The early transcribed adenovirus proteins E1A and E1B display a variety of functions in the transformation of primary rodent cells and the regulation of apoptosis and transcription. We have recently shown that the E1B 19 kDa protein from Adenovirus 5 (Ad 5) can functionally antagonize the stimulatory effect of E1A 13S on the human transcription factor NF-κB. Here we show that expression of E1B 19 kDa negatively interfered with the activation of NF-κB by different stimuli, such as the E1A 13S protein, and treatment with phorbol ester and tumor necrosis factor α. This suggests that E1B 19 kDa acts on a common upstream signaling event. Band shift experiments showed that expression of E1B 19 kDa impaired the generation of the nuclear, DNA-binding form of NF-κB. Domain mapping experiments employing various E1B 19 kDa mutants revealed the necessity of a hydrophobic Bcl-2 homology region between amino acids 90 and 96 for NF-κB inhibition. Co-transfection experiments showed that the inhibitory effect of E1B 19 kDa on E1A 13S-activated NF-κB transcription was gradually lost in the course of time. Thus the continuous stimulatory action of E1A 13S can finally override the antagonistic effects of E1B 19 kDa on NF-κB activity. In contrast to E1B 19 kDa, expression of the E1B 55 kDa protein did not result in a de novo activation of NF-κB, but co-stimulated the transcriptional potential of activated NF-κB.

Adenoviruses are widespread in vertebrates and have been isolated from amphibians to humans. Human adenoviruses cause a variety of diseases affecting the respiratory, gastrointestinal, and urinary tract and the ocular system (Evans, 1958; Fox et al., 1977). Adenoviruses of the subgenus C, including Ad 2, Ad 5, and Ad 7, often cause diseases of the respiratory tract. The viral genome is a linear, double-stranded DNA molecule encoding at least 30 genes. The left 11% of the adenovirus genome comprises early region 1, which encodes the transcriptional units for the regulatory proteins E1A and E1B (Akusjärvi, 1993; Wold and Goding, 1991). The E1A region codes for two major proteins of 243 (12S) and 289 (13S) amino acids length which have transforming activity. E1A proteins were also found to regulate transcription of some viral and cellular promoters (Akusjärvi, 1993). In the absence of any apparent DNA binding activity, these transcriptional effects are due to the association of E1A to some host proteins, including the transcription factors ATF-2, Oct-4, and Sp1 (Schüler et al., 1991; Liu and Green, 1994). Furthermore the E1A protein can activate cell proliferation and induce apoptosis via a p53-mediated pathway (Debbas and White, 1993; White, 1993).

The E1B-encoding mRNA encodes two major unrelated proteins of 19 and 55 kDa molecular mass, respectively. Viral mutants lacking E1B give rise to large plaques on infected cell monolayers and are strongly defective in neoplastic transformation (Chinnadurai, 1983). The absence of the E1B 19 kDa during the productive infection of human cells induces the degradation of host and viral DNA as well as an enhanced cytotoxic effect (Ezeo et al., 1981; Pildes et al., 1984; Subramanian et al., 1984a, 1984b; White et al., 1984a, 1984b). Both splice variants of E1B were found to strongly cooperate with E1A in cell transformation. This supportive effect of E1B in transformation is most likely due to its antiapoptotic properties (Rao et al., 1992). This idea is reinforced by the finding that adenoviruses defective in E1B can be functionally complemented by the cellular Bcl-2 gene, which also acts as an inhibitor of apoptosis (Tarodi et al., 1993; Chiou et al., 1994). In addition to the functional complementation of E1B 19 kDa by Bcl-2 proteins in the inhibition of apoptosis, both proteins are membrane-anchored, contain three short regions with sequence similarity, and interact with a common set of cellular proteins (Boyd et al., 1994). Both splice variants of E1B inhibit cell death induced by different apoptotic stimuli, such as E1A, the anti-cancer drug cisplatin, TNF-α, or Fas antigen, although the E1B 19 kDa protein does so more effectively (Gooding et al., 1991; Hashimoto et al., 1991; Debbas and White, 1993; Subramanian et al., 1993). Besides other functions affecting mRNA export and intermediate filament organization, both E1B splice variants were also found to interfere with transcription (White, 1993). The E1B 55 kDa protein negatively interferes with p53-mediated gene expression by directly interacting with this transcription factor. E1B 55 kDa binds p53 without displacing it from its cognate DNA and abrogates transcription by a transcription repression domain within E1B (Yew et al., 1994). The smaller 19 kDa splice variant does not block p53-mediated transactivation, but alleviates p53-mediated repression (Shen and Shenk, 1994; Sabbatini et al., 1995). We have recently observed that co-expression of E1B 19 kDa impairs the activation of transcription factor NF-κB by expression of the E1A 13S protein (Schmitz et al., 1996).
Transcription factors belonging to the NF-κB/Rel family are specialized in the transduction of pathogenic signals from the cytoplasm to the cell nucleus. To date, five different DNA-binding subunits have been characterized and cloned in vertebrates. These DNA-binding subunits, including p50, p52, RelB, p65, and c-Rel, contact their cognate DNA-sequence as homodimers or heterodimers (Grilli et al., 1993; Schmitz and Baueurele, 1995). They share a conserved domain of approximately 300 amino acids length in their N terminus, which is necessary for DNA binding, dimerization, and nuclear translocation (Baueurele and Henkel, 1994; Verma et al., 1995). The most frequently detected form of NF-κB is a p50/p65 dimer, which is cytoplasmically retained in most cell types by an inhibitory subunit, called IκB (Baueurele and Henkel, 1994). Also the IκB proteins constitute a gene family and preferentially interact with different members of the NF-κB/Rel family (Beg and Baldwin, 1993; Israel, 1995). NF-κB becomes readily activated upon stimulation of cells with a variety of mostly pathogenic conditions, including inflammatory cytokines, bacterial lipopolysaccharide, T cell mitogen, some viruses, and viral proteins, including E1A 135. Apparently most if not all of the diverse inducers of NF-κB activity have in common that they lead to the generation of reactive oxygen intermediates, the phosphorylation of IκB at serines 32 and 36 and finally to its proteolytic degradation by the proteasome (Schmitz, 1995; Verma et al., 1995). The released DNA-binding dimer subsequently enters the nucleus and binds to its cognate DNA. Transcriptional activation of the NF-κB target genes is mediated by the transactivating subunits c-Rel, RelB, and p65. The target genes include some viral genes such as adenovirus E1B 19 kDa (Deryckere et al., 1995) and many cellular pathogen defense genes such as immunoreceptors, cell adhesion molecules, cytokines, hematopoietic growth factors, and acute phase proteins (Schmitz and Baeueurele, 1995). The NF-κB signal transducing system is an integral part for the proper function of the immune system, since the targeted disruption of IκBα, p50, p65, RelB, or c-Rel subunits deregulated immune responsiveness or resulted in lethal phenotypes (Beg et al., 1995a, 1995b; Kötgen et al., 1995; Sha et al., 1995; Weih et al., 1995).

Since it is largely unknown how the E1B 19 kDa protein interferes with activation of NF-κB, several questions were addressed experimentally. In the present study we show that E1B 19 kDa inhibition of NF-κB activity was only transient and required a short hydrophobic sequence within the E1B 19 kDa protein. E1B 19 kDa from Adenoviruses 2 and 5 inhibited NF-κB activated by the different inducers E1A 135, phorbol ester, or TNF-α. The 55-kDa splice variant of E1B co-activated NF-κB-dependent transcription. The regulatory implications of the adenovirus proteins either activating (E1A 135), co-activating (E1B 55 kDa), or repressing (E1B 19 kDa) NF-κB are discussed.

MATERIALS AND METHODS

Cell Culture and Transfections—Monkey COS7 cells and HeLa cells were grown at 37 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 50 mg of penicillin/streptomycin per ml (all from Life Technologies, Inc., Eggenstein, Germany). Approximately 5 × 106 of exponentially growing COS7 cells were transfected in suspension as described (Lopata et al., 1984). The CaPO4 transfection method was essentially used as described (Traenckner et al., 1995). The amounts of reporter plasmids and expression vectors used are given in the figure legends.

Plasmids—The eucaryotic expression vectors pHPR-E1A 135 (Wiegler et al., 1990), CMV 19 kDa, and CMV 55 kDa were described previously (White and Cipriani, 1991; Subramanian et al., 1993; Yew et al., 1994). The plasmids encoding E1B 19 kDa 1–40 and 1–88 were made by deleting the PstI/HindII and BssHII/HindII fragments, respectively. The mutant E1B plasmids E1B 19 kDa 2.3 EA-AS, E1B 19 kDa 14,15 RN-AS, E1B 19 kDa 29,30 RF-AS, E1B 19 kDa 50,51 EF-AS, E1B 19 kDa 75,76 AE-AS, E1B 19 kDa 190–96, E1B 19 kDa 123,124 WR-AS, and E1B 19 kDa 1–146 have been described (Subramanian et al., 1993). The NF-κB-dependent luciferase reporter plasmids used were HIV-1 LTR luciferase (Israel et al., 1992) and 6x B-tk-Luc, which contains three repeats of the human immunodeficiency virus type 1 (HIV-1) tandem NF-κB sites in front of a minimal thymidine kinase promoter (Meyer et al., 1993). The CAT reporter plasmid J16 is controlled by two wild-type NF-κB binding sites (Pierce et al., 1988). The RSV-luciferase plasmid and CMV-IκB-α were published previously (de Wet et al., 1987; Zabel et al., 1993).

Transactivation Assays—Cells were harvested and analyzed for the activity of the reporter genes after a minimum of 24 h post-transfection, as specified in the figure legends. Cells were washed once with ice-cold phosphate-buffered saline and harvested by scraping with a rubber policeman. Subsequently the protein concentration was determined by the method of Bradford (Bradford, 1976), and equal amounts of proteins were assayed for chloramphenicol acetyltransferase (CAT) activity. Acetylated and nonacetylated forms of [14C]chloramphenicol were separated by thin-layer chromatography and the incubation conditions were chosen to result in conversion of [14C]chloramphenicol within the linear range. Transfections were performed at least in duplicate, and the results were quantified by liquid scintillation counting. Cells tested for luciferase activity were washed once in phosphate-buffered saline, scraped off with a rubber policeman, and transferred to Eppendorf tubes. After centrifugation for 3 min at 2000 × g, the pellet was lysed by addition of 150 μl of 1% (v/v) Triton X-100, 25 mM glycylglycine (pH 7.8 adjusted with KOH), 15 mM MgSO4, 4 mM EGTA (pH 8 adjusted with KOH), and 1 mM dithiothreitol. The lysates were centrifuged at 4 °C, and 50 μl of the supernatant was assayed for luciferase activity. This assay was performed by admixture of 150 μl of reacting glycglycine (pH 7.8), 15 mM MgSO4, 30 mM potassium phosphate (pH 7.6), 4 mM EGTA, 1 mM dithiothreitol, and 3 mM ATP and measuring the light emission in a Microtrol LB96 P luminometer (Berthold). The luminometer was programmed to inject 100 μl of 0.3 mg/ml luciferin (Sigma) and to measure light emission for 30 s after injection.

Band Shift Assays—5 × 106 HeLa cells were transfected with the appropriate expression plasmids and harvested 24 h later. Nuclear extracts were prepared essentially as described (Baeuerle and Henkel, 1994). Briefly, cells were washed and harvested in TBS buffer (25 mM Tris/HCl (pH 7.4), 137 mM NaCl, 5 mM KCl, 0.7 mM CaCl2, 0.1 mM MgCl2). After lysis of cells in a buffer containing Nonidet P-40, the cell nuclei were isolated by centrifugation. After extraction of nuclear proteins in a high salt buffer and a subsequent centrifugation, the supernatant was used for band shift assays. These were performed by incubation of 10 μg of nuclear extract, 2 μg of poly(dI–dC) (Sigma), and 10,000 cpm of a labeled oligonucleotide on ice in binding buffer (10 mM Hapes (pH 7.9), 25 mM KCl, 4% Ficoll 400 (w/v), 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 5 mM CaCl2, 0.5 mM spermidine). The free and protein-bound oligonucleotides were separated on a 4% polyacrylamide gel. Gel and running buffer were identical and contained 25 mM Tris, 25 mM boric acid, and 0.5 mM EDTA. The gel was dried after electrophoresis and exposed to a Kodak XAR5 film. The oligonucleotide used for electrophoretic mobility shift assays contains a single NF-κB-binding site from the HIV-1 LTR, which is shown underlined: 5′-AGTTAGGGGGATTCCCCCAGG-3′ and 3′-TCAGGACTCCCTTGAAGGGTCAGC-5′. The oligonucleotide was labeled with [γ-32P]ATP using T4 polynucleotide kinase (Boehringer Mannheim).

Western Blotting—COS cells were transfected using the DEAE-dextran method. Cells were harvested after 36 h and lysed in 1 × SDS buffer and proteins were separated on a reducing 10% SDS-polyacrylamide gel. Subsequently the proteins were transferred onto a nitrocellulose membrane (Bio-Rad) in a semidry blot apparatus (Schteicher & Schüll) according to the instructions of the manufacturer. The detection of E1B 19 kDa proteins was performed by first washing the membrane twice in TBST (10 mM Tris/HCl (pH 8), 150 mM NaCl, 0.05% Tween 20) and a subsequent incubation in TBST containing 5% non-fat dry milk powder for 1 h. The membrane was then incubated in a small volume of TBST, containing 1:5000 dilution of the α-E1B 19 kDa antibody. After 4-h incubation at room temperature, the membrane was washed eight times in TBST and incubated for another hour in TBST containing a 1:3000 dilution of the second anti-rabbit antibody coupled to horseradish peroxidase (Bio-Rad). After extensive washing the bound antibodies were detected using the ECL system (Amersham Corp.), according to manufacturer's instructions. The generation of the antisera, which is specific for the p65 subunit of NF-κB, was described previously (Schmitz et al., 1996).
RESULTS

E1B 19 kDa from Ad2 and Ad5 Inhibit NF-κB Activity to the Same Extent—To monitor the effects of E1B 19 kDa from Ad2 and Ad5 on NF-κB-dependent transcription, reporter gene assays were performed. COS7 cells were co-transfected with a NF-κB-dependent HIV-1-LTR luciferase reporter gene construct, a plasmid encoding the Ad5 E1A 13S gene, and various amounts of expression vectors encoding E1B 19 kDa genes of Ad2 or Ad5. The stimulatory effect of E1A 13S on the luciferase reporter construct was dependent on the integrity of the NF-κB binding sites in the HIV-1 LTR (Fig. 1, compare lanes 2 and 3). Co-expression of increasing amounts of E1B 19 kDa of either Ad2 or Ad5 dose-dependently inhibited NF-κB activation to almost the same extent (Fig. 1, compare lanes 3-7). Expression of both E1B 19 kDa forms did not interfere with the activity of a constitutively active RSV promoter (data not shown), indicating that E1B 19 kDa expression is not a general inhibitor of gene transcription. Because of the similar behavior of these two proteins, the further analyses were performed with the Ad2 E1B 19 kDa protein.

E1B 19 kDa Also Inhibits NF-κB Activated by TNF-α and Phorbol Ester—We next studied whether E1B 19 kDa is also able to inhibit NF-κB activated by induction pathways different from the E1A 13S protein. As an example for a receptor-mediated pathway we stimulated HeLa cells transfected by a κB-dependent luciferase reporter gene with human TNF-α. The induction of κB-dependent transcription by human TNF-α was efficiently impaired by co-expression of increasing amounts of E1B 19 kDa (Fig. 2A). In a further experiment cells were stimulated with phorbol 12-myristate 13-acetate (PMA), an inducer of NF-κB which requires the action of protein kinase C isozymes. COS7 cells were transfected with the κB-dependent CAT reporter gene J16 and increasing amounts of E1B 19 kDa expression plasmid. One day after transfection, cells were stimulated with 30 ng/ml PMA. Subsequently cells were harvested and equal amounts of protein were assayed for CAT activity. Results from a CAT assay are shown, the positions of acetylated (Ac) and nonacetylated (Non-Ac) [14C]chloramphenicol are indicated.

Fig. 1. NF-κB inhibiting activity of the E1B 19 kDa protein from two adenovirus strains. Increasing amounts (0.5 and 1 pmol) of expression vectors encoding E1B 19 kDa were co-transfected into COS7 cells together with 2 pmol of the HIV-1-LTR luciferase reporter plasmids and 0.25 pmol of an E1A 13S expression vector, as indicated. One day after transfection, cells were harvested and assayed for luciferase activity.

Fig. 2. Effect of E1B 19 kDa expression on NF-κB activated by various stimuli. A, inhibition of NF-κB activated by TNF-α. HeLa cells were co-transfected with 2 pmol of the 6 × κB-tk-luc reporter gene and 0.5 or 1 pmol of E1B 19 kDa expression vector as indicated. One day after transfection, cells were stimulated for 8 h with 200 units/ml recombinant human TNF-α. Subsequently cells were harvested and assayed for luciferase activity. B, inhibition of NF-κB activated by PMA. COS cells were transfected with 2 pmol of the κB-dependent CAT reporter gene J16 (Pierce et al., 1977) and increasing amounts (0.125, 0.25, 0.5, and 1 pmol) of E1B 19 kDa expression vector. One day post-transfection the cells received for 8 h 30 ng/ml PMA. Subsequently cells were harvested and equal amounts of protein were assayed for CAT activity. Results from a CAT assay are shown, the positions of acetylated (Ac) and nonacetylated (Non-Ac) [14C]chloramphenicol are indicated.
activation, which is common to TNF-α, PMA, and E1A 13S.

NF-κB Inhibition Requires a Hydrophobic Domain Homologous to Bcl-2 in the E1B 19 kDa Protein—The E1B 19 kDa domain responsible for the inhibition of NF-κB was mapped by a series of E1B 19 kDa mutants in co-transfection experiments by testing their ability to repress E1A 13S-induced NF-κB transcription of the HIV-1-LTR luciferase reporter gene in COS7 cells. As seen in Fig. 3A, mutant proteins altered in the N-terminal portions of E1B were still active in repression. Likewise the E1B 19 kDa 50,51 EF-AS protein (Subramanian et al., 1995), which is mutated in one of the Bcl-2 homology domains, and a mutant lacking the C-terminal 29 amino acids, which contains a domain with homology to the polyoma middle T antigen, also efficiently suppressed NF-κB activity. However, the deletion of the Bcl-2 homology domain between amino acids 90 and 96 strongly impaired the ability of E1B 19 kDa to interfere with NF-κB-dependent transcription. Accordingly, a mutant containing only amino acids 1–88 was found to substantially lack inhibitory potential (data not shown). Similarly, amino acids 1–40 of E1B displayed no inhibitory activity. In order to ensure that the observed loss of the inhibitory activity of the mutant E1B 19 kDa 90–96 protein was not due to its lower expression in comparison with the wild-type protein, the relative protein levels were compared. COS7 cells were transfected with the indicated expression vectors for E1B 19 kDa 2,3 EA-AS, E1B 19 kDa 123,124 WR-AS, E1B 19 kDa 90–96, and E1B 19 kDa wild type. Cellular proteins were separated on a reducing SDS gel, and E1B proteins were analyzed by Western blotting using α-E1B 19 kDa antibodies. The arrowhead points to the E1B 19 kDa proteins.

Fig. 3. Mapping of the E1B 19 kDa region required for NF-κB inhibition. A, COS cells were transfected with 2 pmol of the HIV-1-LTR luciferase reporter plasmid and 0.25 pmol of an E1A 13S expression vector. E1A 13S-induced transcription was repressed by co-transfection of 1 pmol of the indicated E1B 19 kDa expression vectors. The relative repression seen upon co-expression of the wild-type E1B protein was set as 100%. The positions of the deleted or mutated amino acids are given in the left part of the figure. The sequence and the position of the wild-type amino acids that were replaced by alanine and serine are indicated. The three striped boxes highlight the regions with homology to the Bcl-2 protein, the C-terminal region resembling the polyoma middle T antigen is shown by dark shading. The bars indicate the mean deviation, which was obtained from five independent experiments. B, protein expression analysis of E1A 19 kDa proteins. COS cells were transfected with the indicated expression vectors for E1B 19 kDa 2,3 EA-AS, E1B 19 kDa 123,124 WR-AS, E1B 19 kDa 90–96, and E1B 19 kDa wild type. Cellular proteins were separated on a reducing SDS gel, and E1B proteins were analyzed by Western blotting using α-E1B 19 kDa antibodies. The arrowhead points to the E1B 19 kDa proteins.
One day after transfection, nuclear extracts were prepared and tested for DNA binding activity of NF-κB. The expression of E1A 13S induced the nuclear, DNA-binding form of NF-κB, which migrated more slowly than two constitutive complexes. Co-expression of E1B 19 kDa significantly reduced the amount of the DNA binding, nuclear form of NF-κB (Fig. 4A, compare lanes 2 and 3). Co-transfection of a plasmid encoding the E1B 19 kDa 1–40 mutant had no effect on NF-κB activation (Fig. 4A, lane 4). Western blot experiments were performed in order to resolve the question whether E1B 19 kDa prevents NF-κB from entering the nucleus or keeps it there in an inactive form. HeLa cells were transfected with the same combinations and amounts of E1A 13S and wild-type E1B 19 kDa expression vectors as described in the legend to Fig. 4A. Nuclear extracts from these transfected cells were tested for the occurrence of the strongly transactivating NF-κB p65 subunit in Western blots. The expression of the E1A 13S protein led to an increase in nuclear NF-κB p65 (Fig. 4B, lane 1). The co-expression of E1B 19 kDa significantly reduced the amount of nuclear p65 (Fig. 4B, lane 3). These experiments identify the reduction of the amount of nuclear NF-κB as at least one of the mechanisms of E1B 19 kDa action on this host transcription factor. There is recent evidence that E1A-dependent superactivation of p65 in the nucleus can be counteracted by E1B 19 kDa co-expression (data not shown), suggesting that E1B 19 kDa can also reduce the transcriptional activity of nuclear NF-κB p65.

Expression of E1B 19 kDa Impairs Activation of NF-κB

The kinetic of E1B 19 kDa counteraction on E1A 13S-mediated NF-κB activation was tested in time course experiments. A HIV-1-LTR luciferase reporter gene was co-transfected with expression vectors for E1A 13S and E1B 19 kDa into COS7 cells. Starting with 24 h post-transfection, cells were harvested and assayed for luciferase activity. E1A 13S-induced transcription of the HIV-1-LTR luciferase gene alone was set as 100%. The bars indicate the mean deviation obtained from six independent experiments. The displayed control repression experiment with IκB-α was performed two times, the mean values are shown.
counteract viral infections. One strategy is based on the elimination of infected cells by programmed cell death, another strategy is to elicit cellular and humoral immune responses. The pathogen-inducible NF-κB transcription factor family is a key factor in the cellular immune response because many of its cellular target genes, such as interferon-β, TNF-α, IL-2, IL-6, and IL-8, are immunologically of high relevance. Interferon-β is an important molecule conferring protection against viruses through induction of an intracellular antiviral state (Pellegrini and Schindler, 1993). It also activates natural killer (NK) cells. Another NF-κB regulated molecule essential for NK cell activity is major histocompatibility class I. Activation of NF-κB also leads to an increased expression of ELAM-1, VCAM-1, ICAM-1, and IL-8. These proteins are required for the attachment and transmigration of leucocytes to the locus of infection. The cytokine TNF-α was found to synergize with interferons in the antiviral response (Wong and Goeddel, 1986). We found here that at least the two different serotypes Ad2 and Ad5, belonging to subgenus C of human adenoviruses, are equally capable of suppressing NF-κB activated by different inducers such as the E1A 13S protein, PMA, and TNF-α by means of preventing the generation of the active, nuclear form.

We suggest that it is beneficial for the virus to efficiently counteract the various aspects of the immune reactions simultaneously by impairing NF-κB activity. The E1B 19 kDa protein is expressed within the first 10 h post-infection (Green et al., 1983; Wold and Gooding, 1991; Akusjärvi, 1993). We therefore speculate that it might be especially important for the virus to counteract NF-κB activity early in the course of the viral infection in order to escape an antiviral response. This might be the reason why the virus evolved a protein with conserved function between various adenovirus strains being able to counteract NF-κB activity.

Alternatively higher vertebrates eliminate virus-infected cells by programmed cell death (White, 1994; Eick and Hemekeing, 1996). The antiprotective properties of the wild-type and numerous mutant forms of E1B 19 kDa have been investigated in some detail (White et al., 1992; Subramanian et al., 1993; Chiou et al., 1994). Using cisplatin, TNF-α, and the E1A 13S protein as inducers of apoptosis, the regions required to inhibit cell death within E1B 19 kDa have been defined. Interestingly, the ability of E1B 19 kDa mutants to inhibit apoptosis can be separated from their ability to counteract NF-κB activation. The mutant E1B 19 kDa 50,51 EF-AS was fully active in NF-κB inhibition, but was found in a previous study to be incapable of inhibiting apoptosis (Subramanian et al., 1993). This finding is consistent with previous studies, showing that activation of NF-κB and apoptosis by TNF-receptor 1 are separate pathways (Hsu et al., 1995, 1996). Yet, there is evidence for different apoptotic pathways in viral infections requiring NF-κB activity (Lin et al., 1995). It is conceivable that NF-κB activated during adenovirus infection (Bergmann and Shavit, 1988) contributes to virus-induced cell death. In this scenario inhibition of NF-κB activity by E1B 19 kDa would also negatively interfere with apoptosis and thus ensure a prolonged replication time for the virus.

In contrast to IκB-α, the co-expression of E1B 19 kDa protein blocked NF-κB activity not completely. A full repression of NF-κB might be detrimental for the adenovirus, since the expression of some adenovirus genes requires various host transcription factors, including NF-κB. An example for a NF-κB-regulated adenovirus gene is E3/19 KDa (Deryckere et al., 1995). E3/19 KDa down-regulates major histocompatibility class I surface expression. For this immunosuppressive mechanism, it is essential for the virus to activate NF-κB upon E1A 13S expression. The transcription of the early adenovirus genes E1A and E1B is independent from NF-κB, rendering these
virus proteins independent from their regulatory target. The incomplete inhibition of NF-κB by E1B 19 kDa leaves presumably enough NF-κB activity for the expression of its viral target genes. Furthermore, time course experiments showed that E1B 19 kDa impaired NF-κB activity only transiently. The transient nature of the repressing effect might simply be explained by altered stability of the E1B 19 kDa protein. A reversible, transient inhibition has the advantage that NF-κB activity can be determined solely by the relative amounts of E1A 13S and E1B 19 kDa. Another level of influencing NF-κB activity is provided by the slightly co-activating function of E1B 55 kDa.

We suggest that it might be advantageous for the virus to possess a subtle system of regulatory proteins allowing a fine-tuned nature of the repressing effect mightsimply be explained by altered stability of the E1B 19 kDa protein. A reversible, transient inhibition has the advantage that NF-κB activity can be determined solely by the relative amounts of E1A 13S and E1B 19 kDa. Another level of influencing NF-κB activity is provided by the slightly co-activating function of E1B 55 kDa.

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