Granzyme B-induced Cell Death Involves Induction of p53 Tumor Suppressor Gene and Its Activation in Tumor Target Cells*5

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In this study we investigated the involvement of p53 in cytotoxic T-lymophocyte (CTL)-induced tumor target cell killing mediated by the perforin/granzymes pathway. For this purpose we used a human CTL clone (LT12) that kills its autologous melanoma target cells (T1), harboring a wild type p53. We demonstrated initially that LT12 kills its T1 target in a perforin/granzyme-dependent manner. Confocal microscopy and Western blot analysis indicated that conjugate formed between LT12 and T1 resulted in rapid cytoplasmic accumulation of p53 and its activation in T1 target cells. Cytotoxic assay using recombinant granzyme B (GrB) showed that this serine protease is the predominant factor inducing such accumulation. Furthermore, RNA interference-mediated lowering of the p53 protein in T1 cells or pifithrin-α-induced p53-specific inhibition activity significantly decreased CTL-induced target killing mediated by CTL or recombinant GrB. This emphasizes that p53 is an important determinant in granzyme B-induced apoptosis. Our data show furthermore that when T1 cells were treated with streptolysin-O/granzyme B, specific phosphorylation of p53 at Ser-15 and Ser-37 residues was observed subsequent to the activation of the stress kinases ataxia telangiectasia mutated (ATM) and p38K. Treatment of T1 cells with pifithrin-α resulted in inhibition of p53 phosphorylation at these residues and in a significant decrease in GrB-induced apoptotic T1 cell death. Furthermore, small interference RNAs targeting p53 was also accompanied by an inhibition of streptolysin-O/granzyme B-induced apoptotic T1 cell death. The present study supports p53 induction after CTL-induced stress in target cells. These findings provide new insight into a potential role of p53 as a component involved in the dynamic regulation of the major pathway of CTL-mediated cell death and may have therapeutic implications.

Antigen-specific CD8+ T cells play a crucial role in host defense against malignancies in both mouse and human models (1). In the T cell-mediated cytotoxicity process, two major pathways are involved after T cell receptor recognition of silver-major histocompatibility complex complexes expressed on target cells. The first one is an alternate pathway based on T cell receptor-induced surface expression of death receptor ligands (FasL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), and TNF) on effector cells, which cross-links the corresponding receptors (Fas/CD95, TRAIL receptors, and TNF-RI/p55, respectively) on target cells (2). The second, which is undoubtedly the major pathway, is a secretary pathway involving receptor-triggered exocytosis of preformed secretory lysosomes, termed lytic granules (3, 4). On the basis of findings in genetically manipulated mice, human genetic disease, and in vitro studies, the granule exocytosis pathway seems to have the dominant role in eliminating virus-infected cells and in tumor immunosurveillance (5). The cytoytic granules contain the pore-forming protein perforin and a family of highly specific serine proteases know as granzymes. In mice and humans, A and B are the most abundant granzymes and have received the most attention, in particular granzyme B. It has been suggested that the latter induces target cell death by cleaving and activating the pro-apoptotic Bcl-2 family member Bid (6). Truncated Bid disrupts the outer mitochondrial membrane to cause release of proapoptotic factor cytochrome c and endonuclease G (7). It has also been suggested that granzyme B (GrB)5 may induce target cell death by activating caspase 3 directly, by cleaving caspase substrates like poly ADP-ribose polymerase or inhibitor of caspase-activated DNase (CAD) to free CAD, and/or by cleaving several non-caspase substrates (8). GrB also directly disrupts the mitochondrial transmembrane potential in a caspase- and Bid-independent manner (3, 9). However, despite these advances, the functional relationship between GrB and the tumor suppressor protein p53 remains unknown.

It is well established that an appropriate response to stress stimuli is crucial for preventing cellular transformation as well as for maintaining normal tissue function. The tumor suppressor protein p53 has a central role in protecting cells from a variety of stress stimuli such as DNA damage, nucleotide deple-

* This work was supported by grants from the INSERM, Association pour la Recherche contre le Cancer Grants 4744, 3501, and 3922, Ligue contre le Cancer Grant SR2005-430 (comité des Hauts de Seine), and by the Cancéropole Ile-de-France and the Institut National du Cancer. The costs of publishing this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.

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3 Supported by a fellowship from La Ligue contre le Cancer.

4 Supported by a fellowship from L’Association pour la Recherche contre le Cancer and La Société Française du Cancer.

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5 The abbreviations used are: GrB, granzyme B; wt, wild type; CTL, cytototoxic T-lymphocyte; Ab, antibody; CD45R0, 3,3’-dihexyloxacarbocya-nine; CMA, concanamycin A; PFT-α, pifithrin-α; ROS, reactive oxygen species; siRNA, small interference RNA; PBS, phosphate-buffered saline; SLO, streptolysin-O; ATM, ataxia telangiectasia mutated.
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p53 has a short half-life and is often undetectable in normal cells. It is activated as a transcription factor through numerous post-translational modifications that allow its stabilization and accumulation in the nucleus to regulate target gene expression (11). Activated p53 induced transcription from promoters that harbor a p53 consensus binding site of genes involved in the maintenance of genetic stability and cellular homeostasis (12). Many apoptosis-related genes are regulated by p53, such as those encoding death receptors (13) and the proapoptotic Bcl-2 proteins p53-up-regulated modulator of apoptosis (Puma) (14) and Noxa (15). As an additional mode of apoptotic activity, p53 also accumulates in the cytoplasm, where it directly activates those encoding death receptors (13) and the proapoptotic Bcl-2 proteins p53-up-regulated modulator of apoptosis (Puma) (14) and Noxa (15). As an additional mode of apoptotic activity, p53 also accumulates in the cytoplasm, where it directly activates those encoding death receptors (13) and the proapoptotic Bcl-2 proteins p53-up-regulated modulator of apoptosis (Puma) (14) and Noxa (15).

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The physiological consequences of p53 activation essentially lead to cell cycle arrest, senescence, DNA repair, or apoptosis; thereby, p53 prevents cells from replicating a genetically compromised genome. Moreover, the ability of p53 to regulate the cell cycle and apoptosis has been reported to contribute to drug sensitivity induced by many anti-cancer agents (21). Nevertheless, the role of this tumor suppressor protein in the control of apoptosis mediated by cytotoxic T-lymphocyte (CTL) is not well documented. In this regard we have previously shown that p53 is a key determinant in anti-tumor CTL response as it regulates induction of Fas receptor expression, cellular FLICE/caspase-8 inhibitory protein (cFLIP) short protein degradation, and CD95-induced activation of mitochondrial pathway in tumor cells (22, 23). The present studies were designed to delineate the relationship between p53 and GrB during tumor-specific lysis. We first demonstrated that CTL-tumor target cell interaction resulted in p53 accumulation and activation. Such activation is mediated by GrB and contributes at least in part to GrB-induced apoptosis. The current study emphasizes that in addition to its role in controlling irradiation and drug responses, p53 also plays a key role in the regulation of CTL-mediated apoptosis of tumor cells.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Reagents**—Antibodies directed against p53 (DO-1, mouse IgG2a), Mdm2 (SMP14, mouse IgG1), Bid (FL195, rabbit IgG), and actin (C11, goat IgG) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-caspase 3 (8G10) rabbit monoclonal Ab, phospho-p53 antibody (Ser-6, Ser-9, Ser-15, Ser-20, Ser-37, Ser-46, and Ser-392), phospho-p38K (Thr-180/Tyr-182), and phospho-ATM (Ser-1981) polyclonal antibodies were from Cell Signaling Technology (Beverly, MA). Recombinant human GrB was purchased from Alexis Biochemicals (Lausen, Switzerland).

**Tumor Cell Line and CTL Clone**—The T1 tumor cell line was established from the primary lesion of a patient suffering from a melanoma (24) and was cultured at 37 °C (5% CO2) in RPMI 1640 with GlutaMaxTM (Invitrogen) supplemented with 5% of fetal bovine serum (Invitrogen) and 5% Ultroser® G (BioSera, France). The LT12 CTL clone was isolated from autologous tumor infiltrating lymphocytes as described previously (24) and was maintained at 37 °C (5% CO2) in complete medium (RPMI 1640 with GlutaMaxTM (Invitrogen) supplemented with 1% sodium pyruvate (Invitrogen), 5% human serum (Institut Jacques Boy, Reims, France), and recombinant interleukin-2 in the presence of the autologous tumor cell line and irradiated LAZ and allogenic peripheral blood mononuclear cells.

**Cell Death Analysis**—T1 tumor cell sensitivity to LT12 was evaluated after interaction lasting 30 min and 1 h by 3,3’-dihexyloxacarbocyanine iodide (DiOC₆(3)) and propidium iodide labeling (Molecular Probes, Eugene, OR). Inhibition of the perforin/granzymes-mediated cytotoxicity was performed using LT12 cells preincubated for 2 h with 100 nm concanamycin A (CMA) (Sigma). Cells were analyzed on a FACScalibur flow cytometer, and data were processed using Cell Quest software (BD Biosciences).

**Cytotoxicity Assay**—Cytotoxicity assays were performed using a standard 4-h chromium release assay. Briefly, 2 × 10⁵ ⁵¹Cr-labeled T1 target cells were incubated for 4 h at 37 °C with effector cells (LT12) at different effector/target ratios in a final volume of 200 μl in 96-well microplates. Experiments were performed in triplicate. At the end of the incubation 40 μl of the supernatant was transferred into 96-well Lumina Plate solid scintillation plates (Packard Instrument Co.) and, after overnight drying, counted in a Top Count β counter (Packard). Data were expressed as the percentage of specific lysis at the T1/LT12 cell ratio indicated. The percentage of specific ⁵¹Cr release (specific lysis of target cells) was calculated as (experimental release – spontaneous release)/(total release – spontaneous release) × 100. Lytic units present in 10⁵ effector cells were then assessed according to Pross et al. (25) using a computer program. One lytic unit was defined as the number of effector cells required for 30% lysis of 3 × 10⁴ target cells.

**Western Blot Analysis**—Total cellular extracts were prepared by lysing cells in ice-cold buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin). Equivalent protein extracts (30–50 μg) were denatured by boiling in SDS and β-mercaptoethanol, separated by SDS-PAGE, and transferred onto Hybond™ membranes (Amersham Biosiences). The efficiency of the electrotransfer was assessed by Ponceau Red staining. Blots were blocked overnight with Tris-buffered saline containing 5% nonfat dry milk and probed with appropriated antibody for 1 h (anti-p53 (DO-1), anti-Mdm2 (SMP14), anti-Bid (FL195), and actin (C11)) or overnight (anti-caspase 3 (8G10), anti-phospho-p53, anti-phospho-ATM, and anti-phospho-p38K). After washing, blots were incubated with appropriate horseradish peroxidase-conjugate secondary Ab. The complexes were detected using SuperSignal® West Pico Chemiluminescent Substrate (Pierce).

**Inhibition of p53 Activation**—p53 activation was inhibited by preincubating the T1 tumor cell line with 20 μM pifithrin-α.
positive control resulted in the induction of 50% of apoptotic cells. Moreover, preincubation of T1 target cells with CMA, an inhibitor of cytotoxic granules exocytosis by chelating free calcium, resulted in cell death being dramatically inhibited, indicating that the apoptotic death observed is mediated by the perforin/granzymes pathway.

p53 Accumulation in T1 Target Cells after Their Interaction with the LT12 CTL Clone—To gain more insights into p53 implication in the control of CTL-mediated lysis, we asked whether tumor T1 cell interaction with the CTL LT12 clone constitutes cellular stress sufficient to induce p53 activation in tumor cells. To this end we co-incubated T1 tumor target cells with LT12 CTL clone at an effector/target ratio of 2/1 for 10, 30, or 60 min. Western blot analysis consistently revealed that although low level expression of p53 was maintained in non-stressed T1 control target cells, T1/LT12 conjugation resulted in rapid p53 accumulation in T1 tumor target cells (Fig. 2A). Using another melanoma cell line and its specific CTL clone, we obtained data confirming the p53 accumulation in target cells (supplemental Fig. S1). Because p53 activity depends on its expression level and its cell localization, p53 expression in T1 target cells was examined by confocal laser scanning microscopy. Data provided in Fig. 2B show rapid nuclear and cytoplasm p53 accumulation in T1 target cells after CTL-target conjugation. These results underline that CTL hitting of T1 target cells effectively represents significant stress sufficient to induce p53 accumulation. To determine the possible involvement of the cytotoxic granules exocytosis-dependent pathway in cytoplasmic and nuclear p53 accumulation and activity after T1 recognition by LT12, we inhibited the perforin/granzyme-mediated pathway using CMA. Western blot (Fig. 2C) or confocal microscopy (Fig. 2D) analysis showed that preincubation of the LT12 CTL clone with CMA resulted in the inhibition of cytoplasmic and nuclear p53 accumulation and activity at the time indicated. These data show that p53 accumulation and activation observed after T1/LT12 interaction is induced by the

NOVEMBER 9, 2007 • VOLUME 282 • NUMBER 45  JOURNAL OF BIOLOGICAL CHEMISTRY  32993
perforin/granzymes pathway, further supporting the notion
that it constitutes an effective stress in target cells.

The Inhibition of p53 Activity or Lowering of Its Expression
Induced a Decrease in LT12-mediated T1 Target Lysis—To
determine whether the cytoplasmic and nuclear p53 accumu-
lation observed and its activation were involved in LT12-medi-
ated lysis, we performed experiments to inhibit either p53
expression using gene silencing or its activation using PFT-α
(27). T1 cells were transfected with siRNA targeting the p53
gene. As shown in Fig. 3A, Western blot analyses indicate
that both p53 siRNA (siRNA 2 and siRNA JT) were effective in sig-
ificantly lowering the p53 level (70%), whereas siRNA control
in p53 that was not accompanied by induction of apoptotic cell death (see Fig. 4A).

Given that post-translational modifications such as serine and threonine phosphorylation are fundamental for p53 activation, we asked whether the concomitant SLO/GrB exposure interferes with p53 phosphorylation. Western blot analyses were performed using specific antibodies to evaluate the seven sites of p53 most commonly phosphorylated. Although no phosphorylation was detected in whole cell lysates of control cultures and in cells treated with SLO alone, specific bands corresponding only to Ser-15 and Ser-37 could be observed early on at 30 min and 1 and 2 h after exposure of T1 cells to SLO/GrB (Fig. 5B). These observations suggest that GrB can effectively induce p53 activation at least in part by a mechanism involving Ser-37 and Ser-15 phosphorylation after stress kinase activation. Given that ATM and p38K are involved in the phosphorylation of Ser-15 and Ser-37, respectively, we wondered whether SLO/GrB interfere with the activation of these stress kinases. The results illustrated in Fig. 5C indicate that whereas SLO alone had no effect, the SLO/GrB combination leads to the specific phosphorylation of ATM and p38K in T1 cells at 30 min and 1 and 2 h.

Granzyme B-mediated Target Cell Death Involves p53 Phosphorylation—Because p53 phosphorylation is essential in the regulation of its activity and to further investigate how p53 activity impacts on GrB-induced killing of T1 cells, we examined the relationship between p53 phosphorylation and GrB-induced apoptotic cell death. In this aim, to explore this, we preincubated T1 cells with the specific p53 inhibitor PFT-α (20 μM) for 48 h. Data shown in Fig. 6A indicate that such treatment resulted in a significant decrease in GrB-induced apoptosis of T1 cells, which correlated with inhibition of Bid and caspase 3 cleavage (Fig. 6B). These data were confirmed using another melanoma cell line (supplemental Figs. 2 and 3). More interestingly, as depicted in Fig. 6C, the latter event correlated with inhibition of GrB-induced p53 phosphorylation at residues Ser-15 and Ser-37 especially at 1 and 2 h. These observations indicate that GrB-induced phosphorylation of p53 is a key event in coordinating the magnitude of apoptotic target killing.

siRNA Targeting p53 Induced Inhibition of SLO/GrB-mediated Apoptotic T1 Cell Death—To investigate the functional consequence of p53 silencing on SLO/GrB-induced apoptotic cell death in T1 cells, p53 was silenced in these cells by RNA
**DISCUSSION**

Approaches to treatment of cancer based on the immune system have often focused on specific cytolytic effector cells such as CTLs (29). The present study was intended to provide insight into the functional relationship between GrB and p53 during tumor-specific lysis mediated by CTLs. Several phenomenological studies on T cell-mediated cytotoxicity in vitro have been extensively reviewed (30), and major advances have been made in understanding CTL-mediated apoptosis. However, increasing evidence from studies in patients and on cultured cells has highlighted the possibility that the induction of CTLs may be essential but not sufficient to the control of tumor progression (31). It is assumed that tumor cell growth in vivo is influenced not only by the ability of CTLs to recognize and
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Induced cytoplasmic and nuclear accumulation of p53. These observations suggest that the granule exocytosis pathway contributes in the generation of stress signals to induce p53 accumulation that may be a key determinant in regulating target cell death mediated by CTL. More importantly, pharmacological suppression of p53 activity with PFT-α (36) or lowering of p53 using RNA interference resulted in significant inhibition of target cell lysis either by CTL or after treatment with GrB. This further confirms the crucial role of p53 in CTL-induced specific target cell death.

It is well established that the wtp53 protein is a critical transcription factor that responds to signals from a wide range of cellular stresses and allows the cell to cope with these stimuli by activating a set of target genes, facilitating adaptive and protective responses. It integrates cellular stresses, such as DNA damage and oncogenic transformation, to trigger either cell cycle arrest and senescence, DNA repair, or apoptosis (37). In the present studies, using TaqMan real-time quantitative reverse transcription-PCR analysis, we have shown that when LT12 were conjugated with autologous tumor targets, the transcription of p53 target genes (Mdm2, Noxa, PUMA, p21) was observed (supplemental Fig. S4). Delivery of GrB resulted in p53 accumulation in both the cytoplasm and nucleus and induced its phosphorylation at Ser-15 and Ser-37. This fits well with our findings demonstrating that GrB activates stress kinases including ATM and p38K involved in the phosphorylation of p53 and influencing its stabilization. As well as occurring with genotoxic signals, p53 induction may also occur in response to several stimuli (38). In this context it has been reported by Takaoka et al. (39) that the p53 gene is transcriptionally induced by interferon (IFN) α/β and that one mechanism of the anti-tumor action of IFNa/β may involve p53 induction. The authors suggested that treating human cancer with interferon α/β in combination with chemotherapeutic drugs that activate p53 might be useful. It would be, therefore, of major interest to examine the ability of some chemotherapeutic drugs to potentiate the susceptibility of tumor cells to CTL or GrB.

p53 peptide epitopes have been shown to be endogenously processed and presented by the human major histocompatibility class I and class II molecules of tumor cells. Given the high specificity of the CTL clone used in these studies to the melanoma-associated antigen MART-1, the possibility that wild type p53 peptide-derived epitopes contribute to the recognition of T1 cells by LT12 cells is unlikely.

Recent evidences suggest that granzyme A, the second most important granzyme, induces caspase-independent mitochondrial damage and rapid increase in reactive oxygen species (ROS). This latter process is a first step toward granzyme A-induced apoptosis that is blocked by superoxide scavengers (40). Considering evidence showing that GrB can cause rapid mitochondrial damage in the absence of Bid, Bax, and Bak (9) and recent data suggesting that stress-induced ROS generation induces a strong p53 activation (41), we can hypothesize that a rapid first step in GrB-induced apoptosis generates a ROS increase depending on GrB-induced mitochondrial damage, independently of Bid cleavage. In a second step, ROS generation may activate p53 that participates and/or regulates classi-

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**FIGURE 7. Inhibition of SLO/GrB induced apoptosis cell death in p53 siRNA treated T1 cells.** A. inhibition of p53 expression was demonstrated by Western blot analysis. T1 cells were preincubated with siRNA against wtp53 (siRNA 2) or with control siRNA (siRNA Sc) for 72 h. Actin was used as the protein level control. These data are representative of three independent experiments. B. T1 cells were incubated with siRNA against wtp53 (siRNA 2) or with control siRNA (siRNA Sc) for 72 h. Preincubation of T1 cells with siRNA 2 resulted in a significant decrease in GrB-mediated apoptosis at the times indicated. Early apoptosis was assessed by Diodc6(3)/propidium iodide labeling. Results are expressed as the mean ± S.D. for three independent determinations (*, p < 0.01).

respond to the tumor but also by the susceptibility of tumor cells to host-mediated anti-tumor immune responses (32). Such susceptibility involves not only the effector and target cell features but also their reciprocal interaction, which so far remains not clearly understood. Recently we provided evidence indicating that tumor killing by autologous CTLs can be enhanced by targeting degranulation-independent mechanisms via restoration of wtp53, a key determinant of apoptotic machinery regulation (22, 23). In this report we took advantage of the use of a human melanoma cell line displaying a wild type p53 and its autologous CTL clone. The latter is able to induce p53 accumulation in both the cytoplasm and nucleus and induced its phosphorylation at Ser-15 and Ser-37. This fits well with our findings demonstrating that GrB activates stress kinases including ATM and p38K involved in the phosphorylation of p53 and influencing its stabilization. As well as occurring with genotoxic signals, p53 induction may also occur in response to several stimuli (38). In this context it has been reported by Takaoka et al. (39) that the p53 gene is transcriptionally induced by interferon (IFN) α/β and that one mechanism of the anti-tumor action of IFNα/β may involve p53 induction. The authors suggested that treating human cancer with interferon α/β in combination with chemotherapeutic drugs that activate p53 might be useful. It would be, therefore, of major interest to examine the ability of some chemotherapeutic drugs to potentiate the susceptibility of tumor cells to CTL or GrB.

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cal GrB-induced apoptosis, implicating Bid-induced mitochondrial outer-membrane permeabilization. In our experimental model, antioxidant N-acetyl cysteine had no effect on p53 accumulation in T1 target cells in response to CTL (data not shown), ruling out the involvement of ROS in p53 accumulation and subsequent activation. Ongoing experiments will elucidate the putative role of the Mdm2 pathway in the accumulation and activation of p53 in our experimental model. It should also be noted that several proteins other than Mdm2 regulate the stability of p53, some by influencing the interaction between Mdm2 and p53 and others by mechanisms independent of Mdm2 (42).

Our results clearly point to a potential role of p53 in CTL-induced apoptosis of target harboring wild type p53. Given the fact that mitochondria is a central death regulator in response to DNA damage and is critical for p53-dependent cell death, it would seem crucial to determine how the death signal, GrB, and mitochondrial pathway are interconnected through p53 during the CTL-induced killing of target cells. Mihara et al. (19) have reported that p53 protein can directly induce permeabilization of the outer mitochondrial membrane by forming complexes with the protective Bcl-XL and Bcl-2 proteins, resulting in cytochrome c release. In parallel, p53 also accumulates in the cytoplasm, where it directly activates the proapoptotic protein Bax to induce mitochondrial release of apoptogenic factors (16). In this context, p53 seems to function like some of the proapoptotic members of the Bcl-2 superfamily called BH3-only proteins. In a recently published report, Chipuk et al. (43) suggested that Puma couples the nuclear and cytoplasmic proapoptotic functions of p53, where Puma is an enabler and p53 is an activator in a model in which Puma functions to release p53 from Bcl-XL, thereby freeing p53 to activate Bax. Moreover, in light of recent studies showing that p53 translocates to mitochondria in response to stress, Vousden (20) proposed that mitochondrial p53 could function as an enabler BH3-only protein to release an activator like Bid to interact with the antiapoptotic protein Bcl-X1. Given the fact that GrB induces target cell death by cleaving and activating the proapoptotic Bcl-2 family member Bid, which disrupts the outer mitochondrial membrane to cause release of the proapoptotic factor cytochrome c, it is tempting to speculate that p53 in our model acts by promoting GrB-induced truncated Bid release from the truncated Bid:Bcl-2/Bcl-X1 complex and also promotes cytochrome c release. It is conceivable that, to induce target killing, CTLs require both an apoptosis-sensitive phenotype and also a functional p53 pathway, which is crucial in the potentiation of target susceptibility to cell death.

Using another melanoma target cells, we demonstrated that an accumulation of p53 and its phosphorylation were observed after co-culture with the autologous CTL clone or treatment with recombinant GrB (data not shown), indicating that the reported observations are not the peculiarity of the LT12/T1 system.

Accumulating evidence has been provided indicating that p53 and nuclear factor-κB (NF-κB) modulate each other in response to stress to stimulate gene expression and that this process is controlled by relative levels of each activated transcription factor and by competition for limiting pools of the transcriptional co-activators p300 and CBP (cAMP-response element-binding protein (CREB)-binding protein) (44). This interaction could, therefore, have many implications regulating the transcriptional decision-making mechanisms that govern cellular processes such as apoptosis. Here we demonstrated that although NF-κB can be activated after effector/target conjugation or target treatment with recombinant GrB, inhibition of its activation has neither an influence on p53 accumulation nor on the killing of target cells (data not shown). Our findings suggest that, although raising the question about the potential molecular link between GrB and p53 accumulation, targeting the p53 pathway by GrB into tumor target cells may represent a novel biological end point for CTL-mediated apoptosis. Better understanding of the molecular insights into regulation of CTL-induced tumor cell death and its relationship with the p53 pathway may provide novel approaches to defining the sensitivity or resistance of tumor cells to anti-tumor therapy and new targets for rational therapeutic immune interventions.

Acknowledgments—We thank F. Faure for the LT12 CTL clone and T1 cells and M. Zylicz for helpful discussions. We are grateful to J. Benard and F. Mami-Chouaib for critical reading of the manuscript.

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