The Adenovirus E3 Promoter Is Sensitive to Activation Signals in Human T Cells

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The group C adenoviruses typically cause acute respiratory disease in young children. In addition, a persistent phase of infection has been observed in which virus may be shed for years without producing overt pathology. Our laboratory recently reported that group C adenovirus DNA can be found in tonsil and adenoid T lymphocytes from the majority of pediatric donors (C. T. Garnett, D. Erdman, W. Xu, and L. R. Gooding, *J. Virol.* 76:10608-10616, 2002). This finding suggests that immune evasion strategies of human adenoviruses may be directed, in part, toward protection of persistently or latently infected T lymphocytes. Many of the adenoviral gene products implicated in prevention of immune destruction of virus-infected cells are encoded within the E3 transcription unit. In this study, the E3 promoter was evaluated for sensitivity to T-cell activation signals by using a promoter reporter plasmid. Indeed, this promoter is extremely sensitive to T-cell activation, with phorbol myristate acetate (PMA) plus ionomycin increasing E3-directed transcription 100-fold. By comparison, in the same cells E1A expression leads to a 5.5-fold increase in transcription from the E3 promoter. In contrast to induction by E1A, activation by PMA plus ionomycin requires the two E3 NF- κ B binding sites. Interestingly, expression of E1A inhibits induction of the E3 promoter in response to T-cell activation while increasing E3 promoter activity in unactivated cells. Collectively, these data suggest that the E3 promoter may have evolved the capacity to respond to T-cell activation in the absence of E1A expression and may act to upregulate antiapoptotic gene expression in order to promote survival of persistently infected T lymphocytes.

Human group C adenoviruses (serotypes 1, 2, 5, and 6) typically infect the respiratory tract of young children and cause a mild illness (9). A persistent phase of adenovirus infection has also been observed, during which live virus is intermittently excreted into the feces of healthy individuals long after the initial acute infection has been resolved (24, 25). Recently, our laboratory demonstrated that the adenovirus genome can be found in CD3⁺ T lymphocytes from human tonsils or adenoids in the absence of virus replication, suggesting that adenovirus is capable of establishing a quiescent or latent infection in human mucosal T cells (29).

The adenovirus E3 transcription unit encodes several proteins that protect infected cells from apoptosis by mediators such as Fas ligand, tumor necrosis factor (TNF), and TNF-related apoptosis-inducing ligand (TRAIL) (3, 20, 32, 33, 57, 66, 74, 75). Interestingly, these same three mediators play an important role in the homeostatic regulation of T cells through a process called activation-induced cell death (40, 46, 50). Fas ligand, TNF, and TRAIL have also been shown to mediate killing of target cells by CD4⁺ cytotoxic T lymphocytes (27, 44, 51, 54, 73). The adenovirus E3 transcription unit also encodes the gp19K protein, which binds to class I major histocompatibility complex (MHC) molecules in the endoplasmic reticulum and prevents their translocation to the cell surface, where they present peptides to CD8⁺ T cells (4; reviewed in reference 69).

Thus, expression of the E3 transcription unit could function to preserve adenovirus persistence in human T cells in three different ways: (i) inhibition of intrinsic activation-induced apoptotic mechanisms, (ii) suppression of class I MHC antigen presentation of immunogenic adenovirus epitopes, and (iii) prevention of CD4⁺-T-cell killing of infected cells.

Transcription of the E3 region is directed by a single promoter that requires the expression of the adenovirus immediate early gene E1A for optimal activity during lytic infection (5). The 105 bp immediately upstream of the transcriptional start site are sufficient for E1A induction of E3 transcripts (48, 77, 78). DNase I footprinting, electrophoretic mobility shift assays (EMSAs), and site-directed mutagenesis reveal binding sites for the transcription factors AP-1 and ATF in this 105-bp region (28, 38). Immediately upstream of this E1A-responsive element are two NF- κ B binding sites (17, 79). This upstream region is not required for basal or E1A-induced activity of E3 (48, 77); however, these NF- κ B binding sites are conserved among adenoviruses, suggesting that they play an important role in the adenovirus life cycle.

NF- κ B is a family of transcription factors made of hetero- and homodimers of five different proteins: NF- κ B1 (p50), NF- κ B2 (p52), c-Rel, RelA (p65), and RelB (reviewed in reference 41). NF- κ B plays an important role in upregulating gene expression in T cells upon stimulation of the T-cell receptor (TCR) (reviewed in references 2 and 42). Furthermore, NF- κ B can also be activated by both TNF and TRAIL, which are themselves upregulated upon T-cell stimulation (31, 56, 72). NF- κ B controls the expression of several genes known to be important in determining the fate of activated T cells, includ-

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TABLE 1. Primers used in this study

Primer	Sequence
5' E3 promoter	TAGAGCTAGCCCCGTAGTTGGCCCCGCTG
3' E3 promoter	TAGACTCGAGCCTCGCCCTCTGATTTTCAG
5' E3 κ B WT sense	CCCTGGTGTACCAGGAAAGTCCCGCTCCCACCACTG
5' E3 κ B WT antisense	CAGTGGTGGGAGCGGGACTTTCTCTGGTACACCAAGG
3' E3 κ B WT sense	CACCACTGTGGTACTTCCCAG
3' E3 κ B WT antisense	CTGGGAAGTACCACAGTGGTG
5' E3 κ B mutant sense	ACGATATCAGGCTCCCACCACTGTGGTACTTCCCA
5' E3 κ B mutant antisense	CTGATATCGTTGGTACACCAAGGGAGCGGGCCA
3' E3 κ B mutant sense	ACGATATCAGAGAGACGCCAGGCCGAAGTT
3' E3 κ B mutant antisense	CTGATATCGTACAGTGGTGGGAGCGGGACT
5' E3 κ B mutant2 sense	ACGATATCAGGCTCCCACCACTGTACGATATCAGA
E3 AP-1 mutant sense	CCAGGCCGAAGTTCAGACTCGAGACTCAGGGGCGAGCTTGCGGGC
E3 AP-1 mutant antisense	GCCCCGAAGCTGCGCCCCTGAGTCTCGGAGTCTGAACCTCGGCCTGG

ing TRAIL (1), Fas (11), Fas ligand (43), and the antiapoptotic proteins Bcl-x_L (45), c-FLIP (58), and cIAP2 (13).

The observation that the adenovirus genome can be found in human tonsillar T cells in the absence of live virus production suggests the existence of an alternate, nonlytic mode of viral gene expression. Given the importance of Fas, TNF, and TRAIL proapoptotic signaling pathways in determining T-cell survival, we hypothesized that the E3 promoter would be up-regulated by T-cell activation in an E1A-independent manner to ensure efficient production of E3 antiapoptotic proteins. Here we report that the E3 promoter is exquisitely sensitive to T-cell activation signals, displaying up to 200-fold induction compared to the 5- to 10-fold induction seen when E1A induces E3 transcription in the same cells. Most of the E3 transcription in activated T cells appears to be driven by NF- κ B in addition to AP-1.

MATERIALS AND METHODS

Cell culture. Jurkat and CEM-CCRF (referred to here as CEM) are both CD4⁺ acute T-cell leukemia cell lines and were obtained from the American Type Culture Collection. HeLa, a cervical carcinoma-derived cell line, was also obtained from the American Type Culture Collection. CEM and Jurkat cells were grown in RPMI medium containing 10% fetal calf serum (HyClone, Logan, Utah) supplemented with 2 mM glutamine (Gibco BRL); HeLa cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum supplemented with 2 mM glutamine (Gibco BRL) and 100 U of penicillin G per ml, 100 μ g of streptomycin sulfate per ml, and 0.25 μ g of amphotericin B (Gibco BRL) per ml.

Reagents. Anti-NF- κ B p65 antibody was obtained from Santa Cruz Biotechnology (catalog no. SC-109X); anti-CD3 antibody was from Pharmingen (catalog no. 555329). Phorbol myristate acetate (PMA) was from Sigma (catalog no. P8139), and ionomycin (Ca²⁺ salt) was from Calbiochem (catalog no. 407592). Recombinant human TNF was obtained from BD Pharmingen (catalog no. 554618).

Plasmids. The wild-type E3 promoter luciferase reporter (pE3-luc) was constructed by amplifying the 200 bp comprising the E3 promoter from adenovirus type 2 using the primers "5' E3 promoter" and "3' E3 promoter" (see Table 1 for the sequences of all primers used in this study), digesting the product with *Nde*I/*Xho*I, and ligating it into pGL3-basic (Promega). Single-site mutant E3 constructs were generated from the pE3-luc wild-type plasmid through PCR-mediated site-directed mutagenesis using the mutagenic primers in Table 1. Briefly, limited-cycle PCR was performed with *Pfu* polymerase (Stratagene) followed by digestion with *Dpn*I to digest the input (unmutated) DNA. *E. coli* strain XL1-Blue (Stratagene) was transformed with the resulting reaction products, and colonies were screened for the presence of the mutation by digestion with *Eco*RV. Positive clones were then recloned into pGL3-basic in order to eliminate second-site mutations outside the E3 promoter region. The E3 double- κ B mutant promoter construct ("both κ B mutant") was generated by the same process with the 5' κ B mutant plasmid as the template and primers "5' E3

κ B mutant antisense" and "5' E3 κ B mutant2 sense." The sequences of all constructs were verified by double-strand sequencing. pCMV-E1A was obtained from Eileen White (Rutgers University); pN1EGFP was obtained from Clontech. All plasmids were purified by CsCl density gradient centrifugation.

Transient transfections and luciferase assays. CEM or Jurkat cells were diluted to 0.5×10^6 cells/ml 18 h prior to transfection, electroporated at 290 mV and 960 μ F in a Bio-Rad gene pulser, and immediately transferred to 50% conditioned growth medium. For each transfection of HeLa cells, cells from one ~80% confluent 10-cm plate were harvested with trypsin, washed with serum-free DMEM, resuspended in 800 μ l of serum-free DMEM containing the amount of plasmid DNA indicated in the figure legends, and electroporated at 270 mV and 960 μ F. Electroporated cells were allowed to recover for 18 to 24 h and then treated as described in the figure legends. For anti-CD3 treatment, 12-well plates were coated with 10 μ g of UCHT1 (Pharmingen) per ml in coating buffer (0.1 M NaHCO₃, pH 8.2) for 10 h at 4°C or 2 h at 37°C. Luciferase activity was determined with a Promega dual luciferase assay kit by following the manufacturer's protocol with the following changes: cells were lysed in 150 μ l of passive lysis buffer, and 5 μ l of cleared lysate was assayed against 50 μ l of luciferase substrate in a Fentomaster FB12 luminometer (Zylux Corporation). Activation (-fold) was calculated by dividing values for treated samples by the untreated value for that transfection.

Preparation of nuclear extracts and EMSAs. Nuclear extracts were prepared by the method of Fiering et al. (21), using protease inhibitors from Boehringer Mannheim (Indianapolis, Ind.). Protein concentrations were determined with the Bio-Rad DC protein assay kit. The double-stranded 5' E3 κ B probe was made by annealing oligonucleotides "5' E3 κ B WT sense" and "5' E3 κ B WT antisense"; 3' E3 κ B probe was made by annealing oligonucleotides "3' E3 κ B WT sense" and "3' E3 κ B WT antisense." For each binding reaction, 8 μ g of nuclear extract was incubated for 30 min at 25°C with a ³²P-, end-labeled double-stranded oligonucleotide probe and then separated on a 5% acrylamide (80:1) gel. Competition experiments were performed with a 100-fold molar excess of cold competitor oligonucleotides. Anti-NF- κ B p65 (2 μ g) was used per binding reaction for supershifting bound complexes. The dried gel was exposed to film (Biomax MR; Eastman Kodak Company).

RESULTS

The E3 promoter is sensitive to T-cell activation signals. To facilitate the analysis of E3 promoter regulation, 200 bp of sequence upstream of the E3 transcriptional start site was cloned into a plasmid containing the gene for firefly luciferase (pGL3; Promega) such that expression of luciferase was under the control of the adenovirus type 2 E3 promoter (pE3-luc). This sequence contains both the E1A-responsive element and the upstream NF- κ B binding sites. A diagram of the E3 promoter is shown in Fig. 1.

To investigate the activity and regulation of the E3 promoter in T cells, the wild-type E3 promoter reporter plasmid (pE3-luc) was transfected into CEM, a human CD4⁺ T cell line. PMA (an activator of protein kinase C) and ionomycin (a

FIG. 1. Structure of the adenovirus E3 promoter. Numbers indicate nucleotide positions on the adenovirus type 2 genome. Sequences required for E1A induction of E3 (E1A-RE) are described in the text.

calcium ionophore) were used to mimic activation through the TCR. Treatment of pE3-luc-transfected cells with PMA and with ionomycin alone increased the activity of the E3 promoter 9.1-fold and 5.5-fold, respectively (Fig. 2A); however, when PMA and ionomycin were used together there was a ~100-fold increase in E3 promoter activity. Activation of the E3 promoter reporter plasmid was also investigated in another human CD4⁺ T-cell line, Jurkat. Treatment of pE3-luc-transfected Jurkat cells with PMA or ionomycin alone resulted in a ~2-fold increase in E3 promoter activity. Treatment with a combination of PMA and ionomycin increased E3 promoter activity 10-fold in Jurkat cells (Fig. 2B). In order to show directly that the E3 promoter is responsive to signals transmitted through the TCR, pE3-luc-transfected Jurkat cells were activated with plate-bound anti-CD3 antibody. Treatment of pE3-luc-transfected Jurkat cells with anti-CD3 resulted in a three-fold increase in promoter activity after 12 h of treatment (Fig. 2C). These data indicate that the E3 promoter is sensitive to the activation signals delivered to the T cell.

E3 promoter activation in T cells requires both NF- κ B and AP-1 sites. In order to determine the roles of the E3 NF- κ B and AP-1 sites in the T-cell activation-induced activity of this promoter, mutant reporter constructs were generated in which these sites were mutated by substitution. Wild-type and mutant constructs were transfected into CEM cells which were then treated with PMA plus ionomycin or left untreated. At various times, samples were removed and processed for luciferase activity to assess transcription directed by the E3 promoter. Activity of the wild-type E3 promoter after stimulation with PMA plus ionomycin increased ~12-fold by 3 h and ~45-fold by 10 h (Fig. 3A). The 5' NF- κ B site mutant showed a 75% reduction compared to wild-type activation at 3 h of treatment and 78% by 10 h of treatment. Mutation of the 3' NF- κ B site had a less dramatic effect with a 45% decrease in activation at 3 h and 50% at 10 h. Mutation of both NF- κ B sites did not have a greater effect than mutation of the 5' site only. Mutation of the AP-1 site also significantly reduced E3 activation (62% reduction at 3 h and 67% at 10 h), indicating that the AP-1 site is as important as the 5' NF- κ B site. These data suggest that full activation of the E3 promoter via PMA-plus-ionomycin signals in T cells requires both the 5' and 3' κ B sites and the AP-1 site.

Previous studies have shown that the E3 promoter can also be activated by TNF through its NF- κ B site (17). In contrast, others report that the region adjacent to and including these NF- κ B sites is not involved in E1A-mediated transactivation of E3 (28, 77). To verify a lack of involvement of the E3 NF- κ B sites in E1A-mediated transactivation of the E3 promoter but

FIG. 2. Activation of the adenovirus type 2 E3 promoter reporter by T-cell stimulation. CEM and Jurkat cells were transfected with 50 or 5 μ g (respectively) of the wild-type E3 reporter plasmid, pE3-luc. At 24 h after transfection, cells were treated with PMA (16 nM), ionomycin (0.75 μ M), PMA plus ionomycin, or plate-bound anti-CD3 (UCHT1, 10 μ g/ml), and luciferase activity was determined after 12 h. Error bars were determined from the averages of three independent transfection experiments.

a requirement in TNF induction of E3, activation of mutant reporter plasmids by these stimuli was tested in HeLa cells. While mutation of either or both NF- κ B sites resulted in a small decrease in the basal activity of the E3 promoter, it had no effect on E1A induction of the E3 promoter (Fig. 3B). In fact, NF- κ B mutants appeared to be more sensitive to activation by E1A, as measured by "fold induction," than the wild-type promoter. Mutation of the E3 AP-1 site had a more profound effect on E1A induction, reducing induction of E1A by almost 50%. In contrast, TNF induction of the E3 promoter reporter is completely abolished by mutation of both NF- κ B sites (Fig. 3C). These experiments verify that the E3 NF- κ B

FIG. 3. Transcription factor binding sites required for activation of the E3 promoter. (A) CEM cells were transfected with E3 promoter reporter plasmids containing the indicated mutations and treated with PMA plus ionomycin. Cells were harvested at various time points, and luciferase activity was determined. Results are the averages of three independent transfection experiments. (B) HeLa cells were transfected with 5 μ g of the indicated E3 promoter reporter plasmids together with or without 1 μ g pCMV-E1A; cells were harvested, and luciferase activity determined 48 h posttransfection. Results are the averages of two independent transfection experiments. Induction by E1A is shown. (C) HeLa cells were transfected with 5 μ g of the indicated E3 promoter reporter plasmid and treated with TNF (500 U/ml); cells were harvested, and luciferase activity was determined after 12 h. Induction by TNF is shown.

sites are not involved in E1A induction of the E3 promoter but are required for TNF induction of E3.

Transcription factors in activated T cells that bind E3 promoter sites. Previous research demonstrated that the E3 NF- κ B sites bind nuclear proteins found in several different (unactivated) B- and T-cell lines but not in nonlymphoid cell lines (79). The identity of these proteins was not investigated, nor was the effect of T-cell activation assessed. To identify the

FIG. 4. NF- κ B from activated CEM T cells binds to both E3 κ B sites. Nuclear extracts from unactivated or PMA-ionomycin (Iono)-activated CEM cells were incubated with double-stranded oligonucleotides comprising either the 5' or the 3' E3 NF- κ B binding sites as detailed in Materials and Methods. Unlabeled 5' or 3' E3 NF- κ B ("specific") or HLA-DRA oligonucleotide (36) ("non-specific") were used as specificity controls.

factors mediating upregulation of the E3 promoter in activated T cells, EMSAs were performed with nuclear extracts from unactivated or PMA-ionomycin-activated CEM cells. Unactivated CEM nuclei contained a factor that bound to an oligonucleotide consisting of the 5' E3 κ B site (Fig. 4), consistent with the observation that these cells have a high basal level of NF- κ B activity when assayed by an NF- κ B reporter plasmid (data not shown). A significant increase in this factor is seen in nuclei from cells treated with PMA plus ionomycin. Competition with an excess of the unlabeled 5' E3 κ B oligonucleotide ("specific") completely inhibited formation of this complex, while an unlabeled irrelevant oligonucleotide (HLA-DRA, "nonspecific") did not affect binding, thus showing specificity for the 5' E3 κ B site. Formation of this complex was also completely inhibited by competition with an oligonucleotide consisting of the immunoglobulin κ NF- κ B binding site, indicating that this complex contains an NF- κ B binding activity. Confirmation that this complex contained NF- κ B was obtained by adding anti-NF- κ B p65 antibodies to the binding reaction mixture; this resulted in three supershifted complexes, most likely representing p65 heterodimerized to other NF- κ B proteins (p50, c-Rel, etc.). Similar results were obtained when the 3' E3 κ B oligonucleotide was used (Fig. 4), with the notable difference that this site bound less efficiently to NF- κ B than the

FIG. 5. Inhibition of E3 promoter reporter activation in T cells by E1A. CEM cells were cotransfected with 50 μ g of pE3-luc and increasing amounts of pCMV-E1A (or pN1-EGFP [Clontech] as a control). Transfected cells were split and left untreated or treated with PMA plus ionomycin. Cells were harvested after 4 or 10 h of treatment, and reporter activity was determined. Results are the averages of three independent transfection experiments.

5' site. Thus, NF- κ B induced by activation of CEM cells can bind to both the E3 NF- κ B binding sites.

Inhibition of E3 promoter activation by the adenovirus E1A protein. The adenovirus E1A proteins are known to both transactivate the E3 promoter and to modulate NF- κ B-dependent gene expression; therefore, it was of interest to determine how E1A affected NF- κ B-dependent E3 promoter activation in T cells. CEM cells were transfected with pE3-luc and increasing amounts of the pCMV-E1A plasmid, which expresses both the 243R and 289R E1A proteins (data not shown). As has been shown in other cell lines, E1A expression increased the basal activity of the E3 promoter up to sevenfold in the absence of cellular activation (Fig. 5). The magnitude of E1A-driven E3 promoter activity was far less than that of the PMA-plus-ionomycin induction of E3, which increased E3-directed transcription more than 80-fold. Significantly, E1A strongly inhibited E3 induction by PMA plus ionomycin in a dose-dependent manner. Thus, it appears that T-cell-activation-dependent upregulation of the E3 promoter and E1A-dependent activation of the E3 promoter are mutually exclusive: E1A inhibits induction by PMA plus ionomycin while simultaneously driving basal activity of the E3 promoter. To control for possible interference by the cytomegalovirus promoter (which drives E1A expression in this experiment), pN1-EGFP, from which the cytomegalovirus promoter drives green fluorescent protein expression, was included. Adding the largest amount of pN1-EGFP did not affect pE3-luc activation, indicating that expression of the E1A proteins was responsible for suppression of the E3 promoter.

DISCUSSION

These data support two major conclusions. The first is that the E3 promoter is upregulated upon T-cell activation in the absence of other adenoviral protein expression. This result provides a potential mechanism by which E3 antiapoptotic and immune regulatory genes can be rapidly upregulated to protect

quiescently or latently infected human mucosal T cells from elimination. The second major conclusion is that this upregulation utilizes mechanisms that are separate from those used during the lytic phase of infection (i.e., E1A-dependent E3 promoter transactivation).

Previously, it has been suggested that the E3 NF- κ B sites function to upregulate E3 antiapoptotic gene expression in order to counter the cytotoxic effects of mediators such as TNF (17). Indeed, the TNF-induced increase in transcription from an E3 reporter plasmid is dependent on these NF- κ B sites (Fig. 3C) (17). However, several observations suggest that TNF induction of E3 through NF- κ B is not required during lytic infection. First, TNF induction of NF- κ B is inhibited during adenovirus lytic replication by the E3 RID complex (26). Second, inhibition of protein synthesis during treatment of lytically infected cells with TNF does not repress the ability of E3 proteins to prevent TNF-induced cytolysis, suggesting that basal (i.e., E1A-induced) levels are sufficient for protection (32). Third, E3 protects infected cells from apoptosis mediated by Fas, which does not directly trigger NF- κ B activation (20, 46, 66, 74). Collectively, these observations suggest that the E3 NF- κ B sites perform an important function outside lytic infection.

This study shows that E3 promoter upregulation during T-cell activation depends on its NF- κ B and AP-1 sites. This is consistent with our hypothesis that the E3 NF- κ B binding sites play a role outside of lytic, E1A-dependent E3 expression. The involvement of the AP-1 site was unexpected although not surprising, given that this transcription factor is also activated upon T-cell stimulation (22, 53, 60). The role of AP-1 in the control of other T-cell-activation-dependent promoters is best defined in its interaction with the transcription factor NFAT (reviewed in reference 55); however, we were unable to detect binding of NFAT to the E3 promoter (data not shown). Similar to our results, Deryckere and Burgert observed that TNF upregulates transcription from an E3 reporter plasmid in an NF- κ B-dependent manner (17). However, their studies show that in contrast to the activation of the E3 promoter by T-cell activation, TNF induction of E3 is not greatly affected by mutation of the AP-1 site, implying that the mechanisms of E3 promoter upregulation during T-cell activation are distinct from those during TNF induction of E3.

While E1A expression has been found to inhibit various cellular promoters, only the adenoviral E2 late promoter and the E1A enhancer have been shown to be negatively regulated by E1A from adenovirus group C (which includes serotypes 1, 2, 5, and 6) (8, 34, 61, 67). This is the first report of a homologous E1A interaction with the E3 promoter that leads to inhibition; previously, it was shown that E1A from adenovirus serotype 3 is capable of heterologous inhibition of adenovirus serotype 2 E3 promoter activation by E1A (49). The findings reported here are unique in exposing a situation in which E1A expression simultaneously increases the activity of a promoter while inhibiting activation by a separate stimulus (PMA-plus-ionomycin induction through NF- κ B).

Ascertaining the mechanism through which E1A both drives expression of E3 while inhibiting induction by PMA plus ionomycin of E3 is complicated by the fact that the pCMV-E1A plasmid used here expresses two different E1A mRNAs (12S and 13S). This mimics the situation in lytically infected cells:

the primary RNA transcripts of the E1A region are differentially spliced to produce two major mRNAs, 12S (yielding the 243R protein) and 13S (yielding the 289R protein) (37). These two transcripts differ only in that the smaller protein lacks a 46-amino-acid transcriptional activation domain (TAD) that is required for direct transactivation of all adenovirus early promoters by E1A (reviewed in reference 23). Sequences outside the TAD (and thus present on both the 243R and 289R proteins) have been shown to both activate and inhibit transcription through several different mechanisms (7, 10, 35, 47, 59, 68, 71, 80). Thus, E1A could be actively repressing E3 promoter activation through sequences outside the TAD and/or simply transactivating the E3 promoter in such a manner as to preclude NF- κ B-dependent upregulation.

Previous studies indicate that, in isolation, the two E1A proteins have opposite effects on the activation of NF- κ B-dependent gene expression. The 243R E1A protein potentially inhibits NF- κ B-dependent promoter transactivation (15, 30, 39, 59, 64), whereas the 289R isoform is generally found to activate these processes (52, 63). All of the above-cited studies were performed by transfection of an isolated E1A isoform in the absence of adenovirus infection, so the combined effect of both isoforms expressed during infection remains unknown. These findings are in agreement with our data in Fig. 5 in that the E1A 289R protein (which contains the TAD) could increase basal transcription of E3 while E1A 243R inhibits NF- κ B-dependent transactivation of E3. It is interesting that while the E1A 289R protein in isolation can increase NF- κ B-dependent promoter transactivation (52, 63), we see no evidence of this cooperation. This could indicate that the default pathway is for 289R to act through AP-1 and not NF- κ B and/or that inhibition of NF- κ B-dependent transactivation by 243R is more potent than activation by 289R. It is not clear why E1A would inhibit NF- κ B-dependent E3 transactivation. Given that NF- κ B controls the expression of many proinflammatory genes (6), E1A inhibition of NF- κ B activity could have evolved to avoid the generation of inflammatory mediators which would potentially activate a host immune response to the virus. This inhibition would not affect E3 mRNA expression, since once E1A is expressed it would transactivate this promoter.

Why would adenovirus need an E1A-independent mechanism to upregulate E3 expression? Until recently, there has been very little reason to speculate on the activity of the E3 promoter in the absence of E1A. With the exception of gene therapy applications where the E1A gene has been removed or replaced, it appears that adenovirus infection is invariably accompanied by the expression of E1A and lytic replication of the virus. However, the recent finding in our laboratory that the adenovirus genome exists in the absence of virion production in human donors suggests there may be an alternate pattern of nonlytic adenoviral transcription. One possible explanation for the lack of live virus in human mucosal T cells could be the repression of E1A promoter activity. Preliminary analysis of viral mRNAs present in adenovirus genome-positive human tonsillar lymphocytes failed to detect E1A expression in these cells (unpublished data). Suppression of E1A expression could be very desirable given its ability to sensitize cells to a variety of apoptotic stimuli, including death receptors (Fas, TNF receptor 1, and TRAIL) (18, 19, 62, 65), NK cell-mediated cytotoxicity (76), cytotoxic-T-lymphocyte killing by both

Fas and degranulation (14), chemotherapeutic-drug-induced apoptosis (12, 70), and UV-induced apoptosis (16). We propose that the sensitivity of the E3 promoter to T-cell activation functions to rapidly upregulate E3 antiapoptotic products in an E1A-independent manner in order to counter the proapoptotic effects of E1A expression. These E3-expressing cells would be protected from activation-induced cell death pathways, and presentation of viral peptides to CD8⁺ cytotoxic T lymphocytes would be suppressed, thereby inhibiting premature elimination of the reservoir of human adenovirus.

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