**Rb-independent Induction of Apoptosis by Bovine Papillomavirus Type 1 E7 in Response to Tumor Necrosis Factor α***

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Bovine papillomavirus type 1 (BPV-1) is a small DNA virus that causes fibropapillomas of the host. BPV-1 has served as the prototype for studies of the molecular biology of the papillomaviruses. BPV-1 efficiently induces anchorage-independent growth and focus formation in murine C127 cells. The transforming properties of BPV-1 primarily reside in two genes, E5 and E6. Each of these genes is sufficient to transform cells. Although no independent transformation activity has been detected for E7, it was shown to be required for full transformation of C127 by BPV-1. We investigated the biological activities of BPV-1 E7 in several assays. Our results indicate that expression of BPV-1 E7 sensitizes cells to tumor necrosis factor α (TNF)-induced apoptosis. The TNF-induced apoptosis in E7-expressing cells was accompanied by increased release of arachidonic acid, indicating that phospholipase A2 was activated. Unlike the E7 proteins from the cancer-related human papillomaviruses, the BPV-1 E7 protein does not associate efficiently with the retinoblastoma protein (pRB) in vitro, nor does it significantly affect the pRB levels in cultured cells. Furthermore, BPV-1 E7 sensitizes Rb-null cells to TNF-induced apoptosis. These studies indicate that BPV-1 E7 can sensitize cells to apoptosis through mechanisms that are independent of pRB.

Papillomaviruses are small DNA viruses that infect various epithelial tissues, including the epidermis and the epithelial linings of the anogenital tract. Papillomaviruses replicate in the stratified layers of skin and mucosa and usually give rise to benign lesions such as warts or papillomas. Some animal papillomaviruses, including bovine papillomavirus type 1 (BPV-1),1 induce fibropapillomas. Because of its ability to transform cells and to replicate its genome in established murine cell lines, BPV-1 has served as the prototype for studies of molecular biology of the papillomaviruses (for review see Ref. 1). Specific types (“high risk”) of human papillomaviruses (HPV) infect the anogenital tract and are strongly associated with the development of cervical carcinoma (for review see Ref. 2). The low risk HPV types, such as 6 and 11, are found associated primarily with benign lesions that rarely progress to cancer.

Papillomavirus oncogenes manifest their transforming potential in various cell culture based assays and transgenic models (1). The transforming properties of high risk HPV types, such as 6 and 11, in contrast to HPV-31 E7, have also been identified (reviewed in Ref. 6). HPV-16 E7 induces DNA synthesis in quiescent or differentiated cells (7–9). HPV-16 E7 has been shown to be associated with cellular tumor suppressor pRB (10–12). HPV E7 cooperates with E6 to efficiently immortalize primary human epithelial cells (reviewed in Ref. 6). The expression of high risk HPV E7 is sufficient to transform immortalized rodent cells (11–15). The high risk HPV E7s cooperate with an activated ras oncogene to transform primary baby rat kidney cells (16, 17). The E7 proteins of both the low and high risk HPV types were able to activate the Ad E2 promoter (16, 18, 19). HPV-16 E7 has the ability to overcome p53- and p21-mediated cell cycle arrest (10, 20–27). HPV-16 E7 can abrogate the mitotic spindle checkpoint (28). HPV-16 E7 efficiently induced epithelial hyperplasia and potentiated tumorigenesis in transgenic mice (29). Sensitization of cells to apoptosis by HPV E7 expression has been reported (24, 30–36). Recently, HPV-31 E7 was shown to be required for the maintenance of epimemes during the viral life cycle (37), and HPV-16 E7 was shown to be associated with histone deacetylase activity (38).

The major transforming proteins encoded by BPV-1 are E5 and E6. Each of these proteins is sufficient to induce anchorage-independent growth and focus formation of C127 cells (39–43). In contrast to HPV E7, little is known about BPV-1 E7. Although no independent transformation activity has been detected for BPV-1 E7, it was shown to be required for full transformation of murine C127 by BPV-1 (44). A recent study suggests that BPV-1 E7 together with E6 has significant transforming capability that is repressed by E1 and E2 (45). In addition, conflicting data have been published on the role of BPV-1 E7 in BPV-1 genome copy number regulation (44, 46–50). Immunoprecipitation with antisera raised against bacterially expressed BPV-1 E7 has detected a protein with an apparent molecular mass of 15 kDa from BPV-1 transformed cells (50). In the present study, we investigated the biological and biochemical activities of BPV-1 E7. In particular, the susceptibility of cells expressing BPV-1 E7 in response to tumor necrosis factor α (TNF) treatment and the role of Rb in this process were examined. Our results indicated a Rb-independent mechanism of apoptosis by BPV-1 E7.
EXPERIMENTAL PROCEDURES

Plasmids—The retrovirus vector pBabe Puro is a Moloney murine leukemia virus-based vector containing a puromycin resistance gene (51). Plasmids pBE7 and pBUE7 encode BPV-1 E7 and the AU1 epitope-tagged-E7 fusion in pBabe Puro, respectively. Plasmids encoding HPV-6b E7 and HPV-16 E7 were described (45). GPT-éRB (379–928), which encodes glutathione S-transferase (GST), the "large" pocket of papilloma virus E7 protein (379–928), was described in Ref. 11; plasmid encoding wild-type BPV-1 E7 and AU1 epitope-tagged E7 in pBabe Puro vector were transfected into the amphotrophic retrovirus packaging cell line PA317 (57), respectively, by calcium phosphate-mediated transfection. Transfected cells were selected for puromycin resistance. Viruses were collected and titered on C127 cells to determine the puroycin-resistant colony-forming units. C127 or MEF cells were then infected with retroviruses containing an approximately equal number of colony-forming units. After puromycin selection, populations of infected cells were pooled and used for subsequent experiments. All experiments were performed using cells within 12 passages (7 passages for MEFs).

Colorimetric MTT Assay for Cell Proliferation—Cells were seeded in 96-well plates at a density of 1000 cells/well. The following day, the medium was replaced with fresh medium (untreated cells) or treated medium supplemented with various concentrations of murine recombinant TNF (Sigma) as indicated in the text or figure legend (treated cells). In some experiments, TNF was added to the medium together with 2 μg/ml of cycloheximide as indicated in figure legend. Following treatment with TNF, viable cells were measured using the quantitative colorimetric MTT assay kit (Chemicon International Inc., Temecula, CA) according to the manufacturer's protocol. MTT is cleaved by living cells to yield a dark blue formazan product. Plates were analyzed in an ELISA plate reader at 570 nm with a reference wavelength of 655 nm.

Cell Death Detection ELISA®—Cells were seeded in 96-well plates at a density of 1000 cells/well. The following day, the medium was changed to regular medium (untreated cells) or medium containing 10 ng/ml TNF (treated cells) and incubated for 24 h. Of 200 μl of cell extract collected from each well, 20 μl were used for analysis of nucleosomes in cytoplasmic fractions by a Cell Death Detection ELISA® kit (Roche Molecular Biochemicals) according to the manufacturer's protocol. Enrichment factor represents the absorbance measured at 405 nm with a reference of 492 nm of treated cells divided by that of the corresponding untreated cells.

Flow Cytometry—Cells were seeded in 6-well plates at 2 × 10^5/well. The next day, the medium was changed to regular medium (untreated cells) or medium containing 1 ng/ml TNF plus 1 μg/ml cycloheximide (treated cells) and incubated for hours as indicated in figure legends. For viability assay, both floating and adherent cells were harvested and pelleted. Cells were resuspended in PBS containing 1 μg/ml of propidium iodide (PI), and the fluorescence was measured by flow cytometry on a FACSscan flow cytometer (Becton Dickinson, San Jose, CA) in logarimtic scale. For DNA fragmentation analysis, cells were processed as described previously (58) with slight modifications. Briefly, both floating and adherent cells were harvested and fixed in 50% ethanol at 4 °C overnight. Following fixation, the cells were centrifuged and resuspended in 0.5 ml of sample buffer (one part of PBS and three parts of 0.2 M Na_HPO₄, 0.1 M citric acid, pH 7.8, 0.1% Triton X-100 with 10% of PI). After an incubation of 30 min at room temperature, the cell samples were stored on ice and analyzed for DNA content on the FACScan flow cytometer in linear scale.

Arachidonic Acid Release Assay—Cells were seeded in 96-well plates at a density of 1000 cells/well. After adhering to the plates, cells were labeled overnight in 200 μl of complete medium containing 0.33 μCi/ml [3H]AA (5, 6, 8, 11, 12, 14, 15-[3H]AA, 100 μCi/ml; NEN Life Science Products). The cells were then washed twice with PBS and incubated for 48 h in regular medium or medium containing 10 ng/ml of TNF. The medium was collected, and the amount of released AA was determined by liquid scintillation counting.

Protein Preparation and Association Experiments—GST fusion proteins were expressed in Escherichia coli strain DH5α. One-liter cultures were inoculated with 100 ml of stationary cultures and grown for 1 h before induction with 0.2 mM isopropyl-β-D-thiogalactopyranoside for 3 h. Cells were harvested by centrifugation, resuspended in 50 ml of low salt association buffer (100 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1% Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride) plus 0.03% SDS, 2 mM diethiothreitol, and lyzed by sonication. After centrifugation at 10,000 × g for 10 min., supernatant was collected and mixed with glutathione-Sepharose beads (Amersham Pharmacia Biotech). After rotary shaking for 2 h at 4 °C, the beads were collected by centrifugation at 1000 × g, washed three times with 20 volumes of low salt association buffer, and stored at 4 °C.

In vitro translated E7 proteins were prepared by using the rabbit reticulocyte lysate transcription and translation system (Promega) and 35S-labeled cysteine (ICN Biomedicals, Irvine, CA).

For in vitro binding, 30 μl of glutathione-Sepharose beads containing 2 μg of GST fusion proteins were combined with 2–20 μl of [35S]-labeled in vitro translated proteins in lysis buffer (250 mM NaCl, 20 mM Tris-HCl, pH 7.4, 0.5% Nonidet P-40, 1 mM EDTA, 2 mM diethiothreitol, and 1 mM phenylmethylsulfonyl fluoride) in a total volume of 250 μl. The mixtures were subjected to rotary shaking for 3 h at 4 °C. The mixtures were then washed extensively with lysis buffer, boiled in SDS sample buffer, and electrophoresed on SDS-polyacrylamide gels. Gels were dried and scanned by Molecular Imager (Bio-Rad).

Immunoprecipitation, Western Blot, and Reverse Transcription-PCR—To detect E7 proteins, proliferating cells were metabolically labeled overnight with 1 mCi of [35S]-labeled cysteine/10-cm dish in cytostatic Dulbecco's minimal essential medium (5% dialyzed fetal calf serum). Cells were lysed at 4 °C in 1 ml of lysis buffer. Insoluble pelvis labeled by centrifugation at 10,000 × g for 15 min, and the supernatant was incubated with anti-AU1 antibody (BABCO) and protein A-Sepharose beads. After extensive washes with lysis buffer, the bound proteins were recovered by boiling in SDS sample buffer and loaded onto a 15% SDS-polyacrylamide gel. The epoito-tagged BPV-1 E7 band was analyzed by Molecular Image. To compare p53 levels, PBE7 and PURO cells were metabolically labeled overnight with [35S]methionine. Cell extracts were prepared by lysing cells with 0.1% Nonidet P-40 lysis buffer (34). Lysates containing equal numbers of cpms were immunoprecipitated with a p53 monoclonal antibody (Ab421, Amersham Pharmacia Biotech). Following extensive wash, the bound proteins were subjected to SDS-polyacrylamide gel electrophoresis on a 10% gel and analyzed by Molecular Imager. To examine the stability of pRB, exponentially growing PBE7 and PURO cells were treated with 20 μg/ml of cycloheximide for various time periods, harvested, and lysed in the 0.1% Nonidet P-40 lysis buffer. After removing cell debris by centrifugation, 130 μg of proteins were fractionated on a 6% SDS-polyacrylamide gel. The gel was then blotted simultaneously with an anti-pRB monoclonal antibody (PN2-245 (PharMingen) and an anti-tubulin β antibody (Sigma). The antigen-antibody complexes were detected by chemiluminescence (Ficene).

To detect E7 mRNA expression, 1 μg of total cellular RNA isolated from various cell lines was used as a template to synthesize cDNA using SuperScript II reverse transcriptase and an oligo(dT) primer (Life Technologies, Inc.). HPV-1 E7, specific primers (sense, nucleotides 71–193 of E6BP and antisense, nucleotides 381–365, 5’-TCAGGTGCATCAGGCT-3’) were used to amplify a 381-nucleotide fragment from the E7 cDNA.

Statistical Methods—Kruskal-Wallis test has been used to assess statistical significance of differences in E7-expressing cells and control cells. p < 0.05 was considered significant.

RESULTS

Cell Lines Expressing BPV-1 E7—To investigate the effect of BPV-1 E7 expression on cell growth, a cell line that expresses BPV-1 E7, named PBE7, was established. For this purpose, C127 cells were infected with amphotrophic retrovirus expressing BPV-1 E7. After puromycin selection, populations of infected cells were pooled and used for subsequent experiments. To avoid the possibility of chromosomal instability because of the expression of BPV-1 E7, all experiments described here were performed using cells within 12 passages. To facilitate detection with PAb12 using a 12-well colorimetric assay that expresses BPV-1 E7, with a C-terminal AU1 epitope tag (PBAUE7) was also made.

BPV-1 E7 gene expression was confirmed in the E7 expressing cell lines by PCR amplification of the cDNA after reverse transcription of cellular mRNA (Fig. 1A). To examine the expression of BPV-1 E7 protein, a monoclonal antibody against the AU1 epitope was used to precipitate the epitope-tagged E7
protein from PBAUE7 cells, because antibody to BPV-1 E7 was not available. In agreement with previous observations in BPV-1-transformed cells (50), BPV-1 E7 protein was present at a low level (Fig. 1B). This result indicates that in the PBAUE7 cells, overexpression of the E7 protein did not occur.

Morphologically, PBE7 and PBAUE7 cells were indistinguishable from parental cells or retrovirus vector-infected PURO cells (59). For comparison, C127 cells expressing BPV-1 E6 (PBE6) were also examined (59). Within 24 h of confluence, PBE6 cells piled up and became highly spindle shaped, indicating a loss of contact inhibition as observed in focus formation assays with BPV-1 E6. Unlike PBE6 cells, cell piling-up was not found in PBE7 or PBAUE7 cells for up to 3 weeks (data not shown). This phenotype of E7-expressing cells is consistent with the results of focus formation assays, in which no foci were observed in C127 cells infected with BPV-1 E7-expressing retrovirus. In contrast, BPV-1 E6 efficiently induces focus formation in this assay. This result is also in agreement with the previous report that BPV-1 E7 transformed neither C127 nor NIH3T3 cells (60).

Expression of BPV-1 E7 Sensitizes C127 Cells to TNF-induced Cytolysis—We have previously observed that expression of BPV-1 E6 sensitizes C127 cells to TNF-induced apoptosis (59). To examine the TNF susceptibility of E7-expressing cells, PBE7 and PBAUE7 cells were treated with various concentrations of murine TNF, and cell viability was determined quantitatively by analysis of MTT conversion (61). As shown in Fig. 2A, E7-expressing cells were much more susceptible to TNF treatment compared with the control cells. Although TNF induced less than 4% of the PURO cells to undergo cytolysis at a concentration of 10 ng/ml, 28% of PBE7 cells exhibited cytolysis. The TNF sensitivity of PBAUE7 cells was similar to that of PBE7 cells, suggesting that the AU1 tag did not alter the activity of BPV-1 E7.

The TNF sensitivity of E7-expressing cells was compared with that of PBE6 cells. We previously showed that BPV-1 E6 was a strong inducer of apoptosis compared with other known pro-apoptosis viral oncoproteins such as the polyomavirus middle T antigen (59). As shown in Fig. 2A, the effects of BPV-1 E6 and E7 on cell viability were similar, indicating that BPV-1 E7 is an equally potent inducer of cell death.

In addition to the MTT conversion assay, we employed an alternate assay to evaluate cell viability. This assay is based on the fact that live cells with intact plasma membrane exclude PI because of the charged nature of PI, whereas dead or dying cells with damaged cell membrane uptake PI and thus fluoresce when PI intercalates into DNA. After TNF and cyclohex-
imide treatment, both floating and adherent cells were pooled, resuspended in PBS containing a low concentration of PI, and analyzed on a FACSscan flow cytometer. As shown in Fig. 2B, whereas cell death in TNF-treated PURO cells showed modest increase over the spontaneous cell death in untreated PURO culture, TNF-treated PBE7 cells exhibited bigger increase in cell death.

**B**PV-1 E7 Expressing Cells Undergo Apoptosis after TNF Treatment—TNF kills most cell types by apoptosis rather than necrosis (62). Although the MTT conversion assay and the PI permeability analysis measure cell survival and cytotoxicity, they do not differentiate between cells dying of necrosis or apoptosis. To examine whether the cytolytic of PBAUE7 cells after TNF treatment is apoptotic, we performed the Cell Death Detection ELISAmix assay. This assay provides a qualitative and quantitative determination of cytoplasmic histone-associated DNA fragments resulting from DNA degradation that occurs specifically in apoptotic cells. During the process of apoptosis, a number of cellular proteases and endonucleases are activated and cellular DNA is degraded to characteristic nucleosome-sized fragments. Treatment of PBAUE7 cells with TNF for 24 h resulted in specific enrichment of mono- and oligonucleosomes released into the cytoplasm (Fig. 3A). Approximately 2.5-fold enrichment of nucleosomes in the cytoplasm was observed in PBAUE7 cells as compared with PURO cells. These results demonstrate that E7 expressing cells undergo enhanced apoptosis after TNF treatment.

To assess TNF-induced DNA fragmentation further, we compared the TNF-induced DNA fragmentation in E7-expressing cells and the control cells by flow cytometric analysis. Samples from TNF-treated or untreated cells were stained with PI after fixation, and the DNA content was analyzed on a FACSscan flow cytometer. Both PURO and PBE7 cells showed a sub-Gi population in response to TNF. However, PBE7 cells exhibited approximately 2.5-fold increase of sub-Gi population relative to that of PURO cells after incubation with TNF, indicating that E7 expression enhances TNF-induced DNA fragmentation (Fig. 3B).

**Sensitization of E7-expressing Cells to TNF-induced Apoptosis Is Accompanied by Increased Release of Arachidonic Acid—** The TNF-induced lysis of susceptible cells is usually accompanied by the release of AA into the culture medium (63). The release of AA also accompanies the lysis of cells rendered sensitive to TNF by inhibitors of transcription or translation and some viral proteins (64–67). Our recent study showed that TNF-induced apoptosis in BPV-1 E6 cells was accompanied by increased release of arachidonic acid (59). Previous work has demonstrated that activation of the 85-kDa cytosolic phospholipase A2 (cPLA2) is required for TNF cytolysis (63, 64, 68). Previous work has demonstrated that activation of the 85-kDa cytosolic phospholipase A2 (cPLA2) is required for TNF cytolysis (63, 64, 68). These studies have also revealed that the activity of cPLA2 is necessary for cell death. Phospholipase A2 specifically cleaves AA from the sn2 position of membrane phospholipid, which is thereby released from the cells (68–70). Measurement of [3H]AA released from TNF-treated cells is therefore a measure of cPLA2 activity. To determine whether cPLA2 activation is important in the response of E7-expressing cells to TNF, AA release was measured in this assay. After labeling of cells and treatment with TNF, release of [3H]AA into the culture medium was analyzed. As shown in Fig. 4, TNF caused a modest increase of cPLA2 activity on PURO cells. Importantly, PBE7 cells consistently showed a greater increase in released arachidonic acid. Statistically, there is a significant difference ($p = 0.0001$) for the release of arachidonic acid between PBE7 and control cells. This correlated with increased cytotoxicity as measured by other assays.

**Rb-independent Induction of Apoptosis by BPV-1 E7**—It has been shown that destabilization of pRB and stabilization of p53 contribute to HPV-16 E7-induced apoptosis (26). HPV-16 E7 also binds other Rb family members such as p107 and p130 (71, 72). The sequence motif LXCXE was found to present in the HPV E7 and several other viral proteins that was critical for efficient binding to the Rb family proteins (71, 73). In addition, a low affinity pRB-binding site has been identified in the C terminus of HPV-16 E7 (74). Weak association of pRB with E7 proteins from low risk HPV types has been described previously (5, 19, 75). Although they all contain the Cys-Xaa-Xaa-Cys motifs, the E7 proteins of the BPV-1 and the HPVs are quite different in their amino acid composition (less than 20%-identity). In particular, BPV-1 E7 lacks the pRB-binding motif...
BPV-1 E7 Induces Apoptosis

Figure 4. BPV-1 E7 increases the release of arachidonic acid in response to TNF. Subconfluent PURO or PBE7 cells were labeled overnight in complete medium containing 0.33 μCi/ml [3H]AA. After extensive washing, the cells were incubated for 48 h in standard medium or medium containing 10 or 20 ng/ml of TNF. The medium was collected, and the amount of released arachidonic acid was determined in a liquid scintillation counter. The relative release of arachidonic acid in the presence of TNF relative to that in the absence of TNF was calculated. Data represent the means ± S.D. of three experiments, each performed in triplicate.

LX CXE. However, the sequences in the C-terminal half of E7 proteins are well conserved between BPV-1 and HPVs. We therefore attempted to test the interaction of BPV-1 E7 with pRB and p107. For this purpose, GST-pRB, GST-p107, and in vitro translated BPV-1 E7 were tested for binding in an in vitro association experiment. As shown in Fig. 5, BPV-1 E7 did not bind p107 but associated pRB with low affinity (5% of HPV-16 E7 or ~1% of input). This is similar to what we observed for the low risk HPV-6b E7 protein (10% of HPV-16 E7). The significance of the week in vitro association between BPV-1 E7 and pRB remains to be elucidated.

It has been reported that HPV E7 expression resulted in decreased pRB levels and stabilization of wild-type p53 (26, 76, 77). We therefore investigated the steady state level of pRB and p53 in PBE7 cells. Extracts were prepared from PBE7 cells, and levels of pRB and p53 were examined by Western blot and immunoprecipitation. No significant differences in pRB and p53 levels were observed between PBE7 cells and the control PURO cells were found. In addition, no significant change in the turnover rate of pRB was detected in PBE7 cells compared with the PURO cells (data not shown). We therefore conclude that expression of BPV-1 E7 does not significantly affect the stability of pRB or p53.

Both binding and destabilization of pRB have been shown to contribute to HPV-16 E7-induced apoptosis (34). Inhibition of apoptosis by RB has been observed in several studies (78–81). Although BPV-1 E7 does not bind pRB efficiently in vitro or promote its degradation in vivo, it is intriguing to test the requirement of pRB for BPV-1 E7-mediated sensitization of cells to TNF-induced apoptosis. To assess the role of Rb in BPV-1 E7-induced apoptosis, Rb−/− MEF cells were used. The cells were infected with amphotropic retroviruses expressing BPV-1 E7. After puromycin selection, populations of infected cells were pooled. Similarly, control Rb−/− MEF cells infected by the retrovirus vector was also selected. In addition, Rb+/+ MEF cells were also included as controls. The TNF susceptibility of resulting cells was then analyzed. As shown in Fig. 6, increased cytolysis was observed in cells expressing BPV-1 E7 as compared with the control cells. Although the difference is small, Rb−/− E7-expressing cells are statistically more sensitive than the control PURO cells (p < 0.05). These data indicate that BPV-1 E7-mediated sensitization of cells to TNF-induced cytolysis can occur in the absence of Rb, although it does not rule out the possibility that some of the activities seen in C127 cells involve Rb.

Figure 5. Interaction of BPV-1 E7 with pRB family members. Glutathione-Sepharose beads containing GST-E6BP, GST-pRB, and GST-p107 fusion proteins were individually combined with [35S]-labeled in vitro translated E7 proteins in lysis buffer. After incubation and washes, the bound products were separated by SDS-polyacrylamide gel electrophoresis. E7 binding was analyzed by Molecular Imager (Bio-Rad). E7 proteins are indicated to the left. Input was directly loaded into the well and represents 10% of the [35S]cysteine-labeled E7 proteins used in each binding reaction.

Figure 6. pRB-independent induction of apoptosis by BPV-1 E7 in response to TNF. pRB+/+ or pRB−/− MEFs infected with retroviruses were plated in 96-well plates at 2,000 cells/well 1 day before being treated with 10 ng/ml of TNF in the presence of 2 μg/ml of cycloheximide. PURO, cells infected with retrovirus containing Babe Puro; BE7, cells infected with retrovirus expressing PBV-1 E7. Cell viability was determined 24 h later by analysis of MTT conversion. Data represent the means ± S.D. of three experiments, each performed in triplicate.

DISCUSSION

In this study, we investigated the biological and biochemical activities of BPV-1 E7. Our results indicate that expression of BPV-1 E7 sensitized cells to TNF-induced apoptosis. TNF-induced apoptosis in PBE7 cells was accompanied by increased release of arachidonic acid. BPV-1 E7 protein did not efficiently associate with pRB in vitro or significantly affect the pRB levels in culture cells. Furthermore, BPV-1 E7 sensitized Rb-null cells to TNF-induced apoptosis. These studies indicate...
that BPV-1 E7 can sensitize cells to apoptosis through Rb-independent mechanisms.

Viruses have evolved gene products that modulate apoptotic activity (for review see Refs. 82 and 83). For example, adenovirus E1A-induced apoptosis can be counteracted by the adenovirus E1B 19-kDa and E1B 55-kDa proteins (84, 85). Similar to adenovirus E1A/E1B-regulated apoptosis, HPV-16 E6 inhibited E7-mediated apoptosis in the developing ocular lens of transgenic mice (32). In the case of adenovirus, cooperation between the adenovirus E1A and E1B oncoproteins is required for transformation of primary quiescent rodent cells (84). We have observed that BPV-1 E6 independently induces TNF-mediated apoptosis in C127 cells (59). The extent of TNF-induced apoptosis was similar in the BPV-1-gene transformed ID13 and parental C127 cell lines and substantially less than that of the E6-expressing cells (59). These observations suggest that some BPV-1 gene(s) may protect ID13 cells from E6-mediated susceptibility to TNF-induced apoptosis. Given the fact that BPV-1 E7 increased the efficiency of E6 transformation, one might expect that E7 would inhibit E6-mediated apoptosis. However, our result indicates that expression of BPV-1 E7 leads to induction instead of inhibition of apoptosis.

Co-expression of BPV-1 E7 with E6 slightly increased the susceptibility of cells to TNF (data not shown).

How BPV-1 E7 sensitizes cells to TNF is not clear. The fact that BPV-1 E7 can sensitize cells to apoptosis in the presence of metabolic inhibitors suggests that some newly synthesized proteins are required for this process. Similar to the model proposed for c-Myc-induced apoptosis (86), BPV-1 E7 may repress an inhibitor(s) of TNF-induced apoptosis. This inhibitor may be short-lived, because actinomycin D or cycloheximide may help to remove it.

TNF elicits a wide spectrum of organi...
