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Endogenous Expression of E1A in Human Cells Enhances the Effect of Adenovirus E3 on Class I Major Histocompatibility Complex Antigen Expression

JOHN M. ROUTES,[1,3]* BARBARA A. METZ,[2] AND JAMES L. COOK[1,2,3,4]

Department of Medicine[1] and Robert W. Lisle Laboratory in Immunology and Tumor Cell Biology,[2] National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206, and Departments of Medicine[3] and Microbiology/Immunology,[4] University of Colorado Health Sciences Center, Denver, Colorado 80262

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Group C human adenovirus (Ad) serotypes (e.g., Ad type 2 [Ad2] and Ad5) cause persistent infections in humans. One explanation for Ad persistence is an ineffective cytotoxic T-lymphocyte response due to diminished cell surface expression of class I major histocompatibility antigen (MHC Ag) on Ad-infected cells, an effect mediated by the Ad E3 19-kDa glycoprotein (E3 effect). However, we previously reported that, except for the Ad5 E1-transformed human cell line 293, a variety of human lymphoid, epithelial, and fibroblastic cells are resistant to the E3 effect during Ad5 infection (J. M. Routes and J. L. Cook, J. Immunol. 144:2763–2770, 1990). The present study tested the hypothesis that endogenous expression of E1A proteins in 293 cells sensitizes cells to this E3 effect, resulting in an enhanced downregulation of surface class I MHC Ag expression following Ad5 infection. Human epithelial and fibroblastic cells expressing E1A gene products for at least 72 h exhibited an enhanced E3 effect following Ad5 infection that was independent of baseline levels of surface class I MHC Ag expression and of E1A induction of E3 19-kDa glycoprotein expression. There was a direct correlation between the level of endogenous E1A expressed and the magnitude of the E3 effect. We postulate that the in vivo existence of cells stably expressing either E1A proteins or E1A-like activities in the microenvironment of Ad5 infection provides a reservoir of Ad-infected cells that is relatively protected from the virus-specific cytotoxic T-lymphocyte response, thereby favoring Ad persistence in humans.

In humans, group C adenoviruses (Ad) such as Ad type 2 (Ad2) and Ad5 (Ad2/5) are common pathogens causing self-limited, but frequently persistent, infections (7, 8, 25, 29). Since the prevalence of Ad infections is high but the frequency of disseminated or fatal infections in humans is extremely low, the anti-Ad immune response must be effective in clearing the majority of virus-infected cells. Clinical studies have demonstrated that an intact cellular immune response is of central importance in preventing lethal or widespread Ad infections. The vast majority of fatal or disseminated Ad infections occur in people with defects in cellular, not humoral, immunity (14, 16, 26, 30, 31). Cytotoxic T lymphocytes (CTL) are an important component of the cellular immune response that limits viral infections (32). Therefore, it seems likely that CTL would also play a key role in controlling human Ad infections. However, it has been proposed that CTL may be ineffective in eliminating Ad-infected cells as a result of the function of the Ad-encoded E3 19-kDa glycoprotein (1, 5, 18, 20). The E3 19-kDa protein binds newly synthesized class I major histocompatibility complex (MHC) proteins in the endoplasmic reticulum and blocks their further egress to the cell surface. Viral peptides bound by and coexpressed on the cell surface with newly synthesized class I MHC molecules serve as the ligands for the T-cell receptors on CTL (17, 28). Therefore, a block in expression of newly synthesized class I molecules early after Ad infection could prevent CTL recognition of Ad-infected cells.

In a previous study, we examined the generality, magnitude, and kinetics of the E3-dependent downregulation of class I MHC antigen (Ag) expression (E3 effect) following Ad2/5 infection of human epithelial, fibroblastic, and lymphoid cells (24). With the exception of the Ad5 E1-transformed cell line 293, these types of human cells were resistant to E3-induced downregulation of class I Ag until the terminal stages of Ad infection. The resistance of these human cells (other than 293 cells) to the E3 effect was due to continued surface expression of newly synthesized class I MHC Ag despite the presence of the E3 19-kDa protein. On the basis of these observations, we speculated that CTL should be able to recognize and eliminate the majority of Ad-infected cells in vivo. We further postulated that there may exist certain types of human cells that exhibit increased susceptibility to the E3 effect (analogously to 293 cells) that could be resistant to elimination by CTL upon Ad2/5 infection. On the basis of this premise, the 293 cell line can serve as an in vitro model of human cells that allow Ad2/5 persistence in the face of an active CTL response. There were a number of possibilities for the unusual sensitivity of 293 cells to the E3 effect. Among all of the human cell lines tested, 293 expressed the lowest baseline level of surface class I Ag. Therefore, the unusual sensitivity of 293 cells to the E3 effect could be due to the low baseline level of class I Ag expression, the endogenous expression of E1 gene products, or other characteristics unique to 293 (e.g., kidney epithelium or somatic mutations following multiple passages in culture). The purpose of this study was to test the hypothesis that endogenous expression of E1A gene products in 293 cells causes the enhanced response to the E3 19-kDa protein following Ad2/5 infection and that other E1A-expressing human cells will exhibit this same pheno-

* Corresponding author.

type. These studies show that constitutive expression of E1A gene products in human epithelial or fibroblastic cells results in enhanced, E3-dependent downregulation of class I Ag following Ad2/5 infection independently of the baseline levels of cell surface class I MHC Ag expression.

MATERIALS AND METHODS

Cells and cell lines. 293 is a human embryonic kidney cell line transformed by the E1 region of Ad5 (11). IC4, provided by A. Berk, is a HeLa S3 cell line transfected with the Ad2 E1A gene under the control of the murine mammary tumor virus promoter (4). IC4-D10 is a subclone of IC4 obtained by cloning at limiting dilution. P2AHT2A is an Ad5 E1A-transfected clone of the H4 fibrosarcoma cell line (9). Both H4 and P2AHT2A were provided by S. Frisch. HeLa is a cervical epithelial cancer cell line. Two HeLa strains were tested, one obtained from H. Ginsberg and one (HeLa S3) obtained from T. Puck. Cells were maintained in Dulbecco's modified Eagle medium containing antibiotics, 15 mM glucose, and 5% calf serum. To induce E1A expression in IC4-D10 cells, dexamethasone was added at the indicated concentrations to standard culture medium. Cell lines were tested periodically for *Mycoplasma* contamination by using the Mycotect assay (BRL Laboratories, Gaithersburg, Md.) and were negative.

Viruses and virus infections. Ad5 was grown and titered in A549 cells. The Ad2 deletion mutant H2*dl*801 (*dl*801) (6) was provided by G. Ketner and was grown and titered in 293 cells. *dl*801 has a deletion that spans the E3 19-kDa coding region. H5*dl*312 (*dl*312), an E1A-minus deletion mutant of Ad5 (13), provided by T. Shenk, was grown and titered in 293 cells. The cell lines in which viral stocks were grown were screened for *Mycoplasma* contamination and were negative.

Measurement of class I MHC Ag levels. MB40.5 (19) and W6/32 (2) are mouse monoclonal antibodies that are panreactive against HLA A, B, and C that were obtained from the American Type Culture Collection. Fluorescein-isothiocyanate-conjugated sheep anti-mouse immunoglobulin G antibody (Cappel, Durham, N.C.) was used for indirect immunofluorescence assays.

Human cells were incubated in the presence or absence of dexamethasone for the indicated times, mock or Ad infected (100 PFU/ml) in suspension on a low-speed shaker for 1 h at 37°C, and then maintained for an additional 15 h in stationary culture. Infections were performed in culture medium containing 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer. Cells were stained with appropriate antibodies as described previously (24). Then 3,000 to 5,000 cells were analyzed in a standard fashion on an Epics C flow cytometer (Coulter Corp., Hialeah, Fla.). To control for the nonspecific (i.e., non-E3-dependent) reduction of surface class I MHC Ag due to Ad infection, levels of class I MHC Ag following Ad5 infection were compared with that expressed on cells infected with the Ad2 E3 deletion mutant, *dl*801. The nonspecific reduction of class I MHC Ag following *dl*801 infection was small and equivalent on all tested cell lines (data not shown). Results of fluorescence-activated cell sorting (FACS) analysis to measure surface class I Ag represent the means of at least four experiments.

Quantitation of E1A and E3 protein expression. E1A phosphoproteins were quantitatively immunoprecipitated as described previously (23), using the E1A-specific monoclonal antibody M73 (12), provided by E. Harlow. Briefly, lysates were prepared from cells labeled with $H_3{}^{32}PO_4$ (200 μCi/ml).

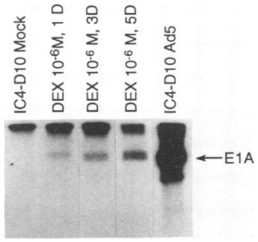

FIG. 1. Time dependence of induction of E1A gene products by dexamethasone in IC4-D10 cells. Shown are quantitative immunoprecipitations of E1A proteins in IC4-D10 cells infected with Ad5 for 16 h or IC4-D10 cells incubated in the presence of 10^{-6} M dexamethasone (DEX) for 1, 3, or 5 days (D). Stimulation of IC4-D10 cells with dexamethasone for increasing periods of time resulted in higher levels of E1A protein expression. The level of E1A protein expression in 5-day dexamethasone-stimulated IC4-D10 cells was far less than that expressed when unstimulated cells were infected with Ad5.

The protein concentration of each lysate was determined (3), and an equal amount of protein from each cell lysate was immunoprecipitated with monoclonal antibody M73, resolved by electrophoresis on sodium dodecyl sulfate–12% polyacrylamide gels, and visualized by fluorography.

For determination of E3 19-kDa protein expression levels, cells were incubated in the presence or absence of dexamethasone and radiolabeled ([^{35}S]methionine, 100 μCi/ml, 4 h) either immediately after Ad5 infection (100 PFU per cell) or 16 h following *dl*312 infection (10 PFU per cell), and E3 19-kDa protein was quantitatively immunoprecipitated as described previously (24).

RESULTS

E1A expression in IC4-D10 cells. IC4 is a HeLa cell line transfected with the Ad2 E1A gene under the control of the murine mammary tumor virus promoter which allows for a dose-dependent induction of E1A gene expression by dexamethasone treatment. Staining of IC4 cells for E1A protein by indirect immunofluorescence revealed that a small percentage of the cells either were noninducible with the addition of 10^{-6} M dexamethasone or continued to express E1A in its absence. Therefore, the IC4 cell line was subcloned by the limiting dilution method. One subclone, IC4-D10, that exhibited strict dexamethasone control of E1A gene expression was chosen for subsequent studies.

The kinetics of E1A gene expression following stimulation of IC4-D10 cells with dexamethasone was measured (Fig. 1). Previous studies had shown that treatment with 10^{-6} M dexamethasone resulted in the maximal stimulation of IC4 cells. Therefore, IC4-D10 cells were incubated with 10^{-6} M dexamethasone for various periods of time, and E1A protein was quantitated. E1A protein expression increased in dexamethasone-treated IC4-D10 cells until treatment day 5, after which no further increase was seen (data not shown). Maximal levels of E1A protein expression in dexamethasone-stimulated IC4-D10 cells were considerably lower than those seen following Ad5 infection of uninduced cells.

E1A protein levels were measured next in IC4-D10 cells following incubation for 5 days with log dilutions (10^{-6} to 10^{-10} M) of dexamethasone (Fig. 2). Stimulation of IC4-D10 cells with 10^{-6} M dexamethasone resulted in maximal induction of E1A. Trace amounts of E1A proteins were still detectable in cells stimulated with 10^{-9} M dexamethasone

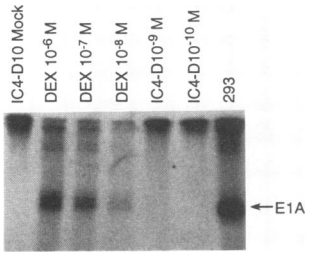

FIG. 2. Concentration dependence of induction of E1A gene products in IC4-D10 cells by dexamethasone. Shown are quantitative immunoprecipitations of E1A proteins in 293 or IC4-D10 cells incubated for 5 days with log dilutions (10^{-6} to 10^{-10} M) of dexamethasone (DEX). Levels of E1A proteins increased in IC4-D10 cells as the concentration of dexamethasone was increased to 10^{-6} M. Trace quantities of E1A proteins were still detectable in IC40-D10 cells stimulated with 10^{-9} M dexamethasone (data not shown), whereas there was no detectable E1A protein in either unstimulated or 10^{-10} M dexamethasone-stimulated cells. 293 cells express approximately twofold more E1A proteins than do maximally induced IC4-D10 cells.

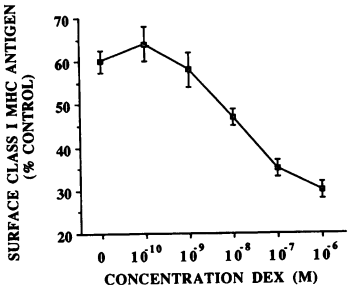

FIG. 4. Evidence that the magnitude of the E3 effect in IC4-D10 cells is dependent on the level of endogenous E1A expression. IC4-D10 cells were incubated for 5 days in the absence or presence of increasing amounts (10^{-10} to 10^{-6} M) of dexamethasone (DEX) and infected with Ad5 for 16 h; then surface class I MHC Ag levels were measured by FACS and compared with levels in identically treated IC4-D10 cells infected with *dl*801. Incubation of IC4-D10 cells with increasing amounts of dexamethasone resulted in a progressive downregulation of class I MHC Ag expression after Ad5 infection.

(data not shown), but there was no detectable E1A at 10^{-10} M. The level of E1A proteins in maximally stimulated IC4-D10 cells was approximately one-half the level measured in 293 cells. These results are similar to those reported for the parental cell line, IC4 (4).

Endogenous expression of E1A gene products sensitizes cells to the E3 effect. Initial experiments compared the downregulation of class I MHC Ag following 16 h of Ad5 infection in untreated and dexamethasone-treated (10^{-6} M, 5 days) IC4-D10, HeLa, and HeLa S3 (a clonal derivative of HeLa and the parental cell line of IC4 cells) (Fig. 3A). To determine whether the reduction of surface class I MHC Ag was dependent on the expression of the E3 19-kDa protein, the level of surface class I MHC Ag in these and subsequent

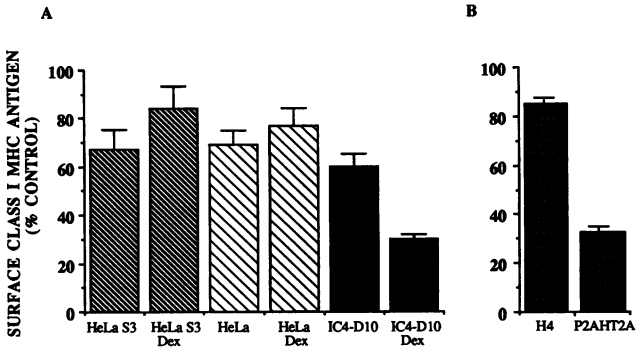

FIG. 3. Evidence that endogenous expression of E1A gene products sensitizes cells to the E3 effect. (A) HeLa, HeLa S3, and IC4-D10 cells were incubated for 5 days in the presence (10^{-6} M) or absence of dexamethasone (Dex) and infected with Ad5 for 16 h; then total levels of surface class I MHC Ag were measured by FACS and compared with levels in equivalently treated IC4-D10 cells infected with *dl*801. Dexamethasone-treated IC4-D10 cells exhibited enhanced downregulation of class I Ag following Ad5 infection compared with untreated IC4-D10. In contrast, Ad5-infected, dexamethasone-treated and untreated HeLa and HeLa S3 cells express similar levels of surface class I MHC Ag. (B) After 16-h Ad5 infections, surface class I MHC Ag levels are substantially lower on E1A-expressing P2AHT2A cells than on H4 cells (E1A-negative, parental cells).

experiments was compared with the level in cells infected with the Ad2 E3 deletion mutant *dl*801. Incubation of HeLa or HeLa S3 cells with dexamethasone did not enhance the E3-dependent downregulation of class I Ag following Ad5 infection. In contrast, the reduction in surface class I MHC Ag was twofold greater in Ad5-infected, dexamethasone-stimulated IC4-D10 cells compared with uninduced control cells.

The prior experiment suggested that endogenous expression of E1A gene products in the epithelial cell line IC4-D10 results in enhanced downregulation of class I Ag following Ad2/5 infection. However, 5-day dexamethasone treatment of HeLa, HeLa S3, and IC4-D10 cells decreased baseline surface class I Ag levels by approximately 30 to 40% compared with untreated control cells (data not shown). Therefore, both low baseline levels of class I MHC Ag expression (resulting in this case from dexamethasone treatment) and endogenous E1A expression might be necessary for the enhanced E3 effect. To address this possibility, an E1A-positive cell line in which E1A expression is constitutive was compared with the parental, E1A-negative control cell line for susceptibility to the E3 effect during viral infection. For these studies, the human fibrosarcoma cell line H4 was compared with the E1A-transfected derivative cell line P2AHT2A (Fig. 3B). H4 cells expressed significantly less surface class I Ag than did P2AHT2A cells (mean ± standard error of the mean of five experiments; P2AHT2A cells expressed 47% ± 3% more surface class I Ag than did H4 cells). However, despite expression of higher baseline levels of surface class I MHC Ag, 16-h Ad5 infections of P2AHT2A cells resulted in a 65% reduction of surface class I Ag, in comparison with a 15% decrease in H4 cells. These data show that endogenous expression of E1A gene products sensitizes cells to the E3 effect independently of baseline class I Ag expression levels.

There is a direct, dose-dependent relationship between the level of endogenous E1A expression and the magnitude of the E3 effect following Ad5 infection in IC4-D10 cells (Fig. 4). For example, IC4-D10 cells stimulated with dexamethasone (10^{-6} M, 5 days) to express maximal levels of endogenous E1A had a 70% reduction of class I Ag following Ad5 infection, compared with the 50% reduction in suboptimally stimulated (10^{-8} M dexamethasone, 5 days) IC4-D10 cells.

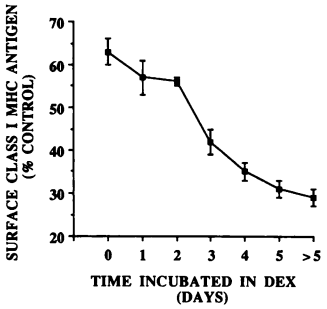

FIG. 5. Time dependence of sensitization of IC4-D10 cells to the E3 effect by E1A gene products. IC4-D10 cells were incubated in the presence (10^{-6} M) or absence of dexamethasone (DEX) for various periods of time and infected with Ad5 for 16 h; then surface class I MHC Ag levels were measured by FACS and compared with levels in equivalently treated IC4-D10 cells infected with *dl*801. Incubation of IC4-D10 cells with dexamethasone for at least 3 days is required for significant enhancement of the E3 effect to be seen.

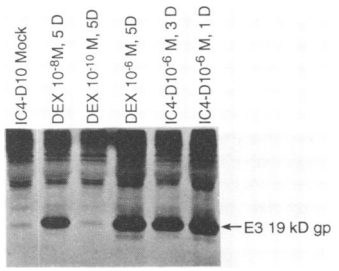

FIG. 6. Evidence that dexamethasone-stimulated IC4-D10 cells complement *dl*312. Dexamethasone (DEX)-stimulated (10^{-6} M for 1, 3, and 5 days; 10^{-8} M for 5 days [D]) but not unstimulated IC4-D10 cells complement *dl*312, resulting in the expression of large amounts of E3 19-kDa protein. gp, gene product.

Thus, higher endogenous levels of E1A protein in IC4-D10 cells result in further reductions in surface class I Ag following Ad5 infection. However, surface class I Ag levels were reduced by almost the same extent in Ad5-infected IC4-D10 cells incubated with either 10^{-7} or 10^{-6} M dexamethasone. This finding may reflect the relatively small difference in E1A protein expression levels in IC4-D10 cells stimulated with 10^{-6} versus 10^{-7} M dexamethasone (Fig. 2). Another possibility is that the level of endogenous E1A in IC4-D10 cells following stimulation with 10^{-7} M dexamethasone is sufficient to maximally sensitize these cells to the E3 effect.

These results show that endogenous expression of E1A gene products sensitize cells to the E3-induced downregulation of class I MHC Ag following Ad5 infection. Furthermore, higher levels of endogenous E1A expression in IC4-D10 cells resulted in greater downregulation of surface class I Ag following Ad5 infection. As previously shown, however, the amount of E1A produced following a 16-h Ad5 infection of unstimulated IC4-D10 cells far exceeded the endogenous expression obtained following maximal (10^{-6} M dexamethasone, 5 days) stimulation of IC4-D10 cells (Fig. 1). These data suggest that endogenous E1A gene products must be expressed for a sufficient period of time prior to Ad5 infection in order for an enhanced E3 effect to be seen. To test this hypothesis, IC4-D10 cells were incubated in 10^{-6} M dexamethasone for various periods of time (1, 2, 3, 5, and 6 to 10 days) and infected with Ad5 for 16 h, and surface class I Ag levels were measured (Fig. 5). An enhanced downregulation of surface class I MHC Ag expression was seen on Ad5-infected IC4-D10 cells only when they were stimulated to express endogenous E1A gene products for at least 3 days prior to infection (Fig. 5).

Endogenous E1A expression does not sensitize cells to the E3 effect by inducing premature E3 expression. The data presented thus far indicate that endogenous expression of E1A gene products for at least 72 h results in enhanced, E3-dependent downregulation of class I MHC Ag following Ad5 infection. Furthermore, there is a direct relationship between the level of endogenous E1A expressed and the magnitude of the enhanced E3 effect. E1A gene products enhance expression of early viral genes following Ad infection. One possible explanation for these results is that the endogenous expression of E1A gene products might induce E3 gene expression earlier in cells infected with Ad5 in comparison with their non-E1A-expressing counterparts. Larger decreases in surface class I levels could occur in E1A-expressing cells because these cells are exposed to the E3 protein earlier after infection than are cells that do not express endogenous E1A gene products. This explanation seemed unlikely since IC4-D10 cells stimulated with dexamethasone (10^{-6} M) for 1 day expressed easily detectable levels of E1A protein (Fig. 1) but were no more susceptible than unstimulated IC4-D10 cells (Fig. 5). However, it was possible that 1-day dexamethasone-stimulated IC4-D10 cells expressed insufficient levels of E1A proteins to induce E3 gene expression. This possibility was tested directly by determining the ability of unstimulated and dexamethasone-stimulated (1, 3, and 5 days at 10^{-6} M; 5 days at 10^{-8} M) IC4-D10 cells to complement the Ad5 E1A deletion mutant *dl*312. As shown in Fig. 6, and in contrast to unstimulated IC4-D10, the levels of endogenous E1A proteins in all of the dexamethasone-treated IC4-D10 cells are sufficient to complement *dl*312, resulting in production of large amounts of the E3 19-kDa protein.

From this result, it seemed likely that the level of endogenous E1A protein expression resulting from these various conditions of dexamethasone treatment of IC4-D10 cells would be sufficient to induce E3 gene expression earlier after Ad5 infection in comparison with unstimulated IC4-D10 cells. To answer this question, E3 19-kDa protein levels were measured during the first 4 h of Ad5 infection in uninduced IC4-D10 cells and in 1- and 5-day dexamethasone (10^{-6} M)-stimulated IC4-D10 cells (Fig. 7). Both 1- and 5-day dexamethasone-treated IC4-D10 cells expressed large, nearly equivalent quantities of the E3 19-kDa protein within 4 h of Ad5 infection. In contrast, the E3 protein was barely detectable in 4-h Ad5 infections of uninduced IC4-D10 cells.

These data establish that sufficient endogenous E1A protein is expressed in 1-day dexamethasone-stimulated IC4-D10 cells to induce E3 gene expression. As a result, these cells express the E3 protein earlier following Ad5 infection in comparison with uninduced cells. However, premature expression of the E3 protein is unrelated to the enhanced E3 effect in E1A-expressing cells, because 1-day dexamethasone-stimulated cells are no more sensitive to the E3 effect than are uninduced cells.

DISCUSSION

Our previous study showed that the magnitude of the E3 effect is most dependent on the cell line infected. Among the 10 cell lines tested, only 293 exhibit greater than a 50%

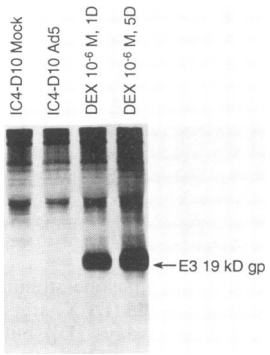

FIG. 7. E3 19-kDa protein expression levels 4 h after Ad5 infection of IC4-D10 cells. IC4-D10 cells stimulated with 10^{-6} M dexamethasone (DEX) for 1 or 5 days (D) express large, nearly equivalent amounts of E3 19-kDa protein 4 h after Ad5 infection. In contrast, there is little E3 19 kDa protein detectable following 4-h Ad5 infection of unstimulated IC4-D10 (IC4-D10 Ad5). gp, gene product.

reduction of surface class I Ag following a 16-h Ad2/5 infection. This report provides evidence that the unusual sensitivity of 293 cells to the E3 effect is due to endogenous expression of E1A gene products and is not due to some other peculiarity of this cell line. Fibroblastic and epithelial human cell lines expressing endogenous E1A gene products exhibited an enhanced E3 effect following Ad5 infection in comparison with parental, non-E1A-expressing controls. For example, 16-h Ad5 infection of the E1A-expressing cell line P2AHT2A resulted in a 65% reduction in surface class I Ag levels, compared with 15% reduction in the parental, non-E1A-expressing cell line H4 (Fig. 3B). Endogenous E1A expression sensitized cells to the E3 effect in both a time- and dose-dependent manner. IC4-D10 cells stimulated with dexamethasone for 5 days to express maximal amounts of endogenous E1A had a greater reduction in surface class I MHC Ag following Ad5 infection than did IC4-D10 cells expressing lower levels of E1A proteins (Fig. 2 and 4). Furthermore, expression of endogenous E1A in IC4-D10 cells for periods of less than 72 h did not result in an enhanced E3 effect (Fig. 5).

Among the fibroblastic, epithelial, and lymphoid human cell lines analyzed in our previous study, 293 cells expressed the lowest baseline level of surface class I MHC Ag. Therefore, one objective of the present study was to determine whether endogenous E1A expression enhanced the E3 effect independently of low baseline surface class I MHC Ag levels. Baseline surface class I MHC Ag levels on the fibrosarcoma cell line H4 were 47% less than levels expressed on the E1A-transfected H4 cell line P2AHT2A. However, P2AHT2A cells were far more sensitive to the E3 effect than were H4 cells despite expressing much higher levels of class I MHC Ag prior to infection. Therefore, the endogenous expression of E1A gene products sensitizes cell to the E3 effect independently of baseline class I MHC Ag levels.

Previous experiments (24; data not shown) showed that E3 19-kDa protein levels 16 h after Ad2/5 infection did not influence the magnitude of the E3 effect. Experiments from this study complement those findings. For example, IC4-D10 cells stimulated with 10^{-6} M dexamethasone for 1 or 5 days both expressed large levels of E3 protein 4 h after Ad5 infection (Fig. 7). In contrast, there were only trace amounts of E3 protein detected in unstimulated, Ad5-infected IC4-D10 cells at this time. However, despite the greater expression of E3 protein 4 h after Ad5 infection, 1-day dexamethasone-stimulated cells were no more sensitive to the E3 effect than were uninduced controls (Fig. 5). These data, in conjunction with our previous studies (24), demonstrate that the levels of E3 19-kDa protein expression early (4 h) or late (16 h) after Ad5 infection do not correlate with the magnitude of the E3 effect in a given cell line.

In our previous study, neither prolonged surface half-life values nor endocytic recycling of class I MHC Ag accounted for the resistance of most human cells to the downregulation of class I Ag following Ad5 infection. Rather, the continued surface expression of newly synthesized class I MHC Ag, despite the presence of large amounts of the E3 protein, maintained surface class I MHC Ag levels on Ad-infected human cells. Class I MHC transcription rates have been shown to increase following Ad5 infection of mouse cells (22), a process dependent on the expression of E1A and E1B gene products. If a similar induction of class I MHC Ag also occurs in infected human cells, this would provide one possible explanation for the inability of the E3 protein to block the surface expression of newly synthesized class I Ag on Ad5-infected human cells.

There are several possible mechanisms by which the constitutive expression E1A gene products may sensitize cells to the E3 effect. For example, E1A-transfected cells may exhibit increased turnover rates of surface class I Ag in comparison with non-E1A expressing cells. Alternatively, class I MHC transcription rates in E1A-transfected cells may be substantially decreased in comparison with control cells following Ad5 infection. This latter hypothesis is consistent with observations by Martin et al. (15) showing that in 293 cells, the endogenous expression of E1A gene products inhibits the ability of transfected E1A to transcriptionally activate a target gene. Other possible mechanisms for an increased E3 effect in E1A-transfected cells include decreased new synthesis of class I Ag by posttranscriptional mechanisms (e.g., increased class I mRNA turnover or inhibition of class I translation), enhanced degradation of newly synthesized class I Ag, or enhanced binding of newly synthesized class I Ag by the E3 protein in the endoplasmic reticulum.

If host CTL are effective mediators of anti-Ad immunity, how might the E3 protein contribute to the propensity of Ad to cause persistent infections and what is the relevance of the enhancement of this effect by endogenous E1A? We have previously suggested that there may be certain human cells that are functionally analogous to 293 cells in their propensity to exhibit an enhanced E3 effect. The existence of a small population of 293-like cells in vivo may allow for a reservoir of Ad-infected cells in the face of an active CTL response, thereby allowing viral persistence. The data presented here show that different types of epithelial and fibroblastic cells stably expressing E1A proteins all can exhibit an enhanced E3 effect.

There are a number of possible mechanisms by which the E3 effect might be rendered more effective in allowing infected cells to escape CTL recognition and destruction. An abortively infected human cell expressing E1A proteins for a sufficient period of time (e.g., $\geq$72 h) could provide an environment in which a second infection of the same cell could result in an exaggerated E3 19-kDa protein-dependent downregulation of class I MHC Ag. Other possible mechanisms might require expression not of E1A itself but of an E1A-like activity in cells prior to infection. For example,

cells that are relatively poorly differentiated (21) or are exposed to cytokines such as interleukin-6 (27) have been shown to exhibit high-level E1A-like activity. The latter hypothesis may be especially relevant, since in a mouse model of Ad pneumonia, high levels of interleukin-6 are detected in both the infected lungs and peripheral blood at early times after Ad5 infection (10). Additionally, an enhanced E3 effect may occur in selected human cells that do not involve E1A or E1A-like activities. For example, cells that inherently express very low levels of cell surface class I MHC Ag may be more sensitive to the E3 effect. This hypothesis is consistent with a study in mice showing decreased CTL recognition of cells that expressed extremely low baseline levels of class I Ag following Ad infection (20).

These data on E1A enhancement of the E3 effect on class I MHC Ag expression provide a new perspective about the possible role of the E3 effect in Ad persistence in humans. This model will be useful for designing studies to test further the importance of the E3 effect on interactions between host CTL and different types of virus-infected target cells. It may also be possible to determine whether cells in which the enhanced E3 effect can be expressed occur naturally in certain sites of Ad infection in humans.

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