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Expression of gp19K Increases the Persistence of Transgene Expression from an Adenovirus Vector in the Mouse Lung and Liver

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Activation of the cellular immune system and subsequent lysis of vector-transduced cells by adenovirus- or transgene-specific cytotoxic T lymphocytes have been shown to limit transgene expression in animal models. The adenovirus gp19K gene product associates with major histocompatibility complex class I proteins and prevents their maturation by sequestering them in the endoplasmic reticulum. gp19K has been shown to block the ability of adenovirus-specific cytotoxic T lymphocytes to recognize virus-infected cells in vitro. To determine if gp19K expression in an adenovirus vector would increase transgene persistence, a vector that replaces the E1 region of adenovirus with an expression cassette encoding both gp19K and β-glucuronidase was constructed. This vector produced high levels of functional gp19K in infected cells. RNase protection analysis revealed efficient expression of the gp19K gene in the mouse lung. Enhanced persistence and increased β-glucuronidase activity were observed in the lung and liver following delivery of the gp19K-expressing adenovirus vector in B10.HTG mice but not in BALB/c mice. Since gp19K binds to both class I alleles on B10.HTG mice but only one allele on BALB/c mice, these results suggest that the major histocompatibility complex class I haplotype of mice is important in determining the effectiveness of gp19K in vivo. Since gp19K has previously been shown to interact with every human major histocompatibility complex class I allele tested, the inclusion of gp19K in gene therapy vectors may increase vector persistence in human gene therapy trials.

A major limitation of adenovirus vectors for use in gene therapy is the transient expression of transgenes, which is associated with vector-induced pathology and problems with repeat administration of the vector. Experiments performed by Wilson and colleagues demonstrated that the transient expression of transgenes observed with adenovirus vectors in the mouse is due to the activation of virus-specific major histocompatibility complex (MHC) class I-restricted responses. $CD8^+$ cells specifically target adenovirus-infected cells (45, 47, 48). A block to repeat administration of adenovirus vectors 28 days following the primary infection was shown to be mediated by MHC class II-restricted responses resulting in the production of neutralizing antibodies (46, 47). An additional problem that may restrict the use of adenovirus vectors in the clinic is a virus-induced inflammatory response that precedes the cytotoxic T-lymphocyte (CTL) response (18–20). This inflammatory response may facilitate CTL generation or the homing of CTLs to the target tissue.

MHC class I molecules are cell surface proteins, found on most cells, that associate with and present endogenously expressed peptides to CTLs. Virus-infected cells are often destroyed by CTLs early after infection. Adenoviruses express a transmembrane glycoprotein from the E3 region, gp19K, which is targeted to the endoplasmic reticulum (ER) (35), associates with MHC class I molecules (32), and prevents the maturation of MHC class I-peptide complexes by trapping them in the ER (4, 7). Differential binding of gp19K to mouse *H-2* alleles has been observed. gp19K interacted efficiently with D^b (13, 41), K^d (8, 9), and L^d (11, 13) *H-2* alleles and associated weakly or not at all with D^k, K^k (8, 25), D^d (5, 11, 13), and K^b (41) *H-2* alleles. gp19K interacted with all of the human MHC class I molecules analyzed, although with different affinities (5, 39).

Since adenovirus antigens that stimulate an immune response are recognized by CTL in the context of MHC class I molecules, reduction of cell surface MHC class I expression by gp19K may reduce the effectiveness of the cellular immune response during adenovirus infection. Rawle et al. (37) developed a system for studying virus-specific CTL generated after a primary adenovirus infection in mice. Those investigators demonstrated that sensitized CTL isolated from spleen cells are capable of lysing syngeneic target cells that have previously been infected with adenovirus mutants carrying large deletions in their early region 3 (E3) genes. However, the efficiency of target cell lysis was dramatically reduced when the target cells were infected with wild-type adenovirus. The protective effect was mapped to the gp19K gene product by using adenoviruses carrying a series of mutations in E3. Interestingly, whether the primary infection was carried out with wild-type adenovirus or the gp19K mutant viruses, adenovirus-specific CTL were efficiently primed, indicating that gp19K expression does not affect the generation of adenovirus-specific CTLs. These results suggested that gp19K expression from an adenovirus vector may protect the transduced cells from lysis by adenovirus-specific CTLs generated after the primary infection. This early study has been corroborated by a more recent investigation utilizing an adenovirus vector that expressed gp19K and β-galactosidase (33). Primary infection with this vector resulted in the generation of β-galactosidase-specific CTLs, and gp19K expression protected target cells from lysis by adenovirus- or β-galactosidase-specific CTLs. Although these results are encouraging, no evidence has been presented that gp19K expression increases the persistence of transgene expression from an adenovirus vector in vivo.

Ginsberg et al. (19) presented evidence that gp19K functions in adenovirus pathology in vivo. They demonstrated that ad-

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enoviruses carrying mutations in the gp19K gene caused a more severe pathology than wild-type adenovirus in the lung of the cotton rat. Specifically, there was a more pronounced perivascular and bronchiolar infiltration of lymphocytes 5 to 7 days after vector administration. This suggests that gp19K may function in vivo to attenuate the immune response against adenovirus. Thus, adenovirus vectors that overexpress gp19K may escape the MHC class I-restricted responses and display increased longevity of transgene expression.

Various strategies to weaken the immune response against adenovirus vectors have been tested. One approach has been to further cripple the vectors with E1 and E3 deletions by removing additional early regions and grow these vectors in newly developed complementing cell lines (3, 6, 15, 17, 26, 30, 44, 52) or to remove all of the virus coding regions and to package a transgene cassette containing the adenovirus packaging sequences (16, 24, 28, 31, 34). However, these new vectors will not affect the CTL response against a foreign transgene, nor will they blunt the humoral immune response. A second approach has been to administer an immune response-modulating drug at the time of vector administration (1, 23, 27, 43, 49). Antibodies directed against CD4 have proven to be effective in reducing both the CTL response and the humoral immune response against the virus (14, 29, 36, 46). Alternatively, it is possible to express an immune response modulator in the viral vector (51). This approach has a potential advantage in that delivery of the immune response modulator would be targeted to the exact location of the vector-transduced cells. In addition, it may simplify in vivo gene therapy by eliminating the need for the separate administration of an immune response-suppressive drug.

We have produced a vector that displays increased persistence of transgene expression in the mouse lung and liver. Ad19K/G expressed high levels of functional gp19K in vitro and in vivo. Differential effects of gp19K expression were observed following administration, depending on the mouse strain used. In B10.HTG mice, *H-2K^{d}D^b*, expression of gp19K following airway or intravenous delivery led to increased persistence of β-glucuronidase expression. However, in BALB/c mice, *H-2^d*, gp19K expression had a minimal effect on the persistence of β-glucuronidase expression. This differential effect of gp19K expression is likely a reflection of the high-affinity binding to *H-2D^b* and low-affinity interaction with *H-2D^d*. These results suggest that in the appropriate setting, gp19K may function to block the cellular immune response directed against a vector and/or a transgene.

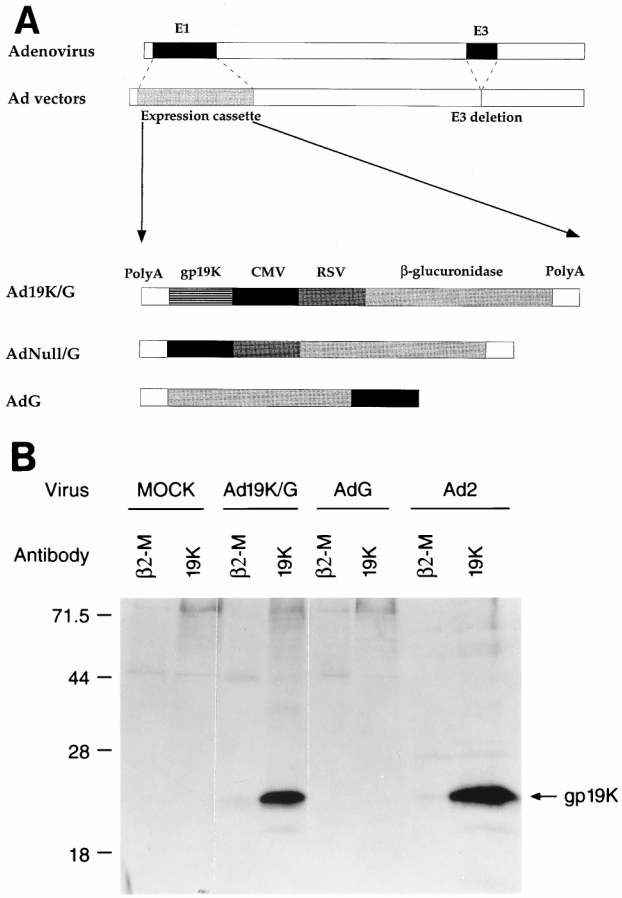

FIG. 1. Ad19K/G virus expresses high levels of gp19K in A549 cells. (A) Schematic view of adenovirus and the adenovirus vectors Ad19K/G, AdNull/G, and AdG. (B) Cells were either mock infected or infected with Ad19K/G, AdG, or Ad2 at a MOI of 20. At 24 h postinfection, the cells were labeled with [^{35}S]methionine and then lysed in digitonin lysis buffer. Cell lysates were immunoprecipitated with monoclonal antibodies specific for gp19K (19K) or β2-M, as indicated. The values on the left are molecular weights in thousands.

MATERIALS AND METHODS

Cells and viruses. A549 cells, a human lung carcinoma cell line, and 293 cells, a human embryonic kidney cell line transformed by sheared adenovirus type 5 DNA, were obtained from the American Type Culture Collection (Rockville, Md.) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum. Adenovirus vector Ad19K/G, with E1 and E3-deletions, expresses β-glucuronidase from the Rous sarcoma virus (RSV) promoter pointing right and gp19K from the cytomegalovirus (CMV) promoter pointing left from an expression cassette inserted at the site of the E1 deletion (Fig. 1A). AdNull/G is similar to Ad19K/G, except that the gp19K sequences were deleted and thus it does not express a transgene from the CMV promoter. AdG expresses β-glucuronidase from the CMV promoter. AdCMV.Null contains the CMV promoter and simian virus 40 poly(A) sequences in place of the E1 region and does not express any transgenes.

All adenovirus vectors were generated by the method of Chinnadurai et al. (10). Briefly, the shuttle vectors were linearized at a unique restriction site adjacent to the left-end inverted terminal repeat and cotransfected into 293 cells with *Cla*I-digested AdLacZ DNA. Virus generated by recombination between the shuttle vector and the adenovirus DNA was plaque purified and propagated on 293 cells (21). Viruses were purified from infected cells at 2 days after vector administration by three freeze-thaw cycles followed by three successive bandings on CsCl gradients. Purified virus was dialyzed against a buffer containing 10 mM Tris (pH 7.8), 150 mM NaCl, 10 mM $MgCl_2$, and 3% sucrose and stored at −70°C until use. All viruses were tested and found to have replication-competent adenovirus levels of less than 1 in 10^7 PFU.

Immunoprecipitations and ^{35}S labeling. Cells grown on 10-cm-diameter plates were infected with adenovirus vectors at a multiplicity of infection (MOI) of 20 in 1 ml of serum-free DMEM. After 24 h, cells were washed twice with phosphate-buffered saline (PBS) and incubated for 30 min at 37°C in 2 ml of DMEM containing 10% dialyzed calf serum and lacking L-methionine. Cells were labeled with 200 μCi of Promix [^{35}S] label (Amersham) per ml for 2 to 3 h at 37°C. Labeled cells were washed three times in PBS and lysed in 1 ml of ice-cold digitonin lysis buffer (20 mM Tris [pH 7.5], 137 mM NaCl, 1% digitonin) containing 1 mM phenylmethlsulfonyl fluoride, 5 μg of aprotinin per ml, 5 μg of leupeptin per ml, and 1 mM sodium vanadate at 4°C for 10 min. Cell lysates were scraped into Eppendorf tubes and clarified. The supernatants were transferred to new tubes and used for immunoprecipitations with either a gp19K-specific antibody (TW1.3) or a β2-microglobulin (β2-M)-specific antibody (W6/32) (American Type Culture Collection). Immunoprecipitates were washed three times in digitonin lysis buffer, Laemmli buffer (100 mM Tris [pH 6.8], 20% glycerol, 7% sodium dodecyl sulfate [SDS], 2 mM EDTA, 5% β-mercaptoethanol, 10 mM sodium pyrophosphate, 2 mM sodium vanadate) was added, and the reaction mixtures were boiled for 5 min and electrophoresed in SDS-polyacrylamide gels. The gels were fixed, enhanced, dried, and exposed to film.

For endoglycosidase H (endo H) digestions, the washed immunoprecipitates were resuspended in 0.1 M sodium citrate (pH 5.5) and incubated at 37°C for 18 h with 2 U of endo H (New England Biolabs Inc., Beverly, Mass.). The reaction mixtures were washed once with 50 mM Tris (pH 6.8) before boiling in Laemmli buffer and electrophoreses in an SDS-polyacrylamide gel.

Animals. Female BALB/c and BIO.HTG mice were obtained from Jackson Laboratories (Bar Harbor, Maine) at 6 to 8 weeks of age. Prior to administration, mice were anesthetized with an intramuscular injection of ketamine (40 mg/kg

and xylamine (12 mg/kg). Vectors were administered intranasally at a single dose of 2.5×10^{10} particles containing approximately 4.5×10^8 PFU per animal in a 75-μl volume. At the time points indicated in Results, mice were given an intraperitoneal injection of a terminal dose of anesthetic. The lungs were removed and washed quickly with PBS. The lungs were flash frozen in liquid nitrogen, ground with a mortar and pestle, aliquoted, and stored at −80°C until use. Intravenous administration was performed by exposing the right jugular vein after making a supraclavicular incision, and the vectors were injected by using a 30-gauge needle over a period of 2 min.

β-Glucuronidase assay. Pulverized lung or liver tissue was lysed in 1× Reporter Lysis Buffer (Promega Corp., Madison, Wis.), and protein determinations were made by using Bradford reagent. A 40-μg protein sample was used to measure β-glucuronidase activity with the β-glucuronidase reporter gene assay system (Tropix, Bedford, Mass.).

RNase protection. Total RNA was made from ground lung tissue with a TRI Reagent kit (Sigma, St. Louis, Mo.) in accordance with the manufacturer's instructions. ^{32}P-labeled gp19K and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antisense RNA probes were synthesized by using T3 and T7 RNA polymerases (Life Technologies, Gaithersburg, Md.), respectively. The KS19K plasmid digested with *Pst*I and the pTRI-GAPDH-Mouse Antisense control template (Ambion Inc., Austin, Tex.) were used as the templates. The transcription reactions were terminated with RNase-free DNase I (Promega Corp.), and the probes were purified by electrophoresis in a 5% denaturing polyacrylamide gel. RNase protection analysis was performed by using the RiboQuant RPA kit (Pharmingen Inc., San Diego, Calif.). RNA hybridization was done with labeled RNA probes and 20 μg of total RNA at 56°C overnight. After RNase A and T_1 treatment and proteinase K digestion, protected RNA probe fragments were purified by phenol-chloroform extraction and ethanol precipitation. Protected fragments were resolved by electrophoresis in a 5% denaturing polyacrylamide gel.

RESULTS

In vitro evaluation of a gp19K-expressing vector. To determine if expression of the adenovirus gp19K protein would increase the persistence of transgene expression in the mouse, we generated a recombinant adenovirus vector termed Ad19K/G (Fig. 1A). This vector carried deletions in the E1 and E3 regions. An expression cassette consisting of the CMV promoter driving gp19K and the RSV promoter driving β-glucuronidase was inserted in place of the wild-type E1 region.

To determine if gp19K was efficiently expressed, A549 cells were infected with Ad19K/G, AdG (a control vector that does not express gp19K but does express β-glucuronidase from the CMV promoter), or wild-type adenovirus type 2 (Ad2). At 24 h after vector administration, cells were labeled with [^{35}S]methionine and cell lysates were analyzed for gp19K expression with a gp19K-specific monoclonal antibody (Fig. 1B). Efficient gp19K expression was observed in cells infected with Ad19K/G and Ad2 but not in mock-infected cells or cells infected with AdG.

Two different assays were employed to test the functionality of gp19K produced from Ad19K/G-infected cells. Since gp19K has previously been shown to bind to MHC class I heavy chains and retain these complexes in the ER, these properties were analyzed. Lysates from vector-infected cells were immunoprecipitated with a β2-M-specific monoclonal antibody (Fig. 1B and 2). This antibody coprecipitated a protein that migrated with the same mobility as gp19K from lysates of cells that were infected with Ad19K/G and Ad2 but not from mock-infected cells or cells infected with AdG (Fig. 1B). Further, upon endo H digestion of the immunoprecipitated complex the cleavage pattern of the coprecipitated protein was identical to that of gp19K (Fig. 2). This analysis suggests that Ad19K/G produces a functional gp19K product that interacts with the MHC class I heavy chain.

Endo H cleaves simple sugars that are attached to proteins in the ER but fails to cleave complex sugars that are added in the Golgi. Thus, digestion with endo H reveals whether proteins have been retained in the ER or have matured through the Golgi. Lysates from cells infected with Ad19K/G or mock-infected cells were immunoprecipitated with the β2-M-specific antiserum. Half of the immunoprecipitate was treated with endo H, and the other half was left untreated. As expected, clear differences in the mobility of the coprecipitating MHC class I heavy chain were observed in mock-infected versus Ad19K/G-infected lysates prior to endo H treatment (Fig. 2). In mock-infected cells, the mobility of the MHC class I heavy chain that coprecipitated with the β2-M-specific antibody was unchanged upon treatment with endo H. In contrast, the MHC class I heavy chain was efficiently cleaved by endo H in cells that were infected with Ad19K/G. These results are consistent with previous findings demonstrating that gp19K functions to interfere with terminal glycosylation and transport of MHC class I molecules (4, 7–9, 11, 32, 35) and indicate that the adenovirus vector Ad19K/G expresses functional gp19K.

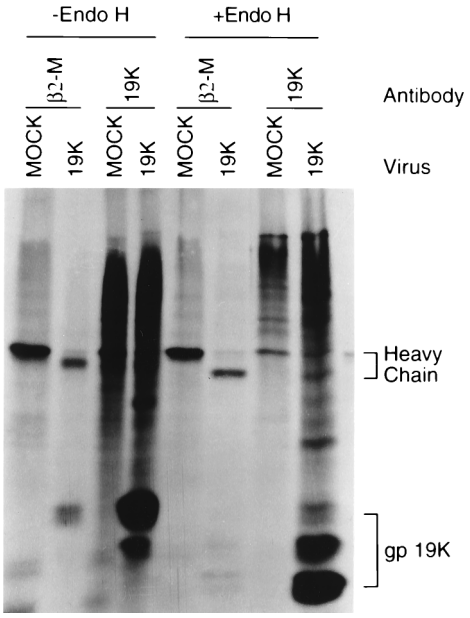

FIG. 2. Vector-produced gp19K blocks cell surface expression of MHC class I molecules in A549 cells. Cells were either mock infected or infected with Ad19K/G at a MOI of 20. At 24 h postinfection, cells were labeled with [^{35}S]methionine and then lysed in digitonin lysis buffer. Cell lysates were immunoprecipitated with the β2-M-specific monoclonal antibody or the gp19K-specific antibody (19K), as indicated. One half of the immunoprecipitate was treated with endo H prior to electrophoresis in an SDS–12.5% polyacrylamide gel.

Ad19K/G expresses gp19K in vivo. To determine if gp19K was expressed in the mouse lung following infection with Ad19K/G, RNase protection analysis was performed on RNA extracted from the lungs of BALB/c mice. Mice were infected intranasally with 5×10^8 PFU of Ad19K/G or AdNull/G. AdNull/G is identical to Ad19K/G, except that it carries a complete deletion of the gp19K gene (Fig. 1A). RNase protection analysis revealed expression of the gp19K gene in the lungs of BALB/c mice at 2, 4, and 7 days after vector administration (Fig. 3). gp19K was not detected at 10, 14, or 21 days after infection with Ad19K/G, and no gp19K expression was observed at any time points in lung tissue from mice infected with AdNull/G. A probe specific for GAPDH was used to control for levels of RNA that were extracted from the lung tissue and used in this analysis. These results reveal transient expression of gp19K in the BALB/c lung following intranasal delivery of Ad19K/G. The finding that gp19K mRNA levels decreased steadily from 2 to 7 days after vector administration and were no longer detectable by day 10 suggested that gp19K

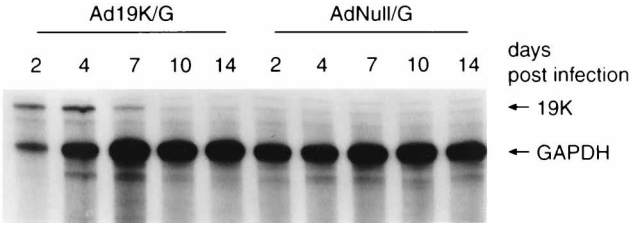

FIG. 3. RNase protection analysis of gp19K expression in the BALB/c mouse lung. Mice were infected intranasally with the indicated vectors at a dose of 5×10^8 PFU. Lung RNA was harvested at 2, 4, 7, 10, and 14 days after vector administration and used for RNase protection assays with gp19K (19K) and GAPDH probes.

expression did not significantly delay or block the CTL-induced clearance of vector-transduced cells in the BALB/c lung.

gp19K expression does not prolong transgene expression in the BALB/c lung. To directly determine if expression of gp19K results in persistence of transgene expression following delivery to the mouse lung, we compared the expression of β-glucuronidase from adenovirus vectors following intranasal delivery to BALB/c mice. Mice were infected with AdNull/G, Ad19K/G, or AdCMV.Null, and β-glucuronidase activity in the lung tissue was monitored over time. As expected, no increase in β-glucuronidase levels was observed in animals that received AdCMV.Null or vehicle. β-Glucuronidase activity peaked at 7 days after vector administration in animals that received AdNull/G and Ad19K/G. In both groups, β-glucuronidase activity declined steadily until, at 21 days after vector administration, the activity in both groups was approximately 5% of the peak activity observed 7 days after vector administration (Fig. 4). Although a marginal and reproducible increase in β-glucuronidase levels was observed at 10, 15, and 21 days after administration in mice that received Ad19K/G over mice that received AdNull/G, it was not statistically significant. These results indicate that expression of gp19K does not prolong β-glucuronidase expression in the BALB/c lung.

gp19K expression prolongs transgene expression in the B10.HTG mouse. BALB/c mice carry the *H-2*^{d} haplotype. Previous studies have shown that gp19K binds to K^d efficiently but does not bind to and block D^d cell surface expression (5, 8, 9, 11, 13). Therefore, if adenoviral or β-glucuronidase peptides

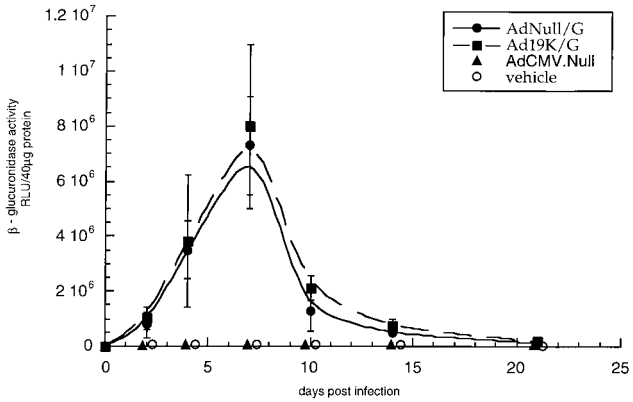

FIG. 4. gp19K function in the BALB/c mouse lung. Mice were infected intranasally with 2.5×10^{10} particles of Ad19K/G, AdNull/G, or AdCMV.Null. At 2, 4, 7, 10, 14, and 21 days after vector administration, the animals were sacrificed and lung tissue was analyzed for β-glucuronidase activity. Error bars indicate the standard errors of the means ($n = 3$). RLU, relative light units.

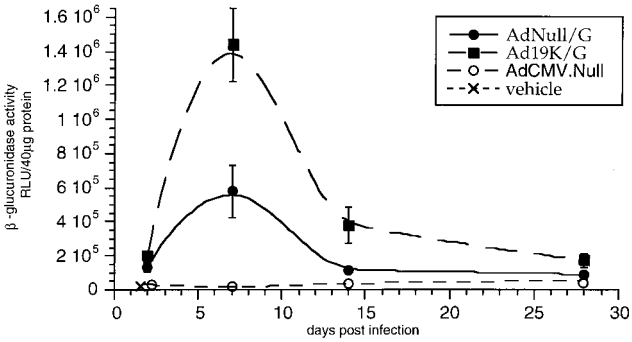

FIG. 5. gp19K function in the B10.HTG mouse lung. Mice were infected intranasally with 2.5×10^{10} particles of Ad19K/G, AdNull/G, or AdCMV.Null. At 2, 4, 7, 10, and 14 days after vector administration, the animals were sacrificed and lung tissue was analyzed for β-glucuronidase activity. Error bars indicate the standard errors of the means ($n = 6$). RLU, relative light units.

were presented by D^d molecules, gp19K would not be expected to alter the CTL response against vector-transduced cells in BALB/c mice.

Since gp19K binds to and inhibits the transport of D^b (41), the effect of gp19K expression on β-glucuronidase expression was studied in the B10.HTG mouse (*H-2K*^{d}*D*^{b}). B10.HTG mice were infected intranasally with 2.5×10^{10} particles of either AdNull/G, Ad19K/G, or AdCMV.Null. Mice were sacrificed at 2, 7, 14, and 28 days after vector administration and β-glucuronidase activity in the lung was analyzed (Fig. 5). As expected, no β-glucuronidase activity was detected in the AdCMV.Null-infected lungs. Significant increases in β-glucuronidase levels were observed in mouse lungs infected with AdNull/G and Ad19K/G. At 2 days after vector administration, approximately equal levels of transgene expression were observed in AdNull/G- and Ad19K/G-infected lungs, indicating equivalent delivery. However, at days 7, 14, and 28, β-glucuronidase activity measured in the lung tissue of B10.HTG mice was elevated between two- and fourfold in the Ad19K/G group compared to the AdNull/G group. These findings indicate that gp19K expression increases and prolongs transgene expression in the B10.HTG mouse lung.

To determine if gp19K expression increases transgene persistence in other tissues, we analyzed the effect of gp19K expression following liver-directed delivery in B10.HTG mice. Mice were infected with either AdNull/G or Ad19K/G by intravenous administration, and β-glucuronidase activity was monitored at 2, 7, 14, and 21 days after vector administration. The results from this experiment mirrored our observations in the lung experiments (Fig. 6). At 2 days after vector administration, approximately equal levels of transgene expression were observed in AdNull/G- and Ad19K/G-infected livers. However, at 7, 14, and 21 days after administration, β-glucuronidase activity in the liver tissue was elevated between two- and fourfold in the Ad19K/G group compared to the AdNull/G group. These findings indicate that gp19K expression prolongs transgene expression in the B10.HTG mouse liver.

DISCUSSION

We have generated an adenovirus vector with E1 and E3 deletions that carries a dual-expression cassette in the E1 region. This expression cassette drives gp19K from the CMV promoter and a reporter gene, β-glucuronidase, from the RSV promoter. In vitro experiments demonstrated that this vector expressed a fully functional gp19K protein. Expression of

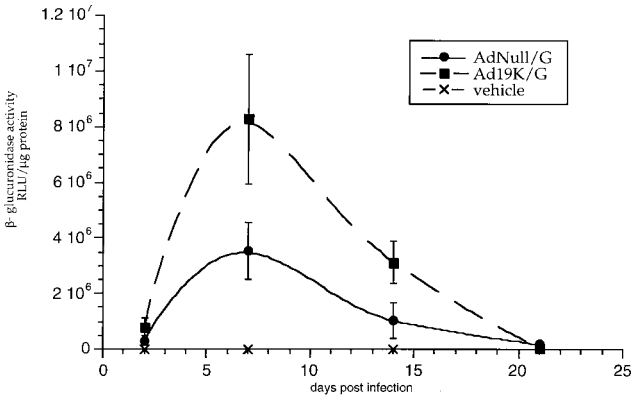

FIG. 6. gp19K function in the B10.HTG mouse liver. Adenovirus vectors were inoculated intravenously into B10.HTG mice. At 2, 7, 14, and 21 days after delivery, the animals were sacrificed and liver tissue was analyzed for β-glucuronidase activity. Error bars indicate the standard errors of the means (days 2 and 14, $n = 6$; days 7 and 21, $n = 3$). RLU, relative light units.

gp19K from this vector, Ad19K/G, in the absence of E1A efficiently blocked the maturation of newly synthesized MHC class I molecules in A549 cells 24 h postinfection.

It is well documented that gp19K expression protects against the lysis of adenovirus-infected cells by adenovirus-specific CTLs in vitro (33, 37, 40). However, this effect has not been observed in vivo in murine pneumonia models. Expression of gp19K in vaccinia virus had no effect on virus lethality, virus replication in vivo, or the generation of a $CD8^+$ infiltrate (22). Similarly, Cox et al. (12) showed that expression of gp19K in vaccinia virus had no effect on CTL responses, natural killer cell responses, or viral replication in B10.HTG mice.

In a recent study, C57BL/10snJ mice received intranasal inoculations with replication-competent adenoviruses that express wild-type gp19K or viruses that carried deletions in the gp19K gene. No differences in the generation of CTLs or in pulmonary infiltrates were observed (40). In addition, treatment of target cells with gamma interferon abrogated the ability of gp19K to block CTL-mediated lysis in vitro. This finding suggests that cellular responses to adenovirus infection may overcome the gp19K effect. However, it appears that gamma interferon does not overcome gp19K protection of cells in vivo since antibody depletion did not uncover a gp19K function in the mouse lung. These findings have led to the speculation that gp19K does not function during an acute infection but, instead, prevents the lysis of adenovirus-infected cells during persistent infections (38, 40). Alternatively, since gp19K has evolved to function in humans and does not bind tightly to many murine MHC class I proteins it may not function effectively in the mouse. In a similar manner, the herpes simplex virus ICP47 protein binds to the transporter associated with antigen presentation (TAP) and prevents the peptide loading of MHC class I molecules in human, but not in mouse, cells (2, 42). Our findings support this idea, since expression of gp19K in the BALB/c strain did not increase transgene expression but gp19K did increase transgene expression in B10.HTG mice. This is the first evidence of an effect of gp19K on the persistence of gene expression in an adenovirus vector. Significantly, this increased persistence was observed concomitant with the expression of the highly immunogenic bacterial β-glucuronidase protein.

It is unlikely that the increase in β-glucuronidase expression observed in B10.HTG mice was due to vector differences, as the gp19K-expressing vector (Ad19K/G) was identical to the control vector (AdNull/G), except for the insertion of 524 bp of gp19K coding sequences. This small increase, representing 1.5% of the adenovirus genome, is unlikely to contribute to increased transgene expression, since the incorporation of other potential immune modulating genes similar in size to gp19K did not increase transgene persistence (6a).

CTLs have been shown to target both virus- and transgene-specific epitopes in mice infected with adenovirus vectors (33, 50). Thus, it is likely that β-glucuronidase-, gp19K-, or adenovirus-specific CTLs were generated in mice infected with Ad19K/G. One potential explanation for the lack of a gp19K effect in the BALB/c lung is that CTLs may have recognized vector-specific peptides in the context of MHC class I molecules that did not interact efficiently with gp19K (e.g., peptides that are presented by $H\text{-}2D^d$). B10.HTG mice, $H\text{-}2K^dD^b$, were utilized to minimize these potential class I-specific effects. Although we did observe a gp19K-dependent increase in transgene persistence in both the liver and lung when using this model, transgene expression decreased over time. It was not unexpected that gp19K expression vectors would not persist longer in this strain, and there are several potential explanations for this result. The kinetics of the loss of transgene expression suggests that gp19K may delay the CTL-induced elimination of the infected cells in this system. B10.HTG mice may express additional MHC class I molecules that do not bind efficiently to gp19K and can thus trigger the elimination of virus-infected cells. Alternatively, there may be other immune or nonimmune mechanisms for the elimination of vector-transduced cells or for the down regulation of transgene expression that are operative in this system. Clearly, additional studies are required to determine the causes of the decrease in transgene expression observed in gp19K-infected B10.HTG mice. However, these results are encouraging and imply that gp19K expression in adenovirus-based gene therapy vectors may prolong gene expression in human clinical trials.

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