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Tumor necrosis factor α stimulates expression of adenovirus early region 3 proteins: Implications for viral persistence

(early region 3/19K protein/HLA antigens/interferon γ)

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ABSTRACT **Human adenovirus (Ad) can cause persistent infections in humans. Early region 3 (E3) of the virus appears to be implicated in this phenomenon. This transcription unit encodes proteins that interfere in various ways with host cell functions, including (*i*) cell-surface expression of histocompatibility class I antigens (HLA), (*ii*) cell-surface expression of the epidermal growth factor receptor (EGF-R), and (*iii*) the biological activity of tumor necrosis factor α (TNF-α). We transfected the human cell line 293 with the entire E3 region of Ad2 and investigated the influence of the cytokines TNF-α and interferon γ (IFN-γ) on cell-surface expression of HLA class I and the EGF-R. Whereas IFN-γ treatment induced expression of HLA to some extent but not that of the EGF-R, TNF-α treatment augmented the reduction of these cell-surface molecules. Subsequent studies on the mechanism of this effect showed a TNF-α-dependent upregulation of E3 protein (E3/19K) and mRNA. The significance of this phenomenon was confirmed in infection experiments. A dramatic increase in the amount of E3/19K, even after short induction with low doses of TNF-α could be demonstrated. The study provides evidence for an interaction between the immune system and Ad in which the virus takes advantage of an immune mediator to escape immunosurveillance of the host.**

Human adenoviruses (Ad) infect epithelial cells of the eye, the respiratory tract, and the gastrointestinal tract (1). Symptoms are in general not severe; however, a certain percentage of infected people develop a persistent virus infection (2). The genes involved in this phenomenon may be encoded primarily in early region 3 (E3) of Ad. Although this region is dispensable for virus replication in tissue culture cells, it is conserved in all Ad subtypes, suggesting an important role during viral pathogenesis (reviewed in ref. 3). The E3 region of Ad2 contains nine open reading frames. For six of these, a protein has been detected in infected cells (ref. 3; see Fig. 1, solid bars). The most abundant E3 protein, E3/19K, is a transmembrane glycoprotein of ≈25 kDa that retains class I major histocompatibility complex (MHC) antigens in the rough endoplasmic reticulum (4, 5). As a consequence, allogeneic and antigen-specific T-cell recognition is drastically reduced (6–9). All Ad, except the highly oncogenic subgroup A, express a MHC-binding protein (10). Subgroup A, however, also decreases MHC expression in transformed cells, albeit by a different mechanism affecting the level of MHC-specific mRNA (11, 12). Thus, modulation of MHC antigens appears to be of utmost importance for Ad. Two other proteins encoded in the E3 transcription unit—E3/10.4 kDa and E3/14.5 kDa—are responsible for downregulation of the epidermal growth factor receptor (EGF-R) during infection of host cells (3, 14).

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In addition, the virus devotes several early gene products to counteract the biological activity of tumor necrosis factor α (TNF-α). Ad-infected cells sensitized to lysis by TNF-α by the expression of the immediate early protein E1A (15, 16) subsequently become resistant to TNF-α-mediated lysis due to the expression of the E1B/19-kDa protein in human, and the E3/14.7-, 10.4-, and 14.5-kDa proteins in murine cells (3). Furthermore, there is evidence that TNF-α and other cytokines are induced in Ad-infected lung tissue of mice (17).

TNF-α is a pleiotropic mediator in the inflammatory response (18) that can, at high doses, inhibit replication of RNA and DNA viruses—e.g., Ad (19, 20)—but also exerts a variety of immunomodulatory activities including upregulation of MHC class I antigens (21).

Another cytokine that induces MHC expression on the cell surface is interferon γ (IFN-γ) (23–25), which seems to influence MHC expression at the level of transcription (23, 24) and at the level of protein processing and MHC/peptide assembly (26).

Here the question we address is whether IFN-γ and TNF-α are able to overcome the inhibiting effect of E3/19K on MHC transport. In stable $E3^+$ cell lines, IFN-γ increased HLA expression, whereas TNF-α augmented the repressing effects of the E3 proteins on HLA and EGF-R expression. Further studies on the mechanism of the TNF-α-mediated effect showed induction of E3 proteins in both transfected and infected cells. The implications for viral pathogenesis of this intriguing interaction between virus and host will be discussed.

MATERIALS AND METHODS

Cloning Ad2 DNA. Ad2 was isolated by cesium chloride banding; DNA was extracted and digested with *Eco*RV (Boehringer Mannheim). The *Eco*RV C fragment comprising the E3 transcription unit was gel-purified and cloned into Bluescript II KS− (Stratagene). The *Eco*RI D fragment has been described (4, 28). The cDNA of E3/19K was amplified by PCR (Cetus) and subsequently cloned into the *Eco*RI site of Bluescript II KS− using primers selected from the 5′ (E3/19K–5′RX) and 3′ part (E3/19K–3′XR) of the E3/19K gene. The sequence was 5′-ATTCGAATTCTCGAGGTCAGCTTTTTAAACGCTGGGGGC-3′ (E3/19K–5′RX) and 5′-TAGTGAATTCTAGACACATAGAGTAAATTGTCCAGGGG-3′ (E3/19K–3′XR). *Eco*RI restriction sites are underlined.

Cell Culture and DNA Transfection. The human cell line 293 has been established by transformation of embryonic kidney cells with Ad5 (29). Cells were propagated and transfected as

Abbreviations: Ad, adenovirus; E3, early region 3; TNF-α, tumor necrosis factor α; MHC, major histocompatibility complex; mAb, monoclonal antibody; EGF-R, epidermal growth factor receptor; IFN-γ, interferon γ; β_2m, β_2-microglobulin; FACS, fluorescence-activated cell sorter.

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described (4, 30). The cell line 293E22.7 was established by transfection with the Ad2 *Eco*RI D fragment (4, 28) using Lipofectin (GIBCO/BRL). All lines were cotransfected with the neophosphotransferase gene (31). The selection was carried out with G418 at 600 μg/ml (GIBCO/BRL).

Virus Propagation and Infection. Ad2 was grown and titrated on HeLa cells as described (32). Briefly, after 32 h of infection the cells were fixed for 10 min with methanol/acetone (1:1), rinsed with phosphate-buffered saline, and then incubated with a mouse anti-Ad hexon monoclonal antibody (mAb) (Serotec) followed by peroxidase-labeled rabbit anti-mouse IgG (Dako, Hamburg, F.R.G.). Infected cells showed blue staining when developed with α-chloronaphthol. In the infection experiment, cells were incubated with virus-containing cell lysate (calculated multiplicity of infection, 3) for 60 min at 37°C in serum-free medium. Then medium was added containing 5% fetal calf serum. The indicated time includes the adsorption time of the virus.

Antibodies. The following mAbs were used: Tw1.3, anti-E3/19K of Ad5 (33) that crossreacts with E3/19K of Ad2, a generous gift of J. Yewdell; BBM.1, anti-human β_2-microglobulin (β_2m; 34); W6/32, anti-human HLA-A, -B, and -C (35). The mAb against the EGF-R and the fluorescein isothiocyanate-conjugated goat anti-mouse IgG were purchased from Dianova (Hamburg, F.R.G.) and from Sigma, respectively.

Cell Labeling, Immunoprecipitation, and SDS/PAGE. Labeling of cells with [^{35}S]methionine (Amersham), immunoprecipitation, and SDS/PAGE have been described in detail (4). Two or three exposed films were scanned by a laser densitometer (Pharmacia-LKB).

Flow Cytometry. The staining procedure for fluorescence-activated cell sorter (FACS) analysis has been described (4). As control for background staining, cells were incubated with the second antibody only. Ten thousand viable cells were analyzed with a FACscan (Becton Dickinson).

Cytokines. Recombinant human TNF-α with a specific activity of 6.6×10^6 units/mg was a kind gift of Knoll (Ludwigshafen, F.R.G.). IFN-γ was purchased from Boehringer Mannheim. The 293 cells and transfected clones were treated with IFN-γ (500 units/ml) and TNF-α (600 units/ml) for the time indicated in the text and figure legends.

RNA Preparation and Northern Blot Analysis. Total RNA was prepared (36), separated in 1.5% formaldehyde agarose gels, and transferred onto Biodyne B membranes (Pall). Hybridization was carried out with cDNA clones of E3/19K (pBS-E3/19K) and E1A (pE1A-neo, a gift of G. Johnson, National Jewish Center, Denver) and with plasmid pX28 specific for mRNA of the ribosomal protein S12 (ref. 37; a gift of P. Nielsen). Probes were labeled by the Multiprime DNA labeling system (Amersham) to a total activity of 1×10^9 cpm/μg and hybridized for 18 h. The blots were exposed to Kodak X-AR 5 films overnight and the films were scanned by laser densitometry (Pharmacia-LKB).

RESULTS

Transfected 293 Cells Express Proteins of the E3A and E3B Region of Ad2. To investigate the function of E3 proteins in more detail, we cloned the whole E3 region of Ad2 contained in the *Eco*RV C fragment (Fig. 1*A*) and transfected it into 293 cells (4, 38). Stable clones were screened for the expression of E3/19K by coimmunoprecipitation with HLA antigens and clone 293E3-45 was selected for further analysis. Similarly, clone 293E22.7, derived from 293 cells transfected with the *Eco*RI D fragment, containing only part of the E3 region of Ad2 (Fig. 1*A*) was chosen (data not shown).

Further evidence for expression and function of E3 proteins in the transfectants was obtained by analyzing the cell-surface density of HLA class I antigens and of the

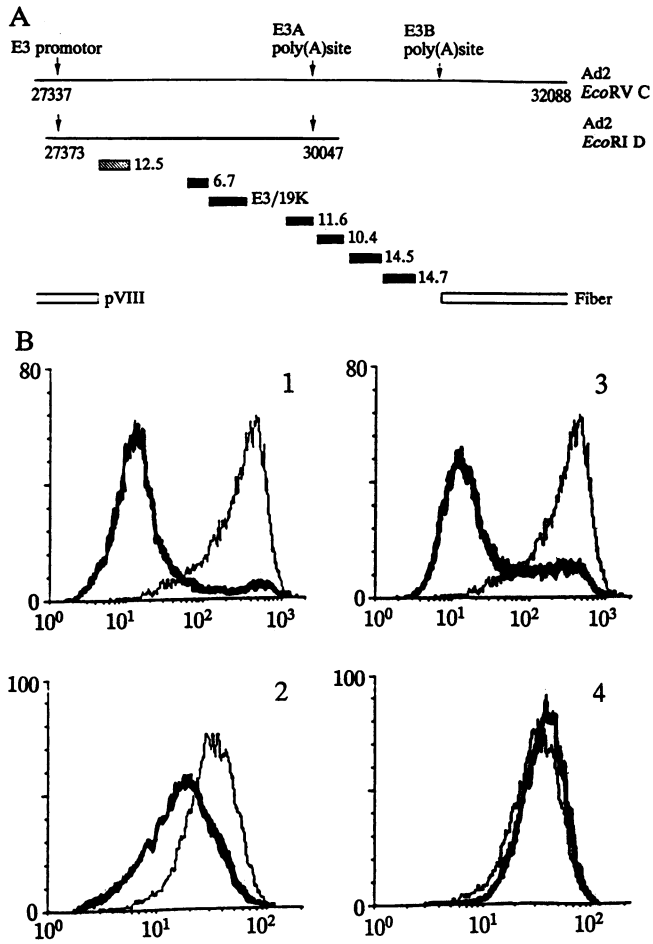

FIG. 1. (*A*) Schematic representation of the cloned and transfected Ad2 DNA. The *Eco*RV C fragment DNA comprises all genes of the E3 transcription unit. Stippled bar, 12.5-kDa protein proposed to exist; solid bars, six proteins identified so far; open boxes, partial coding sequences of the pVIII and fiber proteins. (*B*) Cell-surface expression of HLA and EGF-R as determined by FACS analysis. *Eco*RV C fragment transfectant 293E3-45 (*B1* and *B2*, boldface curves) and *Eco*RI D fragment transfectant 293E22.7 (*B3* and *B4*, boldface curves) as well as untransfected 293 cells (lightface curves) were treated with mAb W6/32, directed to HLA class I antigens (*B1* and *B3*), or mAb 528, against the EGF-R (*B2* and *B4*). Comparing the mean values of fluorescence, HLA and EGF-R expression of 293E3-45 was reduced by 85.2% (*1*) and 40.4% (*2*), respectively, compared to untransfected cells. In the *Eco*RI D transfectant 293E22.7, the reduction was visible for HLA (76.3%, *3*) but not for the EGF-R (*4*; +17%). Histograms *1* and *3* contain a fraction of cells with a mean of 300–400. They may represent cells transiently not expressing E3/19K. The percentage of these cells is characteristic for each clone and seems to be constant even after sorting (H.-G.B. and H.K., unpublished results). The *y* axis depicts the relative number of cells and the *x* axis depicts the fluorescence intensity.

EGF-R (Fig. 1*B*). FACS analysis shows a drastically reduced cell-surface expression of HLA antigens in both types of transfectants (boldface curves) compared to untransfected 293 cells (Fig. 1*B1* and *-3*, lightface curves). In contrast, EGF-R expression is affected only in transfectants containing the whole E3 region, such as 293E3-45 (Fig. 1*B2*), suggesting that, apart from E3/19K, E3/10.4 kDa and E3/14.5 kDa are also expressed in 293E3 cells, producing effects similar to those seen during infection (14).

TNF-α Treatment Augments the Effects of E3 Proteins. We used these E3$^+$ cell lines to study the influence of IFN-γ and TNF-α on HLA and EGF-R expression; 293E22.7 cells, 293E3-45 cells, and, as a control, 293 cells were treated with TNF-α or IFN-γ for 18 and 48 h, respectively. These times

of induction have been previously shown to be sufficient to observe the enhancing effect of these cytokines. Thereafter, HLA and EGF-R expression was analyzed by FACS analysis. Under these conditions, IFN-γ stimulates cell-surface expression of HLA by a factor of 1.75 in all cells (Fig. 2*A*, middle row) compared to untreated cells (Fig. 2*A*, top row), whereas EGF-R expression is not significantly altered (Fig. 2*B*, compare middle row with top row). In contrast, while TNF-α induced HLA expression in 293 cells, it was unable to do so in E3 transfectants (Fig. 2*A*, bottom row; compare numbers in parentheses). Rather, a further decrease in expression of HLA was observed. This downregulating effect of TNF-α was even more pronounced for the EGF-R in 293E3-45 cells ($\approx$40%) but not visible in 293 cells or the E3/14.5K$^-$ transfectant (Fig. 2*B*, bottom row). Other surface molecules, such as the transferrin receptor, were not influenced (data not shown).

TNF-α Treatment Increases Synthesis of the E3/19K Protein in Transfected Cells. We decided to investigate the mechanism of this TNF-α-mediated downregulation of HLA in E3$^+$ 293 cells. TNF-α treatment may (*i*) decrease HLA synthesis, (*ii*) increase expression of E3/19K, and/or (*iii*) increase the interaction between E3/19K and HLA. To distinguish between these possibilities, the biosynthesis and assembly of HLA, E3/19K, and β_2m were studied. Both 293 and 293E3-45 cells were treated with IFN-γ or TNF-α for 20 and 6 h, respectively, labeled with [^{35}S]methionine for 90 min, and lysed. The HLA-E3/19K–β_2m complex was then immunoprecipitated with a mAb, directed against β_2m (Fig. 3*A*). It is obvious that, compared to the control, in IFN-γ-treated cells the amount of HLA and β_2m precipitated increased by a factor of 2–3, in agreement with the FACS data (Fig. 2), whereas that of coprecipitated E3/19K did not change. In contrast, precipitates derived from TNF-α-treated cells contained twice as much E3/19K as untreated 293E3-45 cells, while the amount of HLA increased only by $\approx$30%

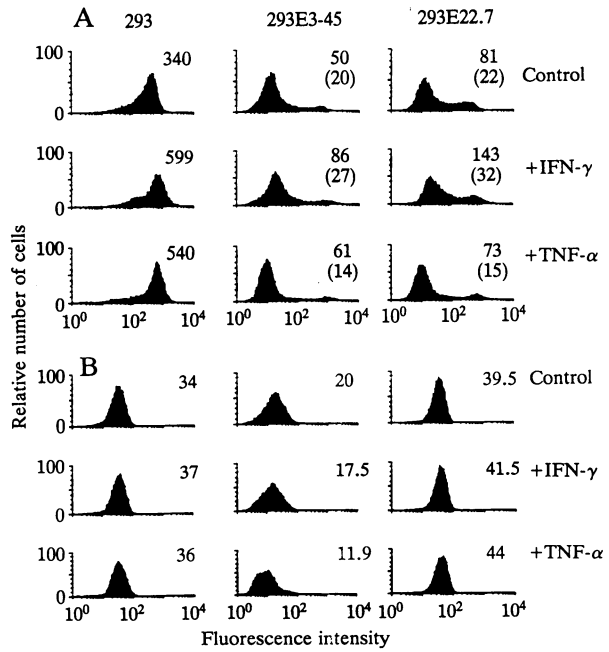

FIG. 2. Cell-surface expression of HLA and EGF-R after cytokine treatment of transfected and untransfected 293 cells; 293, 293E3-45 (*Eco*RV C), and 293E22.7 (*Eco*RI D) were treated for 48 or 18 h with IFN-γ or TNF-α, respectively. After staining for HLA (*A*) or EGF-R (*B*), FACS analysis was carried out as described in Fig. 1. Mean values of fluorescence are indicated for each histogram. Numbers in parentheses below represent mean value of the main peak. Mock-treated cells are indicated as controls.

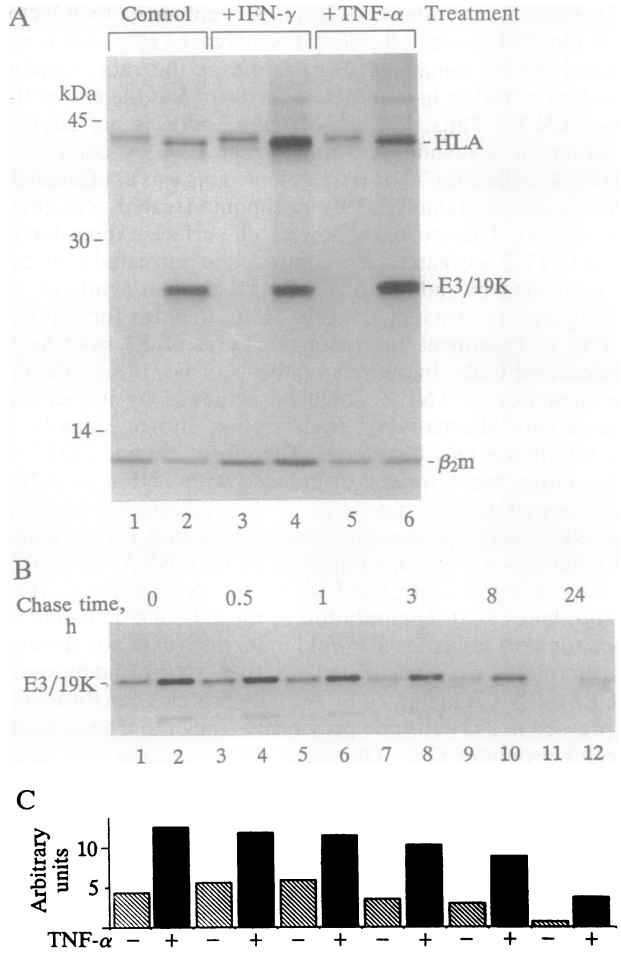

FIG. 3. Synthesis of E3/19K and HLA in transfected 293 cells after induction by different cytokines. (*A*) The 293 cells (lanes 1, 3, and 5) and the transfected clone 293 E3-45 (lanes 2, 4, and 6) were treated with IFN-γ for 20 h (lanes 3 and 4), with TNF-α for 6 h (lanes 5 and 6), or were mock treated (lanes 1 and 2). After biosynthetic labeling, lysates were immunoprecipitated with mAb BBM.1 (anti-β_2m). The positions of HLA, E3/19K, and β_2m are denoted on the right; positions of molecular mass markers (kDa) are on the left. (*B*) Fate of E3/19K in TNF-α-treated or mock-treated cells. The 293E3-45 cells were incubated for 18 h with or without TNF-α, pulsed for 15 min with [^{35}S]methionine (200 μCi/ml) (1 Ci = 37 GBq), lysed (0), or chased with excess unlabeled methionine for the time indicated on the top of the figure. To ensure efficient precipitation of E3/19K, the lysates were incubated once with a mixture of Tw1.3 and W6/32 and twice with Tw1.3 alone. The first two immunoprecipitations were pooled and analyzed in this figure. Residual material contained in the third immunoprecipitation represented $\approx$15% in TNF-α-treated cells. Position of the high mannose form of E3/19K is denoted. (*C*) Quantitative analysis of the E3/19K band. Bars represent amount (in arbitrary units) of E3/19K at each time point as determined by densitometry. Data are the mean of two exposures. Residual amount of E3/19K (15% in TNF-α-treated cells) was not included in the calculation and has to be added. Addition of TNF-α is indicated (+).

(densitometry data not shown). Thus, HLA synthesis is not decreased, but the relative amount of E3/19K in HLA immunoprecipitates is higher than that in untreated cells.

To discriminate between possibilities *ii* and *iii* above, we directly compared synthesis and degradation of the E3/19K protein with or without prior TNF-α treatment in a pulse–chase experiment (Fig. 3*B*). The 293E3-45 cells were mock treated (lanes 1, 3, 5, 7, 9, and 11) or TNF-α treated (lanes 2, 4, 6, 8, 10, and 12) before pulse labeling and then chased for the times indicated in Fig. 3*B*. The quantitative analysis (Fig.

3C) shows that synthesis of E3/19K is enhanced by a factor of 2–3 in TNF-α-treated cells. This increased synthesis is not neutralized by enhanced degradation as the ratio remains constant or rather increases to a factor of 5 at the end of the chase (24 h). Thus, the half-life of E3/19K is not altered, resulting in a sustained, 3-fold higher E3/19K content of 293E3-45 cells after TNF-α treatment. This was confirmed by staining intracellular E3/19K in saponin-treated cells (data not shown). Therefore, the lower cell-surface expression of HLA in TNF-α-treated cells compared to untreated cells can be explained by induction of E3/19K protein synthesis allowing a more efficient E3/19K–HLA complex formation.

TNF-α Treatment Increases the Level of E3 mRNAs in Transfected Cells. Increased synthesis of E3/19K protein in the presence of TNF-α could be achieved by influencing translation. Alternatively, transcription and/or stability of the E3 mRNA may be altered. Therefore, 293 and 293E3-45 cells, either mock treated or induced with TNF-α or IFN-γ were tested for the presence of E3 mRNA by using an E3/19K-specific probe. The results show that TNF-α markedly induces the steady-state level of E3 mRNAs compared to the untreated control and the IFN-γ-treated sample (Fig. 4 *Top*, lanes 1–3). Quantitation of the E3-specific bands by densitometry indicates a 15-fold induction (data not shown). Furthermore, we investigated whether TNF-α induction of the E3 mRNA is mediated by Ad E1A proteins constitutively expressed in 293 cells and previously shown to transactivate the E3 promoter (28). Therefore, the same blot was rehybridized with an E1A-specific probe. As shown in Fig. 4 (*Middle*, lanes 1–6), E1A-specific mRNA is not significantly influenced by any treatment. Thus, in transfected cells the level of E3 mRNAs is specifically upregulated by TNF-α.

Induction by TNF-α of E3/19K Synthesis Is Also Observed in Infected Cells. To investigate the potential relevance of this observation, it was important to determine whether induction of E3 proteins also occurs during infection. Therefore, HeLa cells were infected with a limited number of Ad2 particles in the presence or absence of TNF-α. The same experimental protocol was applied to uninfected cells. Upon TNF-α treatment, immunoprecipitation of β_2m in infected cells reveals a strong increase of E3/19K in the HLA/β_2m–E3/19K complex compared to mock-treated cells, indicative of the induced expression of E3 proteins (Fig. 5, compare lanes 4 and 2). No influence was seen on the biosynthesis of HLA after this short (6 h) induction period. The increased amount of coprecipitated E3/19K was also detected when another mAb (W6/32) was used that recognizes an epitope on HLA heavy chains (data not shown). Moreover, other human cell types like the transformed cell lines A431 (epidermoid carcinoma) and A549 (lung carcinoma), but also the nontransformed diploid lung fibroblast cell line Wi-38, responded with increased E3/19K expression upon infection and TNF-α treatment. While the effect in the lung cells was only moderate, A431 cells were highly susceptible (data not shown). Thus, although the extent of TNF-α-mediated stimulation of E3 proteins varied in different cell types, all cells tested showed the effect.

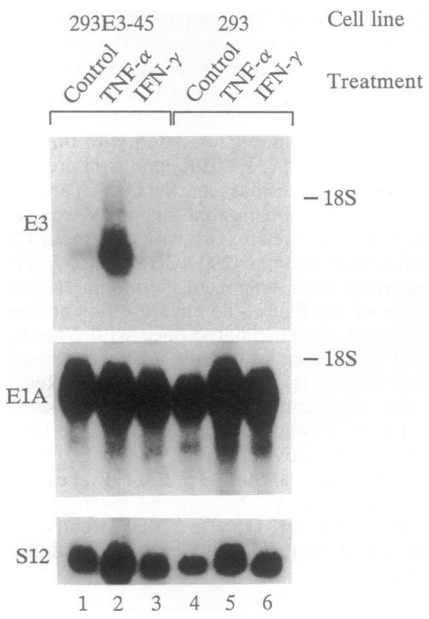

FIG. 4. Northern blot analysis of E3$^+$ and E3$^-$ 293 cells treated with different cytokines. Total RNA was prepared from cells treated with TNF-α (8 h) or IFN-γ (22 h). E3-specific RNA was detected with a probe containing a 587-base-pair cDNA fragment of E3/19K. Specificity of this probe was confirmed by the absence of hybridization to RNA from wild-type 293 cells (lanes 4–6, *Top*). E1A RNA was detected with a 2.3-kilobase probe comprising the viral E1A gene (*Middle*). As a control for the amount of RNA loaded, the same membrane was rehybridized with a probe (pX28) specific for the ribosomal protein S12 (*Bottom*). Position of 18S RNA is denoted on the right.

DISCUSSION

In this communication, we have described transfected cell lines containing a complete E3 transcription unit of Ad2. The drastically reduced cell-surface expression of HLA and of EGF-R indicates the functional activity of the E3/19K and the E3/10.4-kDa/14.5-kDa Ad proteins (Fig. 1), implying that mRNAs with E3A as well as E3B poly(A) sites are transcribed in these cells. Thus, it is likely that other E3 proteins might be expressed as well. Therefore, we established a convenient system for studying E3 functions.

IFN-γ treatment led to a partial release of the E3/19K-mediated block of HLA transport, probably by increasing HLA and β_2m synthesis. Also, IFN-γ-induced components of the machinery that produces and transports peptides into

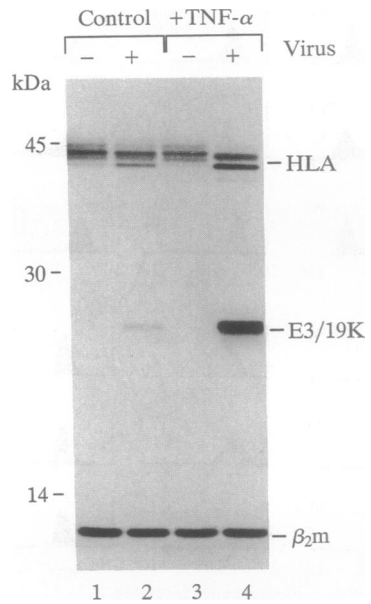

FIG. 5. Increased coprecipitation of E3/19K after TNF-α treatment of Ad2-infected cells. HeLa cells were infected at a multiplicity of infection of 3 (lanes 2 and 4) or were mock infected (lanes 1 and 3). Half the dishes were treated with TNF-α (400 units/ml) for 6 h (lanes 3 and 4) and half were left untreated (lanes 1 and 2). Immunoprecipitation was carried out as described in Fig. 3. Positions of molecular mass markers (kDa) and HLA, E3/19K, and β_2m are denoted on the left and right, respectively.

the endoplasmic reticulum (26) might help to bypass E3/19K. However, in contrast to Ad12-transformed cells, we do not see complete recovery of MHC antigen expression upon IFN-γ treatment (25). Surprisingly, TNF-α, also reported to induce HLA expression (21), caused a further reduction of cell-surface expression (Fig. 2) by stimulating E3/19K synthesis (Fig. 3). By analogy, the reduced amount of EGF-R on the cell surface can be explained by induction of the 10.4- and 14.5-kDa proteins (Fig. 2). The increased steady-state level of the E3 mRNA (Fig. 4) suggests that TNF-α affects either mRNA stability or transcription rate. At present, we cannot distinguish between these possibilities. We ruled out that TNF-α stimulates expression of E1A proteins and thereby enhances E3 expression (28). Neither increased transcription nor an influence on E1A protein synthesis could be found (Fig. 4; data not shown). Thus, it seems that TNF-α specifically activates the E3 promoter. However, a qualitative change in E1A-dependent induction of the E3 promoter cannot be excluded.

Studies of the TNF-α signal transduction pathway demonstrated activation of NF-κB-inducible genes of cellular and viral origin (39). Recently, NF-κB consensus sequences within the E3 promoter and binding of cellular factors to this sequence motif were found (40). In light of these results, we postulate that induction of E3 mRNA and protein by TNF-α may be mediated by NF-κB binding to the E3 promoter.

A number of previous publications demonstrated inducibility by NF-κB and TNF-α of viral genes involved in activation or replication. For example, TNF-α treatment of cell lines persistently infected with human immunodeficiency virus activates the provirus through binding of NF-κB to the long terminal repeat leading to increased viral replication (13). In contrast, the induction of E3 proteins by TNF-α described above has no stimulative effect on Ad replication, but rather influences the interaction with its host. The E3 region is not essential for virus growth in tissue culture cells. However, the mere existence of this region in all Ad as well as the functional data for E3/19K and E3/14.7 kDa and 10.4–14.5 kDa point to a biological role of this part of the viral genome in pathogenesis (3). While the consequences for the virus of downregulating the EGF-R are elusive, its interference with transport of HLA antigens inhibits their antigen presentation function for class I-restricted cytotoxic T cells *in vitro* (6–9). It is likely that this mechanism also operates to rescue virus-infected cells *in vivo*.

More recently, Ginsberg and colleagues (17, 22) developed *in vivo* models for Ad-induced disease and confirmed the crucial role of the E3 region, and in particular that of the E3/19K protein, in viral pathogenesis. Two phases of the inflammatory response were distinguished: an early phase, characterized by the presence of polymorphonuclear leukocytes and induction of TNF-α, interleukin 1, and interleukin 6; and a late phase, with a prominent cytotoxic T-cell response. A mutant Ad with a deletion of E3/19K caused an increased inflammatory response. As TNF-α is present in Ad-induced lesions, we believe that our findings bear some relevance for the natural infection. It is likely that TNF-α is produced by monocytes–macrophages attracted to the site of infection. Alternatively, Ad itself may induce TNF-α in certain cell types upon infection. Thus, it is conceivable that Ad directly or indirectly induces TNF-α production, which in turn supports a viral escape mechanism that on its own may not operate efficiently in all cell types (27).

We hypothesize that in evolution the virus has adapted to use this early defense mechanism to support its own escape. This adaptation may also be advantageous for the host as it may limit immunopathology. Further studies will show whether this kind of cross-talk between persistent viruses and the host is a more common phenomenon.

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1. Horwitz, M. S. (1990) in *Virology*, eds. Fields, B. N. & Knipe, D. M. (Raven, New York), pp. 1723–1740.
2. Fox, J. P., Hall, C. E. & Cooney, M. K. (1977) *Am. J. Epidemiol.* **105**, 362–396.
3. Wold, W. S. M. & Gooding, L. R. (1991) *Virology* **184**, 1–8.
4. Burgert, H.-G. & Kvist, S. (1985) *Cell* **41**, 987–997.
5. Andersson, M., Pääbo, S., Nilsson, T. & Peterson, P. A. (1985) *Cell* **43**, 215–222.
6. Burgert, H.-G., Maryanski, J. L. & Kvist, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1356–1360.
7. Andersson, M., McMichael, A. & Peterson, P. A. (1987) *J. Immunol.* **138**, 3960–3966.
8. Rawle, F. C., Tollefson, A. E., Wold, W. S. M. & Gooding, L. R. (1989) *J. Immunol.* **143**, 2031–2037.
9. Cox, J. H., Yewdell, J. W., Eisenlohr, L. C., Johnson, P. R. & Bennink, J. R. (1990) *Science* **247**, 715–718.
10. Pääbo, S., Nilsson, T. & Peterson, P. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9665–9669.
11. Schrier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A. & van der Eb, A. J. (1983) *Nature (London)* **305**, 771–775.
12. Friedman, D. J. & Ricciardi, R. P. (1988) *Virology* **165**, 303–305.
13. Duh, E. J., Maury, W. J., Folks, T. M., Fauci, A. S. & Rabson, A. B. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5974–5978.
14. Tollefson, A. E., Stewart, A. R., Yei, S., Saha, S. K. & Wold, W. S. M. (1991) *J. Virol.* **65**, 3095–3105.
15. Cook, J. L. & Lewis, A. M., Jr. (1984) *Science* **224**, 612–615.
16. Duerksen-Hughes, P., Wold, W. S. M. & Gooding, L. R. (1989) *J. Immunol.* **143**, 4193–4200.
17. Ginsberg, H. S., Moldawer, L. L., Sehgal, P. B., Redington, M., Kilian, P. L., Chanock, R. M. & Prince, G. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1651–1655.
18. Beutler, B. & Cerami, A. (1989) *Annu. Rev. Immunol.* **7**, 625–711.
19. Wong, G. H. W. & Goeddel, D. V. (1986) *Nature (London)* **323**, 819–822.
20. Mestan, J., Digel, W., Mittnacht, S., Hillen, H., Blohm, D., Möller, A., Jacobsen, H. & Kirchner, H. (1986) *Nature (London)* **323**, 816–819.
21. Collins, T., Lapierre, L. A., Fiers, W., Strominger, J. L. & Pober, J. S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 446–450.
22. Ginsberg, H. S., Lundholm-Beauchamp, U., Horswood, R. L., Pernis, B., Wold, W. S. M., Chanock, R. M. & Prince, G. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3823–3827.
23. Burrone, O. R. & Milstein, C. (1982) *EMBO J.* **1**, 345–352.
24. Rosa, F., Le Bouteiller, P. P., Abadie, A., Mishal, Z., Lemonnier, F. A., Bourrel, D., Lamotte, M., Kalil, J., Jordan, B. & Fellous, M. (1983) *Eur. J. Immunol.* **13**, 495–499.
25. Eager, K. B., Pfizenmaier, K. & Ricciardi, R. P. (1989) *Oncogene* **4**, 39–44.
26. Monaco, J. J. (1992) *Immunol. Today* **13**, 173–179.
27. Routes, J. M. & Cook, J. L. (1990) *J. Immunol.* **144**, 2763–2770.
28. Leff, T., Elkaim, R., Goding, C. R., Jalinot, P., Sassone-Corsi, P., Perricaudet, M., Kédinger, C. & Chambon, P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4381–4385.
29. Graham, F. L., Smiley, J., Russel, W. C. & Nair, R. (1977) *J. Gen. Virol.* **36**, 59–72.
30. Arnold, B., Burgert, H.-G., Hamann, U., Hämmerling, G., Kees, U. & Kvist, S. (1984) *Cell* **38**, 79–87.
31. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341.
32. Green, M. & Wold, W. S. M. (1979) *Methods Enzymol.* **57**, 425–435.
33. Cox, J. H., Bennink, J. R. & Yewdell, J. W. (1991) *J. Exp. Med.* **174**, 1629–1637.
34. Brodsky, F. M., Bodmer, W. F. & Parham, P. (1979) *Eur. J. Immunol.* **9**, 536–545.
35. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F. & Ziegler, A. (1978) *Cell* **14**, 9–20.
36. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
37. Ayane, M., Nielsen, P. & Köhler, G. (1989) *Nucleic Acids Res.* **17**, 6722.
38. Burgert, H.-G. & Kvist, S. (1987) *EMBO J.* **6**, 2019–2026.
39. Lenardo, M. J. & Baltimore, D. (1989) *Cell* **58**, 227–229.
40. Williams, J. L., Garcia, J., Harrich, D., Pearson, L., Wu, F. & Gaynor, R. (1990) *EMBO J.* **9**, 4435–4442.