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Immunology

Activation of an interleukin 1 converting enzyme-dependent apoptosis pathway by granzyme B

[CPP32/Ich-1S/Fas/poly(ADP-ribose) polymerase]

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ABSTRACT **Cytotoxic T lymphocytes (CTL) can induce apoptosis through a granzyme B-based killing mechanism. Here we show that in cells undergoing apoptosis by granzyme B, both p45 pro-interleukin 1β converting enzyme (ICE) and pro-CPP32 are processed. Using ICE deficient (ICE −/−) mice, embryonic fibroblasts exhibit high levels of resistance to apoptosis by granzyme B or granzyme 3, while B lymphoblasts are granzyme B-resistant, thus identifying an ICE-dependent apoptotic pathway that is activated by CTL granzymes. In contrast, an alternative ICE-independent pathway must also be activated as ICE −/− thymocytes remain susceptible to apoptosis by both granzymes. In ICE −/− B cells or HeLa cells transfected with mutant inactive *ICE* or *Ich-1S* that exhibit resistance to granzyme B, CPP32 is processed to p17 and poly(ADP-ribose) polymerase is cleaved indicating that this protease although activated was not associated with an apoptotic nuclear phenotype. Using the peptide inhibitor Ac-DEVD-CHO, apoptosis as well as p45 ICE hydrolysis are suppressed in HeLa cells, suggesting that a CPP32-like protease is upstream of ICE. In contrast, $p34^{cdc2}$ kinase, which is required for granzyme B-induced apoptosis, remains inactive in ICE −/− B cells indicating it is downstream of ICE. We conclude that granzyme B activates an ICE-dependent cell death pathway in some cell types and requires a CPP32-like Ac-DEVD-CHO inhibitable protease acting upstream to initiate apoptosis.**

Induction of apoptosis by cytotoxic T lymphocytes (CTLs) occurs by distinct mechanisms one of which requires their degranulation and the release of the serine protease granzyme B and the pore-forming protein perforin (1, 2). The combined action of these molecules initiates cell death, and the process requires that the proteolytic activity of granzyme B is intact (1). The similarity of the aspartate specificity of this granzyme to members of the CED-3/interleukin 1β converting enzyme (ICE) family proteases which are important for cell death (3, 4) has led to the speculation that granzyme B may either mimic the effects of ICE proteases on substrates required for apoptosis, or it may activate one or more members of the ICE family which require processing at aspartic acid residues to be converted from an inactive pro-form as a single inactive polypeptide chain to an active protease (5).

It is now clear that the CED-3/ICE family in mammals exists as a large family (6) unlike *Caenorhabditis elegans* where only a single molecule appears to control developmental cell death (34). The reason for this multiplicity remains unexplained, but comparative analysis suggests that the family may be divided into subgroups. TX/ICErelII/ICH-2 and ICErelIII are more homologous to ICE, while CPP32/Yama/Apopain, MCH-2, MCH-3/ICE-Lap3/CMH-1 are more homologous to each other and CED-3 plus a third subgroup of ICH-2/Nedd-2 (6). The functional relationship between members within or outside these groups is not clear but it is possible that members of the family act in sequence or parallel. For example, ICE can hydrolyze and activate CPP32 (7), thromboxane can activate ICE (8), and pro-ICE cannot cleave itself (4) and may require another protease to activate it. Although it is not known if this occurs *in vivo*, members of the ICE family are often expressed in inactive forms in many tissues, so the ability of one protease to activate another may be important for apoptosis.

ICE induces apoptosis when expressed in cells (9) and crmA, an ICE inhibitor suppresses apoptosis (10); however, ICE is not a granzyme B substrate (11). Granzyme B can hydrolyze and activate CPP32 and CPP32-like proteases *in vitro* (12, 13). Furthermore, the protease is cleaved in cells undergoing CTL-induced apoptosis (12). Nevertheless, it remains unclear if either CPP32 is an intracellular substrate of granzyme B and whether CPP32 activation is necessary for granzyme B-induced apoptosis. Recently, gene-targeted ICE-deficient mice were found to have no significant defect in developmental cell death (14, 15); however, the thymocytes of ICE −/− cells were partly resistant to Fas-induced apoptosis suggesting that ICE may also participate in other forms of apoptosis (15). In the following study we report that granzyme B-induced apoptosis is defective in some ICE-deficient cell types and that CPP32 or a CPP32-like protease likely acts upstream of ICE.

METHODS

Cells. Embryonal fibroblasts (14 days) were cultured in DMEM with high glucose (GIBCO/BRL) supplemented with 4 mM L-glutamine and 10% fetal calf serum. Cells were examined within three cell passages of explantation. Thymocytes were examined directly from organs removed from the mice. B lymphoblasts were derived by incubation of splenocytes in 5 μg/ml *Escherichia coli* lipopolysaccharide (0127:B8; Sigma) for 72 h. Rat-1 and HeLa cells transfected with mutant inactive ICE (*mut-ICE*) or *Ich-S* have been described (9, 16).

Apoptosis Assays. Cells derived from ICE-deficient mice or normal controls (fibroblasts, thymocytes, and B lymphoblasts) or HeLa or Rat-1 cells were incubated in increasing concentration of granzyme B or 3 and a constant amount of perforin (125 ng/ml) for 3 and 6 h, respectively, as described (17). Cells incubated in anti-Fas antibody (JO2; PharMingen) were incu-

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Abbreviations: ICE, interleukin-1β converting enzyme; CTL, cytotoxic T lymphocytes; PARP, poly(ADP-ribose) polymerase; IL-1β, interleukin 1β.

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bated for 12–16 h at the indicated concentration, then apoptotic cells were counted with Hoechst dye staining (18). Each experimental point represents the percentage of apoptotic cells detected on the basis of chromatin condensation of 300 or more cells counted. The experiments were all repeated two or three times with similar results.

In assays using peptide inhibitors, 100 μM Ac-DEVD-CHO or Ac-YVAD-CHO (Bachem) were incubated with HeLa cells for 30 min at 37°C prior to the addition of granzyme B and perforin, then incubated an additional 3 h before enumerating apoptotic cells by Hoechst dye.

Detection of ICE Proteases, Poly(ADP-Ribose) Polymerase (PARP), and interleukin 1β (IL-1β) by Western Blotting. Postnuclear lysates were prepared as described (17) from cells incubated in the presence or absence of granzymes or anti-Fas antibody with fresh protease inhibitors (10 μg/ml phenylmethylsulfonyl fluoride, 2 mg/ml pepstatin, 1 mg/ml leupeptin, and 1 mg/ml aprotinin). Following electrophoresis on 15% SDS/PAGE and transfer to nitrocellulose or Immobilon-P, membranes were blocked with 2% BSA and incubated with either rabbit anti-ICE p45, rabbit anti-ICE p20 antibody, rabbit anti-p17 CPP32 antibody (provided by D. Nicholson, Merck Laboratories, Montreal), or rabbit anti-PARP 422 antibody which can detect murine PARP, provided by G. Poirier (Laval University, Sherbrook, Canada). Blots were then developed with either goat anti-rabbit alkaline phosphatase (Bio-Rad) or horseradish peroxidase-conjugated goat anti-rabbit IgG antibody and ECL detection kit according to manufacturers instructions (Amersham).

To detect IL-1β, HeLa cells (2×10^6) were incubated with granzyme B (1 μg/ml) and perforin (200 ng/ml) in 1 ml of granzyme buffer (17) for 6 h or the times indicated at 37°C; then supernatants were collected and centrifuged with fresh protease inhibitors as above. Samples were lyophilized, then dissolved in 50 μl SDS/PAGE sample buffer, and electrophoresed and transferred to nitrocellulose as described above. IL-1β was detected by rabbit anti-human IL-1β antibody (Calbiochem) and goat anti-rabbit IgG-AP and Bio-Rad Immun-lite chemiluminescence assay kit.

$p34^{cdc2}$ Kinase Assay. $p34^{cdc2}$ kinase activity was assayed using 2×10^6 cells following incubation with 1.25 μg/ml granzyme B or granzyme 3 and 1.25 μg/ml perforin. Cytosolic lysates were immunoprecipitated using an antibody to the C terminus of $p34^{cdc2}$ and kinase activity measured with a CKII peptide substrate as described (17). Data are given as pmol/10 min/10^6 cells based on three experiments and then statistically analyzed by the Student's *t* test.

RESULTS

Processing of p45 Pro-ICE and CPP32. We first determined whether p45 ICE was hydrolyzed in cells undergoing granzyme B-induced apoptosis. Although ICE protein cannot be directly processed by granzyme B *in vitro* (12, 13), an upstream signal could initiate processing if it were on an apoptosis pathway. We found that p45 pro-ICE was hydrolyzed in cell lysates of THP.1 or HeLa cells when incubated with granzyme B plus the pore forming protein perforin, but not either alone which are ineffective in inducing apoptosis (1) (Fig. 1 *a* and *b*). ICE

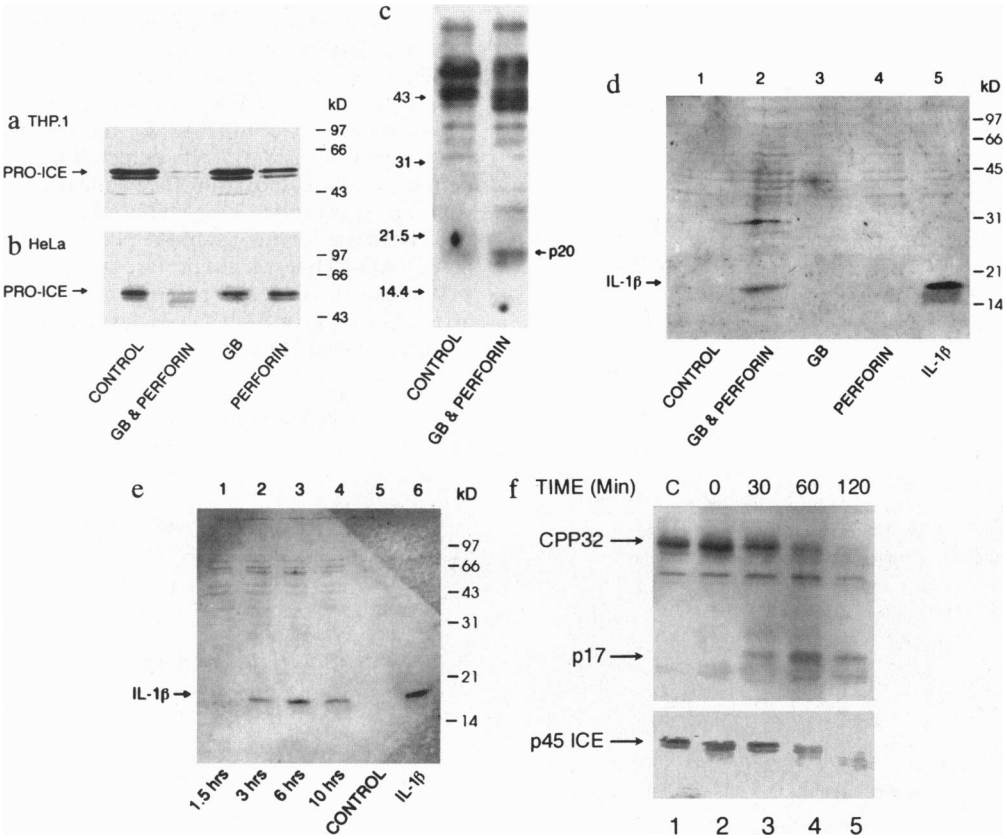

FIG. 1. Granzyme B induces p45 pro-ICE and CPP32 hydrolysis *in vivo* during apoptosis. THP.1 (*a*) or HeLa (*b*) cells were incubated with granzyme B and perforin or each agent alone for 2 h, then lysed, and then supernatants were electrophoresed and blotted with anti-p45 antibody. (*c*) Lysates from HeLa cells treated with granzyme B and perforin or untreated controls were Western blotted with antibody to p20/p10 ICE. p20 ICE is indicated on the right and the standards (kDa) on the left. (*d*) IL-1β released by HeLa cells incubated with granzyme B and perforin, or either alone. (*e*) Time course of the release of IL-1β following granzyme B and perforin treatment. (*f*) Time course of CPP32 and p45 ICE hydrolysis. CPP32 and ICE decrease and the CPP32 p17 fragment increases with time after treatment of HeLa cells with granzyme B and perforin. Lane C, untreated control.

activation requires the hydrolysis of p45 to p20 and p10 to form the active protease (4). Following granzyme B treatment, the p20 subunit was observed using an anti-p20/p10 ICE antibody (Fig. 1*c*). We also observed that mature IL-1β was released from cells treated with granzyme B and perforin reaching a maximum 6 h later (Fig. 1 *d* and *e*), indicating that normal ICE activation and processing had occurred (4). We simultaneously examined CPP32 and p45 ICE in cells undergoing granzyme B-induced apoptosis and found processing of CPP32 to p17 within 30 min of treatment; this slightly preceded the hydrolysis of p45 ICE (Fig. 1*f*).

Resistance of ICE −/− Cells to Granzyme B and Granzyme 3. To determine whether ICE is required for apoptosis, we examined cells from ICE-deficient mice for sensitivity to granzyme B as well as the tryptase granzyme 3. A pattern of resistance was observed which was unique to each cell type and for each granzyme when comparing B lymphoblasts, embryonic fibroblasts, and thymocytes. Both B cells and fibroblasts were highly resistant to granzyme B while resistance to granzyme 3 was limited to fibroblasts (Fig. 2). ICE −/− thymocytes, on the other hand, underwent apoptosis when treated with either granzyme while exhibiting partial resistance to JO-2 anti-Fas antibody, similar to earlier observations (16). Although the inefficient apoptosis of certain cells to granzyme treatment demonstrates the existence of an ICE-dependent pathway, it is also evident that ICE is not used by granzymes in some tissues. In addition, low levels of apoptosis were observed even in the most resistant ICE −/− cells, suggesting that ICE independent pathways may function within the same cell type.

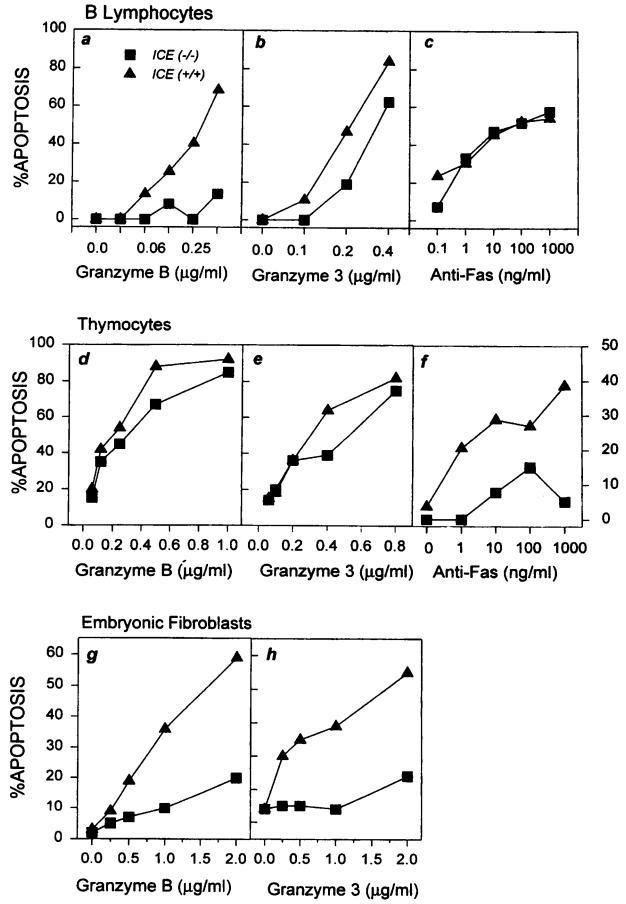

FIG. 2. ICE −/− cells resistant to apoptosis by granzyme B 3 or Fas. B lymphoblasts (*a–c*), thymocytes (*d–f*), or embryonic fibroblasts (*g* and *h*) were incubated with either granzyme B or 3 and perforin, or JO-2 anti-Fas antibody. Cells were derived from either ICE −/− or ICE +/+ mice as described.

Processing of CPP32 in Granzyme B-Resistant Cells. As shown above, CPP32 is hydrolyzed to p17 *in vivo* during granzyme B-induced apoptosis (Fig. 1*f*). It has been observed that ICE can directly cleave CPP32 to p17 and activate it *in vivo* (7), so it was of interest to determine whether or not CPP32 was processed in B lymphoblasts from ICE −/− mice which are resistant to granzyme B. Surprisingly, the hydrolysis of both CPP32 and a CPP32 substrate PARP were observed at equal levels in both normal and ICE −/− B cells (Fig. 3).

We next determined whether CPP32 was activated in other cells that were resistant to granzyme B. Rat-1 and HeLa cells, which were transfected with either mutant inactive *ICE (mut-ICE)*, in which the Cys in the conserved pentapeptide QACRG domain was converted to Gly, or *Ich-1S*, the inhibitory form of *Ich-1S/Nedd2* (9, 16), were examined. These cells, although highly resistant to granzyme B and serum withdrawal (Fig. 4 *a–c*), again exhibited hydrolysis of CPP32 to p17 and PARP was converted to p85 (Fig. 4*d*). These experiments were repeated at earlier time points and with lower doses of granzyme B and perforin with similar CPP32 hydrolysis patterns (not shown). Transfected cells were not completely resistant to granzyme B and became slowly more apoptotic at later time points although they did not reach the level of the control HeLa cells.

Inhibition of Granzyme B Apoptosis, CPP32 and p45 Processing by Ac-DEVD-CHO. PARP cleavage is an indicator of the activation of CPP32-like proteases (7, 18, 19). A peptide based on the PARP sequence DEVD was developed into an effective apoptosis inhibitor that can block CPP32 and CPP32-like protease activation and PARP cleavage (20). We next observed the effects of the inhibitor Ac-DEVD-CHO to determine whether a CPP32-like protease may be regulating p45 ICE hydrolysis and apoptosis. Ac-DEVD-CHO completely suppressed granzyme B induced apoptosis in HeLa cells (Fig. 5*a*); PARP cleavage was blocked and p45 ICE hydrolysis was reduced (Fig. 5*b*). Interestingly, CPP32 hydrolysis to p24 but not p17 was detected in the presence of the peptide inhibitor, indicating that while it blocked conversion of p24 to p17, granzyme B had induced the conversion of CPP32 to p24 and was unaffected by the peptide (Fig. 5*b*). The peptide Ac-YVAD-CHO, which preferentially inhibits ICE-related proteases (3), reduced apoptosis by 6-fold (Fig. 5*a*) without affecting CPP32 or PARP hydrolysis, while ICE hydrolysis was slightly reduced (Fig. 5*b*).

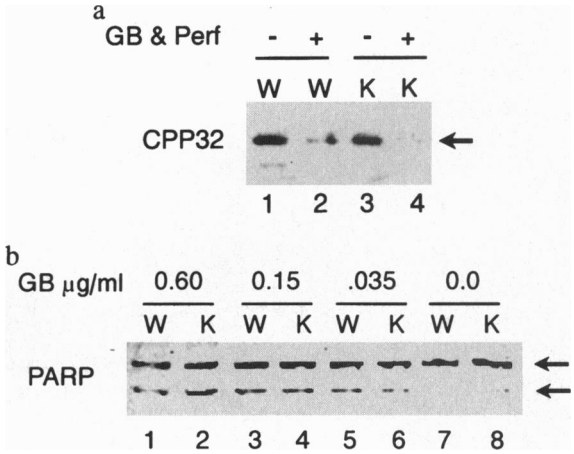

FIG. 3. CPP32 and PARP hydrolysis in granzyme B-resistant ICE −/− B lymphoblasts. (*a*) Hydrolysis of CPP32 in B cells from ICE −/− (K) and ICE +/+ (W) mice treated with granzyme B (1.0 μg/ml) and perforin (0.2 μg/ml) (lanes 2 and 4) and untreated controls (lanes 1 and 3). (*b*) Hydrolysis of PARP in B lymphoblasts from ICE −/− (K) (lanes 2, 4, 6, and 8) and ICE +/+ (W) mice (lanes 1, 3, 5, and 7) treated with granzyme B at the indicated doses and perforin (0.2 μg/ml).

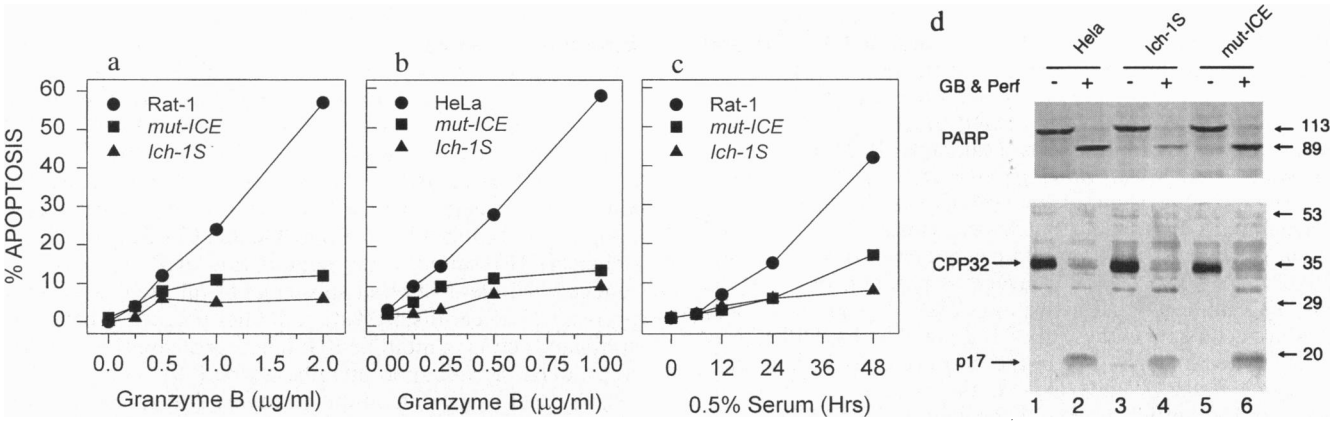

FIG. 4. CPP32 and PARP hydrolysis in granzyme B-resistant cells expressing mutant *ICE* or *Ich-1S*. (*a*) Rat-1 cells expressing mutant inactive *ICE (mut-ICE)* or *Ich-1S*, or control parental cells (Rat-1), were incubated with granzyme B at the indicated concentrations and perforin (100 ng/ml) for 2 h. Apoptotic cells were evaluated by Hoechst dye staining. (*b*) HeLa cells transfected with *mut-ICE* or *Ich-1S* were resistant to granzyme B and perforin. Other transfected HeLa cell clones with a similar resistance pattern were examined but are not shown. (*c*) Rat-1 cells were incubated in 0.5% fetal calf serum for 48 h, and apoptotic cells were counted at the time intervals indicated. (*d*) PARP (*Upper*) and CPP32 (*Lower*) hydrolysis in cells expressing *Ich-1S, mut-ICE* or untransfected HeLa cells following granzyme B and perforin treatment. The p85 product of PARP and the p17 product of CPP32 activation are shown. Molecular weight markers of the CPP32 gel are shown on the right.

p34^{cdc2} Kinase Is Not Inactivated by Granzyme B in ICE −/− Cells. We have previously shown that granzyme B and 3 induce p34^{cdc2} kinase activity (17). Others have recently found that either p34^{cdc2} or related kinases participate in some (21, 22) although not all forms of apoptosis (23). To determine whether p34^{cdc2} activation requires ICE, we examined ICE −/− B lymphoblasts which are resistant to granzyme B (Fig. 2*a*). These cells exhibited no increase in kinase activity following granzyme B treatment, while granzyme 3 was able to induce both apoptosis and kinase activity (Fig. 6).

DISCUSSION

CTLs are important mediators of host resistance. Induction of apoptosis by CTL is now understood to be both granzyme/perforin and Fas-based (24). CTL and natural killer cells from mice made deficient in granzyme B are unable to efficiently induce apoptosis in short-term assays (2), and purified granzyme B in the presence of perforin can induce apoptosis which is morphologically indistinguishable from CTL-induced apoptosis (1).

In the present study, using granzyme B and perforin we have determined that in cells derived from ICE-deficient mice, apoptosis is impaired in some but not all cell types. This demonstrates the requirement for ICE, although it is also evident that other ICE independent pathways must exist. In the context of an ICE-dependent pathway, it is not immediately obvious how apoptosis is initiated as ICE is not a granzyme B substrate (12). However, since the expression of ICE induces apoptosis in a number of cell types (9, 10) and the inhibition or absence of ICE can prevent granzyme B (this study) or other forms of apoptosis (10, 15, 25) in some cells, it is reasonable to hypothesize that the indirect activation of ICE by granzyme B can lead to cell death.

The nature of the *in vivo* substrate for granzyme B is not known, although several ICE family members have been found

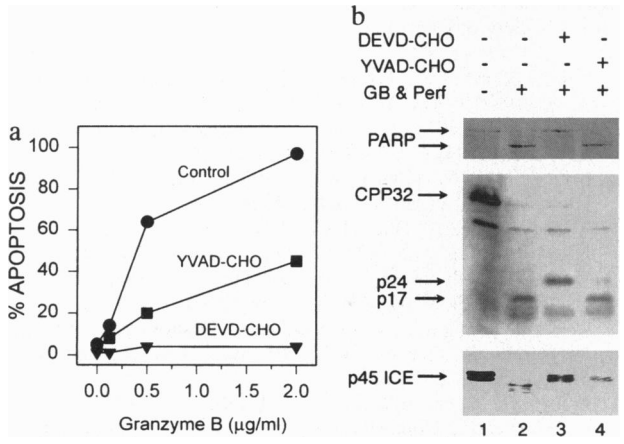

FIG. 5. ICE, CPP32, and PARP hydrolysis in granzyme B-treated HeLa cells blocked by peptide inhibitors Ac-DEVD-CHO or Ac-YVAD-CHO. (*a*) Cells were pretreated with Ac-DEVD-CHO or Ac-YVAD-CHO (100 μM) for 30 min at 37°C; then granzyme B at the indicated doses and perforin (50 ng/ml) were added for an additional 3 h before enumerating apoptotic cells after Hoechst dye staining. (*b*) In Ac-DEVD-CHO-treated cells, hydrolysis of both PARP and ICE are examined following granzyme B (1 μg/ml) and perforin (50 ng/ml) treatment for 2 h. CPP32 conversion from p32 to either p24 (arrow) or p17 (arrow) are shown, along with p45 ICE hydrolysis.

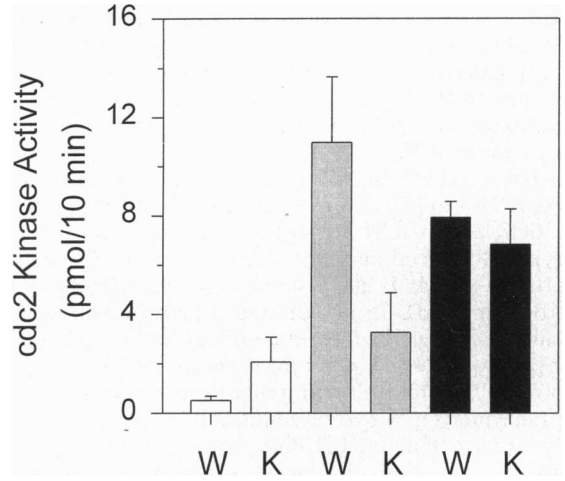

FIG. 6. p34^{cdc2} kinase activity in ICE −/− (K) and ICE +/+ (W) B lymphoblasts undergoing apoptosis induced by granzyme B (gray bars) or granzyme 3 (black bars) and perforin versus controls (open bars). p34^{cdc2} kinase activity (mean ± SD, $n = 3$) in ICE −/− cells was not significantly different from controls after granzyme B treatment ($P < 0.1$) but increased after granzyme 3 treatment ($P < 0.01$). In ICE +/+ cells both granzyme B ($P < 0.02$) and granzyme 3 ($P < 0.003$) significantly increased p34^{cdc2} kinase activity versus untreated controls.

to be correctly processed by granzyme B *in vitro*, CPP32 (12, 13), CMH-1/MCH3/ICE-LAP3 (26) and ICH-3 (35) and therefore could be candidate *in vivo* substrates. Consistent with a role for CPP32 in apoptosis, we have shown that it is processed *in vivo* along with PARP in granzyme B-treated cells. We have also recently found similar CPP32 processing by granzyme B in an *in vitro* reconstituted cell system (27). However, cells that show a high level of resistance to granzyme B, including ICE-deficient B cells and HeLa cells transfected with *mutICE* or *ICH-1S*, have equivalent levels of hydrolysis of CPP32 to p17 and PARP cleavage as parental or wild-type cells. This indicates that activation of CPP32 was insufficient to induce cell death in these cells. It is possible that CPP32 may initiate a death signal, but in the absence of ICE or in cells expressing *mutICE* and *ICH-1S*, the process is kinetically slower and less efficient. Exactly how *mutICE* and *ICH-1S* act is not known, but they may block the action of ICE-like proteases acting downstream of CPP32. They do not seem to alter the processing of CPP32.

One interpretation of how CPP32 might be activated by granzyme B but the cells remain resistant to apoptosis in ICE −/− B cells is that CPP32 lies upstream of ICE. Although ICE is capable of hydrolyzing CPP32 *in vitro* (7), the activation of CPP32 in apoptosis-resistant ICE −/− cells is not consistent with this order of action, at least in these cells. The observation that the inhibitor Ac-DEVD-CHO blocks apoptosis by granzyme B *in vivo* in HeLa cells (this study) as well as in an *in vitro* reconstituted Jurkat cell system (25) indicates that a CPP32-like protease is necessary for apoptosis. The peptide Ac-YVAD-CHO preferentially inhibits ICE-like proteases (3) and partly blocks apoptosis in HeLa cells suggesting that this ICE family subgroup is also required, and is consistent with our observations in ICE −/− B cells. Ac-DEVD-CHO also partially blocks p45 ICE processing and processing of CPP32 p24 to p17. Thus, it is possible that CPP32 lies upstream of ICE. However, we cannot exclude the participation of other CPP32-like proteases, such as MCH-2 (28) or MCH-3/ICE-LAP3 (6, 19), which may also be Ac-DEVD-CHO inhibitable and capable of activating ICE in these cells (3). MCH-3 in particular is very similar to CPP32 in its substrate specificity to PARP and DEVD peptide (19). Interestingly, the ordering of protease activation induced by Fas seems to be somewhat different to granzyme B, where it has been shown in an *in vitro* cell-free system that an ICE-like protease is activated initially and then a CPP32-like protease (25). The different ordering may more apparent than real because of the imprecise characterization of the identity of the CPP32-like proteases in these studies, and the possibility that some may participate upstream and others downstream of ICE.

We have recently shown that granzyme B can correctly process ICH-3 to p20 and p10 and that fibroblasts from mice made deficient in ICH-3 by gene targeting are resistant to granzyme B-induced apoptosis (35) similar to ICE-deficient cells. ICH-3 is an ICE-like cysteine protease. ICH-3 promotes the processing of IL-1β by ICE and is therefore an upstream regulator, although it does not directly cleave ICE. ICH-3 might be another entry point for granzyme B-induced activation of an ICE pathway. In different cell types or within a single cell, granzyme B may hydrolyze more than one substrate and very likely more that one ICE-like protease that participates in apoptosis, perhaps accounting for its rapid and ubiquitous action in inducing apoptosis.

The observation that CPP32 is processed to p17 and PARP is cleaved in granzyme B-treated cells that are not frankly apoptotic is surprising considering the consistent association between apoptosis and CPP32 activation with PARP cleavage observed by others (3, 7, 18). It is known that no defect in apoptosis is observed in PARP deficient mice so this enzyme is not critical to apoptosis (29). Although we have been discussing a model in which members of the ICE family act in a linear pathway, it has been suggested that they may function in parallel and complementary pathways proteolyzing different substrates that together induce apoptosis (30). For example, it has been suggested that the CPP32-like proteases cleave proteins that are essential for DNA repair (31) while ICE protease may release DNase I from actin (32). Nuclear lamins are cleaved by an Ac-YVAD-cmk inhibitable protease that may alter nuclear envelope integrity in the later stages of apoptosis (30). It is therefore possible that CPP32 activation by granzyme B resulted in the degradation of PARP, but with reduced hydrolysis of other substrates by different ICE family proteases, nuclear disintegration did not proceed. That is, the combined action of multiple ICE family proteases are required to produce the full apoptotic phenotype (30).

One other participant in the granzyme B apoptosis pathway is $p34^{cdc2}$ kinase (17). We have shown in earlier work that the absence of $p34^{cdc2}$ (17) or its inactivation by Wee1 kinase (33) prevents apoptosis. In the present study we found that the $p34^{cdc2}$ kinase remains inactive in the ICE −/− cells B lymphoblasts following granzyme B and perforin treatment. $p34^{cdc2}$ was capable of being activated since apoptosis induced by granzyme 3 in the same ICE-deficient B cells resulted in elevated kinase activity. Thus, granzyme B activation of $p34^{cdc2}$ kinase requires the ICE protease in these cells and likely is situated on the same pathway and downstream of ICE. Since granzyme 3 activates the kinase in ICE-deficient cells then $p34^{cdc2}$ must be activated on an ICE-independent pathway by this granzyme. We have not observed $p34^{cdc2}$ hydrolysis during apoptosis (17) and believe that ICE family proteases may target an intermediate substrate that leads to kinase activation. The exact function of $p34^{cdc2}$ in apoptosis by granzymes remains to be elucidated but evidence here indicates that it acts well downstream of the ICE family.

In conclusion, we have observed that some cells of ICE protease-deficient mice are resistant to granzyme-induced apoptosis and suggest that a CPP32-like protease and ICE are sequentially activated on a cell death pathway.

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