) or GrB (*B*). *In vitro* translated ^{3}H-labeled or ^{35}S-labeled-ζ protein were incubated with recombinant caspase-3 (1 μM) or GrB (200 nM) for 3 h at 37°C. The reaction products were resolved by SDS/PAGE. The gels were then fixed, impregnated with Amplify, and vacuum-dried; and proteins were detected by autoradiography (*top panels*). For immunoblotting analyses (*bottom panels*), the proteins were transferred to PVDF membranes and probed with a ζ-COOH terminus-specific Ab capable of detecting full-length, but not cleavage products of the ζ protein. Protein bands were detected by chemiluminescence.

caspase-3 (42). In that report, we considered several aspartic acid residues as potential cleavage sites based on the size of the cleavage products, their recognition by a ζ-NH_2 terminus-specific Ab, and their labeling with [^{35}S]methionine present only in the COOH-terminal portion of the ζ protein (excluding initiator methionine residue; Ref. 42). To positively identify these cleavage sites, in the present study, we used site-directed mutagenesis to replace four aspartic acid residues of the TCR-ζ protein with alanine. Mutations D84A and D87A, but not D28A or D36A, blocked the susceptibility of *in vitro* translated ζ products to caspase-3 (Fig. 5*A*). Because a potential caspase-3 cleavage site is formed by a sequence of 4 amino acid residues with an aspartic acid residue at position P-1, mutations D84A and D87A may be part of a single cleavage site (D84XXD87). None of these mutations, when singly applied, had any effect on the susceptibility of the ζ product to GrB activity. These findings suggest that the ζ degradation mediated by GrB is targeted toward multiple sites, so that a single mutation does not result in any significant blockage of the process. However, it is also possible that the GrB activity is not directed toward any of the four mutated aspartic acid residues. Although the TCR-ζ protein encompasses a total of nine aspartic acid residues (42), only a single 7-kDa radiolabeled protein was detected in GrB-treated ζ protein, which suggests that additional cleavage products are smaller and probably run off the gel.

Our previous studies have identified the TCR-ζ protein as a substrate for caspase activity initiated by a myriad of apoptotic stimuli (42–44). In the present study, we demonstrate that GrB can also cleave TCR-ζ protein directly when caspases are inactivated. GrB is a powerful apoptotic mediator because it can efficiently activate the caspase cascade, commencing with the cleavage of procaspase-3 (48). However, cell death through GrB still occurs when caspases are blocked either by an endogenous inhibitor such as cytokine response modifier A (CrmA), FLICE/caspase-8-like inhibitory protein (FLIP), or inhibitor of apoptosis (IAP) or by exogenous peptide inhibitors such as Z-VAD-FMK. The ability of GrB to mediate the direct degradation of the TCR-ζ protein, even when other apoptotic cascades are blocked, may represent a cellular mechanism developed in T cells to ensure the removal of TCR-ζ protein during apoptosis of either leukemic or virally infected T cells.

Because the TCR-ζ protein is not part of the apoptotic machinery, the significance of its degradation during apoptosis remains unclear. However, the usage of TCR-ζ protein in the pathogenesis of certain T-cell viruses may explain the need for its degradation during apoptosis mediated by cytotoxic lymphocytes. In this regard, it has been reported that the HIV accessory protein, Nef, contributes to the pathogenesis of HIV by binding to the TCR-ζ protein (49, 50). Such an interaction forms a signaling complex that bypasses the requirement for antigen to initiate T-cell activation. Additional studies demonstrated that the utilization of the TCR-ζ protein allows Nef to act as a master switch of a transcriptional program, generating an environment conducive to dynamic viral production (51). When such virally infected T cells are targeted by CTLs, the removal of TCR-ζ protein may be crucial for the cessation of any further viral production. Furthermore, many viruses encode inhibitors of the apoptotic machinery such as vFLIP, CrmA or IAP, which endow the harboring cell with various mechanisms of resistance to apoptotic death (52). The ability of GrB to induce death through a caspase-independent mechanism provides a way around viral strategies for blocking caspase activity. However, in the absence of caspase activity, GrB-mediated apoptosis may be a slower and less efficient process, thus allowing, in the interim period, the harbored viruses to exploit the signaling properties of the TCR-ζ protein. Indeed, it has been reported that the integrity of a single immunoreceptor-tyrosine-based activation motif (ITAM) of the three present in the TCR-ζ protein was required and was sufficient to promote the activation and binding of the Nef-associated kinase (49). Thus, the ability of GrB to directly degrade TCR-ζ protein in HIV or other virally infected T cells may be of importance in ensuring that the viral-derived pro-survival signals via the TCR-ζ protein will not interfere with or reverse the apoptotic process.

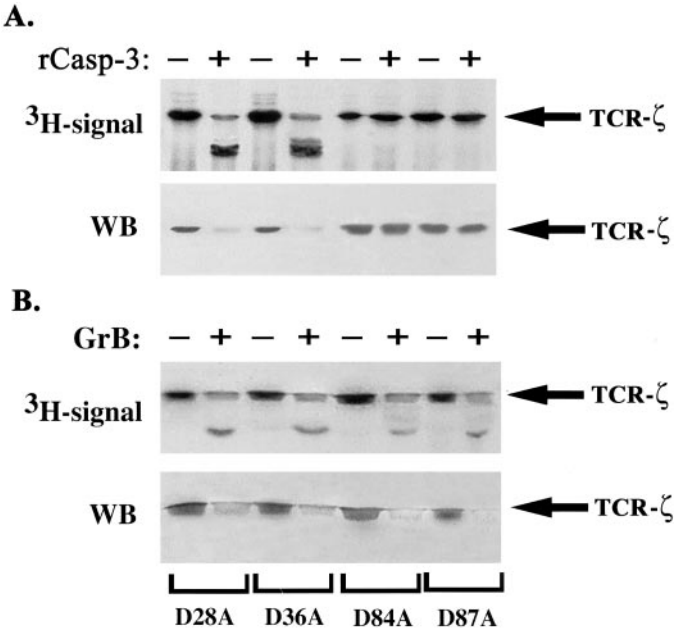

Fig. 5. Differential cleavage of *in vitro* translated TCR-ζ protein by recombinant caspase-3 and GrB. *A*, cleavage of ζ protein by recombinant caspase-3 at D84 or D87. Mutated *in vitro* translated ζ proteins, including D28A, D36A, D84A, or D87A, were incubated with recombinant caspase-3 (1 μM). The reaction products were resolved by SDS/PAGE and detected as in Fig. 4 by autoradiography (*top*) or Western blotting (*WB*, *bottom*). *B*, none of the four aspartic acid to alanine mutations in TCR-ζ blocked the degradation by GrB. The mutated *in vitro* translation products of ζ were subjected to GrB degradation (200 nM) for 3 h at 37°C. Proteins were resolved and detected as described in "Materials and Methods."

Recent studies have demonstrated that activation-induced cell death (AICD) is mediated not only by death receptor-stimulated apoptosis but also by the active involvement of GrB and perforin (53). AICD is one of the mechanisms considered to be involved in the increased apoptosis seen in lymphocytes at the tumor site (54, 55) and may be associated with the observed loss in expression of TCR-ζ protein in tumor-infiltrating lymphocytes or peripheral blood lymphocytes of cancer patients. Thus, after excess or inappropriate activation of T lymphocytes, the T cell's own GrB may contribute either directly or via activation of caspases to the degradation of TCR-ζ protein. Because TCR-ζ protein is essential for the T-cell signal transduction mechanism, including survival and proliferation signals, its removal may serve as a signal to target the cell to an irreversible path of cell death.

In summary, the findings presented in this article have defined a novel mechanism for TCR-ζ protein degradation. By its direct proteolytic effect on TCR-ζ, GrB can overcome the potential inhibition of caspases and promote the degradation of a signaling protein, which might otherwise be exploited by infecting viruses to deliver survival signals.

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