JOURNAL OF VIROLOGY, Sept. 1993, p. 5289–5298
0022-538X/93/095289-10$02.00/0

Vol. 67, No. 9

Deletion Mutation Analysis of the Adenovirus Type 2 E3-gp19K Protein: Identification of Sequences within the Endoplasmic Reticulum Lumenal Domain That Are Required for Class I Antigen Binding and Protection from Adenovirus-Specific Cytotoxic T Lymphocytes

TERRY W. HERMISTON,[1] RALPH A. TRIPP,[2] TIM SPARER,[2] LINDA R. GOODING,[2] AND WILLIAM S. M. WOLD[1]*

Department of Molecular Microbiology and Immunology, St. Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, Missouri 63104,[1] and Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia 30322[2]

Received 22 February 1993/Accepted 4 June 1993

Adenovirus E3-gp19K is a transmembrane glycoprotein, localized in the endoplasmic reticulum (ER), which forms a complex with major histocompatibility complex (MHC) class I antigens and retains them in the ER, thereby preventing cytolysis by cytotoxic T lymphocytes (CTL). The ER lumenal domain of gp19K, residues 1 to 107, is known to be sufficient for binding to class I antigens; the transmembrane and cytoplasmic ER retention domains are located at residues ca. 108 to 127 and 128 to 142, respectively. To identify more precisely which gp19K regions are involved in binding to class I antigens, we constructed 13 in-frame virus deletion mutants (4 to 12 amino acids deleted) in the ER lumenal domain of gp19K, and we analyzed the ability of the mutant proteins to form a complex with class I antigens, retain them in the ER, and prevent cytolysis by adenovirus-specific CTL. All mutant proteins except one (residues 102 to 107 deleted) were defective for these properties, indicating that the ability of gp19K to bind to class I antigens is highly sensitive to mutation. All mutant proteins were stable and were retained in the ER. Sequence comparisons among adenovirus serotypes reveal that the ER lumenal domain of gp19K consists of a variable region (residues 1 to 76) and a conserved region (residues 77 to 98). We show, using the mutant proteins, that the gp19K-specific monoclonal antibody Tw1.3 recognizes a noncontiguous epitope in the variable region and that disruption of the variable region by deletion destroys the epitope. The monoclonal antibody and class I antigen binding results, together with the serotype sequence comparisons, are consistent with the idea that the ER lumenal domain of gp19K has three subdomains that we have termed the ER lumenal variable domain (residues 1 to ca. 77 to 83), the ER lumenal conserved domain (residues ca. 84 to 98), and the ER lumenal spacer domain (residues 99 to 107). We suggest that the ER lumenal variable domain of gp19K has a specific tertiary structure that is important for binding to the polymorphic α1 and α2 domains of class I heavy (α) chains. We suggest that the ER lumenal conserved domain of gp19K may interact with some conserved protein, perhaps the highly conserved α3 domain of class I heavy chains. Finally, the ER lumenal spacer domain may allow the ER lumenal variable and conserved domains to extend out from the ER membrane so that they can interact with class I heavy chains.

Human adenoviruses (Ads) offer an ideal model system with which to study viral persistence. Epidemiological studies have identified individuals shedding Ad years after the initial infection, confirming the virus's ability to establish persistent infections (18). The molecular biology of Ad is well understood, and animal models that mimic the immunopathology of human upper respiratory tract infections have been recently identified (24, 25, 52).

The virus's ability to evade host immune surveillance must be critical to persistence. Four immunoregulatory proteins that are encoded by the E3 region of human Ads have been described (reviewed in references 26, 30, 50, and 66 to 68). The E3 region is conserved among Ad serotypes yet is not required for viral replication in vitro (42) or in vivo (24, 47). Three of the E3 genes code for proteins, termed 14.7K (molecular weight of 14,700 MW), 14.5K, and 10.4K, that prevent cytolysis by tumor necrosis factor (27–29, 35, 36, 54). The fourth protein, gp19K (also termed E3/19K), binds heavy (α) chains of the major histocompatibility complex (MHC) class I antigens and inhibits their transport to the cell surface (30, 50, 66–68).

The MHC class I molecule is a heterodimer consisting of an integral membrane glycoprotein (the α or heavy chain) and a noncovalently associated light chain (β_2-microglobulin). Crystallographic analysis and subsequent in vitro analysis has revealed that the α1 and α2 domains of the heavy chain fold to form a cleft (4, 19) that is occupied by a short (eight to nine amino acids) peptide (15, 19, 37, 39, 45, 62). Viral infection of class I-bearing cells results in viral peptide association with the class I molecules on the cell surface, and this leads to the subsequent destruction of the virus-infected cell by cytotoxic T lymphocytes (CTL). Consequently, events that alter viral peptide processing, class I-viral peptide association, or expression of viral peptide-class I complexes on the cell surface could serve a major role in the establishment and maintenance of a persistent viral infection.

gp19K is an abundant transmembrane glycoprotein (Fig. 1) that has two key properties that enable it to block cell

* Corresponding author.

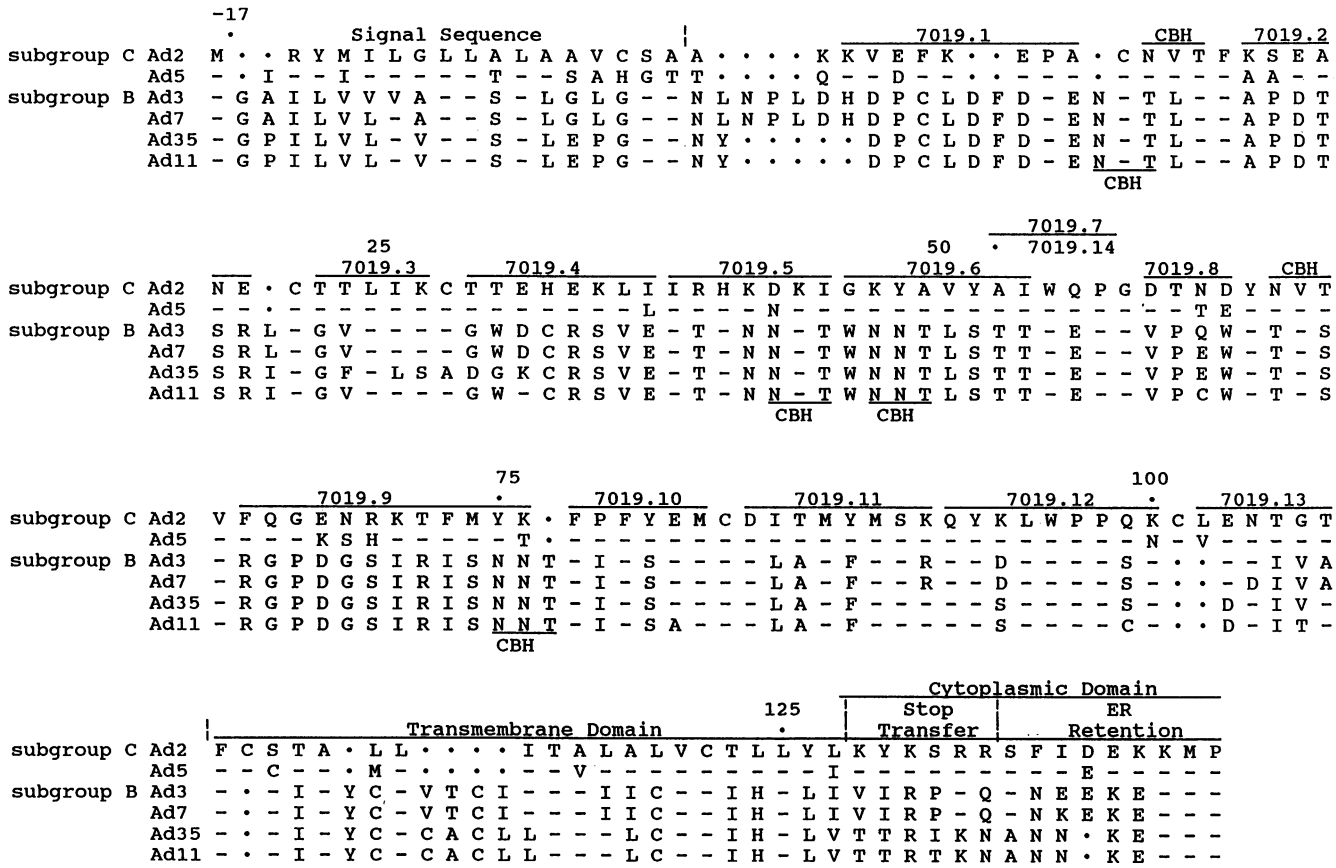

FIG. 1. The gp19K amino acid sequences from Ad2, Ad3, Ad5, Ad7, Ad35, and Ad11. For Ad3, Ad5, Ad7, Ad35, and 11, residues that are identical to the Ad2 gp19K protein are indicated by dashes. Dots indicate that the residue is absent. Mutations in the Ad2 gp19K sequence are depicted as a continuous line that is above the amino acid sequence and borders the amino acids deleted, with the mutant name listed above. The *dl*7019.14 mutation is a frameshift mutation with the insertion of five amino acids (VCCIC) following the alanine residue at amino acid 53. The amino acid sequences are predicted from the DNA sequences as determined for Ad2 (33), Ad5 (11), Ad3 (58), Ad7 (34), and Ad11a (respiratory isolate) (46).

surface expression of class I antigens complexed with Ad peptides (reviewed in references 30, 50, and 66 to 68). First, gp19K has an endoplasmic reticulum (ER) retention signal at its C terminus that extends into the cytoplasm and anchors gp19K in the ER. The mechanism of ER retention of gp19K is unknown (12, 21, 38) and may involve binding to microtubules (14). The second key property of gp19K is that it can bind to certain class I antigens of human, monkey, rat, and mouse. These two properties of gp19K cause class I antigens to be retained in the ER in the form of a complex with gp19K (2, 7). Consequently, gp19K-expressing cells are not killed by Ad-specific CTL (55) or other types of CTL (1, 9, 12, 13). The inhibition of cell surface class I expression by gp19K is conserved among Ad serotypes in subgroups B to E (49). The subgroup A Ads, acting through their E1A gene, inhibit synthesis of the mRNA for class I antigens (20, 57, 61). The gp19K-class I binding is noncovalent, does not require other Ad proteins, and can occur in the absence of β_2-microglobulin (8, 21, 34, 41, 51).

gp19K binds the α1 and α2 domains of the mouse class I molecule (8, 40). The region(s) of gp19K involved in binding the class I molecule is unclear. The ER lumenal domain of Ad2 gp19K, alone, will bind class I antigens (22, 51), as will gp19K lacking the ER retention signal at its C terminus (12, 16, 22, 48). In this report, we extend these observations by using deletion mutation analysis of the entire ER lumenal domain of gp19K in an attempt to define the amino acid region(s) involved in binding class I molecules, blocking their transport to the cell surface, and inhibiting cytolysis by Ad-specific CTL. Interestingly, we find that all deletions except one at the C-terminal end of the ER lumenal domain preclude binding of gp19K to MHC class I molecules.

MATERIALS AND METHODS

Cells and viruses. Human 293 cells (31) were grown in Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum. Viruses were grown in human KB cells and banded in CsCl, and titers were determined in human A549 cells as previously described (32). All of the gp19K viral mutants are derivatives of the parent virus, *rec*700. *rec*700 is an Ad5/Ad2/Ad5 recombinant which consists of the Ad5 *Eco*RI A (map positions 0 to 76), Ad2 *Eco*RI D (76 to 83), and Ad5 *Eco*RI B (83 to 100) fragments (65). Mutants *dl*739 (6) and *dl*754 (27) have been described elsewhere. Mutant *dl*327, which lacks the *Xba*I D fragment at map positions 78.5 to 84.3 and is isogenic with *dl*324 (60), was obtained from T. Shenk (Princeton University, Princeton, N.J.).

Deletions were introduced into the gp19K gene by using

the Amersham (Arlington Heights, Ill.) site-directed oligonucleotide mutagenesis kit. Briefly, 20-bp oligonucleotides (Operon, Almeda, Calif.), consisting of 10 bp 5′ and 10 bp 3′, respectively, of the site to be deleted were annealed to M13 stocks containing either the Ad2 *Hin*dIII L fragment or the 1,122-bp *Sac*I-to-*Stu*I fragment from the Ad2 *Eco*RI D fragment (33). M13 gp19K mutant phage plaques were picked, propagated, and sequenced, and the mutated sequences were exchanged for wild-type sequences in plasmid pED, which contains the *Eco*RI D fragment of Ad2. The mutation was sequenced in the plasmid, and the mutant *Eco*RI D fragment was transferred to the *rec*700 genome by the ligation procedure (65). Plaques were propagated on A549 cells and analyzed by a modification of the method of Hirt (65). Mutant viruses were plaque purified twice before high-titer stocks were prepared by banding in CsCl.

To confirm the mutation within the viral genome, CsCl-purified virus was lysed, and a polymerase chain reaction was performed (34) with a 20-bp oligonucleotide (5′-CG TTT GTA CCT ATT GTC AGC-3′) for 5′ priming and a 20-bp oligonucleotide (5′-GA GCA TAT CCC ACA TAG AGT-3′) for 3′ priming. The polymerase chain reaction-derived DNA was sequenced by using a femtomole sequencing kit from Promega (Madison, Wis.).

Indirect immunofluorescence. A549 cells were grown on glass coverslips and either mock infected or infected at 50 PFU of virus per cell. The cells were fixed in methanol (−20°C) for 8 min at 8 h postinfection (p.i.), rehydrated in phosphate-buffered saline (PBS), and reacted with a gp19K-specific rabbit antiserum diluted 1:100 in PBS containing 1% bovine serum albumin (BSA) and 0.1% NaN_3. The gp19K antiserum, P129-143, is directed against a peptide corresponding to the C-terminal 15 residues of the Ad5 version of gp19K (64). Goat anti-rabbit immunoglobulin G conjugated to fluorescein isothiocyanate (Organon Teknika, Malvern, Pa.) was used as a second antibody, diluted 1:50 in PBS containing 1% BSA and 0.1% NaN_3, and the coverslips were mounted in polyvinyl alcohol containing 1 mg of *p*-phenylenediamine (Sigma Chemical Co., St. Louis, Mo.) per ml. A Nikon epifluorescence microscope was used for photography, utilizing a 60× Plan apo objective and Kodak T-Max 400 film. Diafine developer (Acufine, Inc., Chicago, Ill.) was used to process the film.

Fluorescence-activated cell sorting (FACS) analysis. Mock- or gp19K mutant virus-infected 293 cells (200 PFU per cell) were harvested from 60-mm-diameter dishes (Corning) at 16 h p.i. by washing the cells in ice-cold PBS containing 0.025% EDTA. Cells were resuspended to a single-cell suspension in PBS containing 1% BSA, and then they were divided equally. Half of the cells were incubated at 4°C with W6/32 (American Type Culture Collection, Rockville, Md.), a mouse monoclonal antibody that reacts with all human class I molecules; the other half of the cells did not receive primary antibody. Following three washes in ice-cold PBS containing 1% BSA, fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G2A (Organon Teknika) was added and allowed to incubate at room temperature. Cells were washed four more times in ice-cold PBS containing 1% BSA and then fixed in 1.5% (vol/vol) *p*-formaldehyde. Fluorescence profiles were determined by analyzing 10,000 cells on a Becton Dickinson FACScan flow cytometer. A fluorescence percentage was calculated by taking the median peak of fluorescence intensity of each virus-infected culture, dividing it by the median peak of fluorescence intensity of the mock-infected culture, and multiplying the resulting value by 100.

Immunoprecipitation and endo-β-*N*-acetylglycosaminidase (endo H) H analysis. A549 cells were either mock infected or infected at 100 to 200 PFU of virus per cell and were labeled with 50 to 100 μCi of Tran^{35}S-label (>1,000 Ci/mmol; ICN Biomedical, Inc., Costa Mesa, Calif.) from 4 to 8 h p.i. in Cys-free, Met-free minimal essential medium. Cells were collected by trypsinization or washing in ice-cold PBS containing 0.025% EDTA, rinsed in ice-cold PBS, and lysed in Iso-Hi-pH buffer (0.14 M NaCl, 1 mM $MgCl_2$, 10 mM Tris-HCl [pH 8.5]) containing 0.5% Nonidet P-40. Nuclei were removed by centrifugation. To examine wild-type and mutant gp19K, cell supernatants (10^7 cpm) were mixed either with the gp19K P129-143 antipeptide antiserum or with a gp19K-specific monoclonal antibody, Tw1.3 (kindly provided by J. Cox, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md.) (12), bound to protein A-Sepharose (Sigma). To examine MHC class I-gp19K coimmunoprecipitation, supernatants were mixed with W6/32-bound protein A-Sepharose. Prior to addition of the supernatants, W6/32, Tw1.3, or the P129-143 antipeptide antiserum was absorbed to protein A-Sepharose for 1 h in the presence of NET (0.15 M NaCl, 50 mM Tris-Cl [pH 7.4], 5 mM EDTA) on ice and then washed twice in NET. Supernatants were added, the samples were incubated for 16 h on a rotating wheel, and then the beads were washed five times in high-salt buffer (0.5 M NaCl, 1 mM EDTA, 10 mM Tris-Cl [pH 7.4], 0.5% Nonidet P-40, 1% sodium deoxycholate) and washed twice in ice-cold 50 mM Tris-Cl (pH 6.8). Immunoprecipitates were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) on 15% gels (0.75 mm by 16 cm; acrylamide-*N*,*N*′-methylenebisacrylamide, 29.2:0.8 [wt/wt]). All gels were fluorographed. ^{14}C-labeled molecular weight markers were obtained from Bethesda Research Laboratories (Gaithersburg, Md.).

To determine the sensitivity to endo H (Dupont, NEN Research Products, Boston, Mass.), the coimmunoprecipitation mixtures, while still bound to the protein A-Sepharose beads, were resuspended in buffer (0.1 M sodium citrate [pH 5.5]). One half of the beads was mixed with 2 U of endo H, and all samples were incubated at 37°C for 18 h, washed in 50 mM Tris-Cl (pH 6.8), and then analyzed by SDS-PAGE.

CTL activation and ^{51}Cr release assay. Male mice of strains C57BL/10SnJ (*H-2^b*) and BALB/cJ (*H-2^d*) were obtained from the Jackson Laboratory (Bar Harbor, Maine) and were immunized intraperitoneally with 2×10^7 PFU of Ad5 at least 3 weeks prior to use. Secondary in vitro sensitization of immune spleen cells for production of Ad5-specific CTL was performed as described elsewhere (55), using two Ad-infected simian virus 40 (SV40)-transformed cell lines, SV-BALB (clone A derived from SV40-transformed embryo fibroblasts of a BALB/cJ mouse) and SV-B6KHA (derived from SV40-transformed kidney cells of a C57BL/6Kh mouse). T-cell cytotoxicity was determined by a 5-h ^{51}Cr release assay (55) using either SV-B6KHA or SV-BALB cells infected with Ad mutants and labeled with 200 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, Mass.). Results were calculated as means ± standard errors of the means of triplicate samples and expressed as E − C/T − C = % specific lysis, where E equals counts per minute released by sensitized spleen cells and T equals total releasable counts per minute, determined by addition of 0.05 ml of 1 N HCl to 0.05 ml of target cells. Percent specific lysis of uninfected SV-B6KHA cells ranged from 2 to 10%.

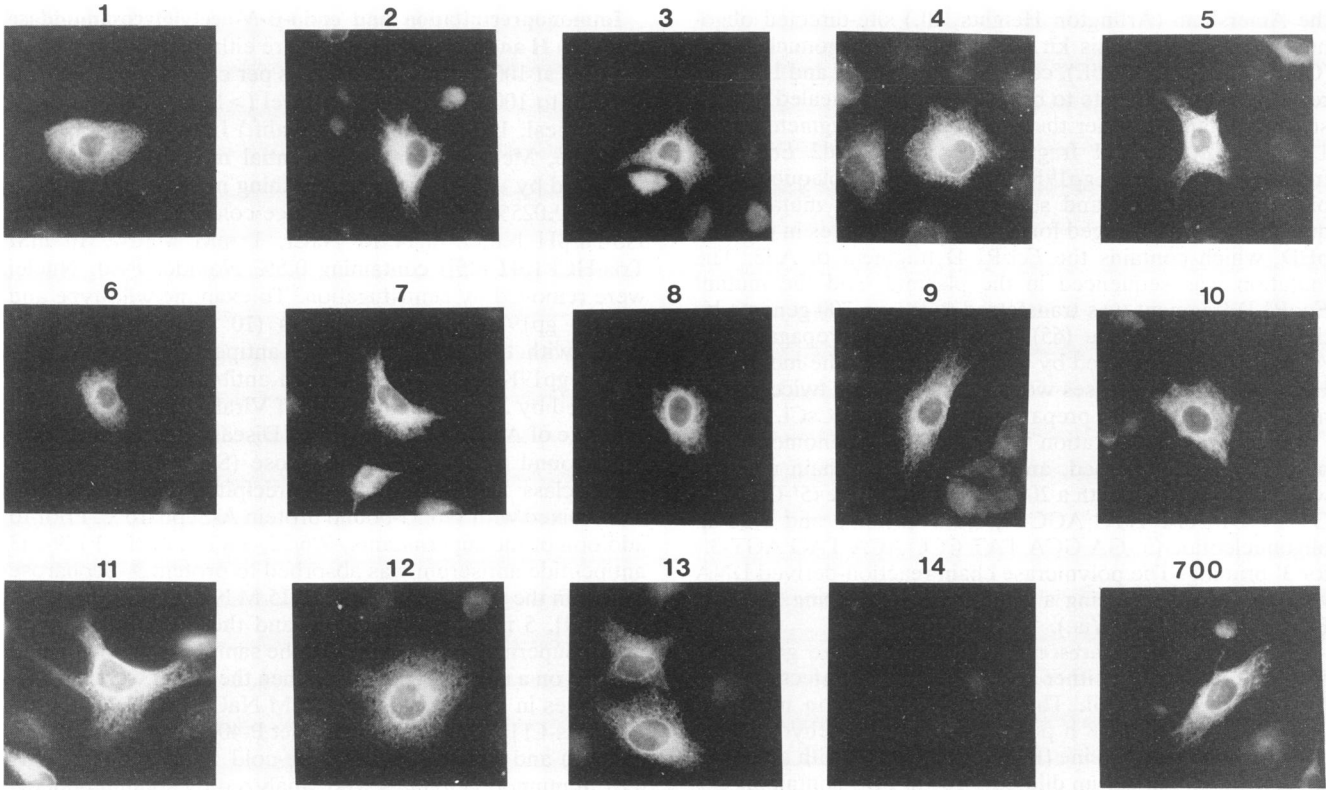

FIG. 2. Immunofluorescence of the gp19K wild-type and mutant proteins in human A549 cells. Cells were fixed and permeabilized at 8 h.p.i., incubated with the gp19K P129-143 antipeptide rabbit antiserum, and then exposed to fluorescein isothiocyanate-conjugated goat anti-rabbit antibody. Panels 1 to 14 correspond to viral mutants *dl*7019.1 to *dl*7019.14. In all cases except mutant *dl*7019.14, ER staining was evident and similar in pattern to the wild-type virus, *rec*700 (abbreviated 700).

RESULTS

E3-gp19K mutant proteins are stable and retained in the ER. gp19K of Ad2 is an ER membrane glycoprotein of 142 amino acids following cleavage of a 17-amino-acid N-terminal signal sequence (Fig. 1). A 15-amino-acid polar C-terminal sequence, which contains the signal for retention of the protein in the ER, is preceded in the protein by a transmembrane domain of about 20 amino acids. There are two Asn-linked glycosylation sites (44, 64) and five cysteine residues in the ER lumenal domain.

It has been previously shown that residues 1 to 96 in the ER lumenal domain of Ad2 gp19K are sufficient to bind to class I heavy chains (22, 51). To determine which sequences in the ER lumenal domain are required for binding to class I heavy chains, we created a series of in-frame deletions in this domain and analyzed their ability to bind to class I antigens. The deletions range from 12 to 36 bp (4 to 12 amino acids) (Fig. 1) and do not alter the signal sequence, Asn glycosylation sites, cysteine residues, the transmembrane domain, or the ER retention signal. The deletion in *dl*7019.13 (residues 102 to 107) is C terminal to the truncated gp19K protein fragment (residues 1 to 96) that is sufficient for binding to class I antigens. Mutant *dl*7019.14 is truncated approximately halfway into the ER lumenal domain by insertion of a dual stop codon. Mutant *dl*739 retains gp19K but deletes the upstream gene encoding the E3-6.7K protein (63). Considering that the 6.7K and gp19K genes are contiguous and translated from the same mRNAs, it is conceivable that 6.7K could play a role in the gp19K-class I complex (63). Mutant *dl*754 deletes the initiation codon for gp19K. *dl*754 also deletes the stop codon and C-terminal residues of 6.7K, which results in 6.7K having 28 new residues at its C terminus.

To ensure that the deletions had not altered the retention of gp19K in the ER, immunofluorescence studies were performed. As shown in Fig. 2, wild-type *rec*700 and all mutants (panels 1 to 13) with the exception of the negative control, *dl*7019.14 (panel 14), exhibited strong perinuclear staining along with staining of the reticular network extending throughout the cytoplasm, characteristic of the ER. We conclude that none of the deletions in the gp19K lumenal domain affects the retention of gp19K in the ER.

When examined by immunoprecipitation, the mutant proteins were equal in abundance to the wild-type protein encoded by *rec*700 (Fig. 3). Taken together with the results for immunofluorescence staining, this finding indicates that the mutant proteins are stable and expressed to levels equivalent to the wild-type level. As expected, gp19K was not obtained from *dl*7019.14. The deletion of the 6.7K gene in *dl*739 did not affect the level of gp19K.

For some of the proteins, unpredicted shifts in the migration pattern occurred; e.g., the *dl*7019.3 protein migrated more slowly than expected (Fig. 3). Each mutant gene was sequenced after the introduction of the mutation and prior to insertion into the viral genome. Polymerase chain reaction PCR isolation of the gene from each mutant viral genome and subsequent sequence analysis across the deletions confirmed the mutation within the viral genome; thus, any

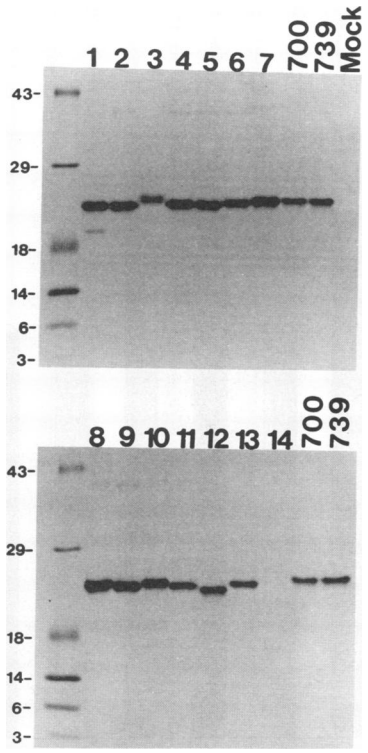

FIG. 3. Immunoprecipitation of wild-type and mutant gp19K proteins from human A549 cells. Infected cells were labeled from 4 to 8 h p.i. with 100 μCi of Trans^{35}S-label, and gp19K was immunoprecipitated with the P129-143 antipeptide antiserum. Lanes designated 1 to 14 correspond to mutants *dl*7019.1 to *dl*7019.14. Lanes 700 and 739 refer to *rec*700 and *dl*739, respectively. Sizes are indicated in kilodaltons.

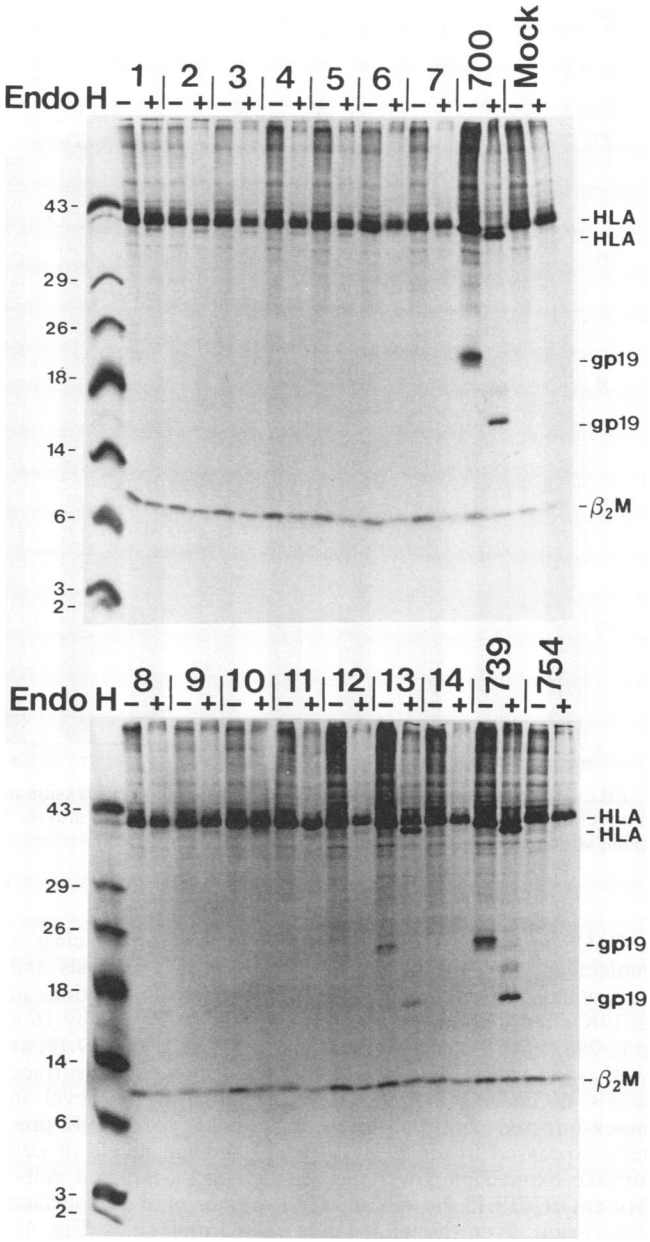

FIG. 4. gp19K-MHC class I coimmunoprecipitation and endo H sensitivity analysis in human A549 cells. Cells were labeled as described for Fig. 3, and MHC class I molecules were immunoprecipitated with W6/32, a monoclonal antibody specific to class I antigens HLA-A, -B, and -C. Immunoprecipitation mixtures were split, and one half was digested with endo H for 16 h. The presence (+) or absence (−) of endo H is depicted above the lane for each corresponding virus. HLA refers to the class I heavy chain. 700, *rec*700; 739, *dl*739. Sizes are indicated in kilodaltons.

aberrant migration of the mutant proteins may be due secondary structure formation or to altered ability to bind SDS.

gp19K-MHC class I coimmunoprecipitation and endo H sensitivity analysis. Monoclonal antibody W6/32 was used to immunoprecipitate class I antigens and examine their ability to coimmunoprecipitate the mutant gp19K proteins. As shown in Fig. 4, all deletions except that in *dl*7019.13 abrogated the ability of gp19K to bind class I molecules. Even in *dl*7019.13, binding to class I is reduced, as judged by the amount of gp19K coimmunoprecipitating relative to wild-type protein.

gp19K binding to class I heavy chains in the ER blocks their transport to the Golgi and, accordingly, blocks their subsequent terminal glycosylation (43). Endo H removes Asn-linked core sugar moieties lacking terminal glycosylation (43). Thus, endo H sensitivity is an assay for gp19K binding and retention of class I molecules in the ER. With wild-type *rec*700, endo H increased the electrophoretic mobility of the HLA heavy chain as well as gp19K (Fig. 4), indicating that these proteins had predominantly (HLA) or exclusively (gp19K) high-mannose oligosaccharides characteristic of glycoproteins in the ER. Similar results were obtained with *dl*7019.13 except that more heavy-chain molecules were resistant to endo H; this finding indicates that *dl*7019.13 may be partially defective in binding class I antigens. For all of the other gp19K mutants, the heavy chains were nearly completely resistant to endo H, indicating that these mutant gp19K proteins do not bind and retain class I antigens in the ER. These results suggest that the entire ER lumenal portion of the gp19K protein plays a role in class I heavy-chain binding.

As expected, gp19K was not obtained from mutant *dl*7019.14 or *dl*754. Deletion of the 6.7K gene in *dl*739 did not detectably abrogate the binding of gp19K to class I antigens or the sensitivity of class I antigens to endo H.

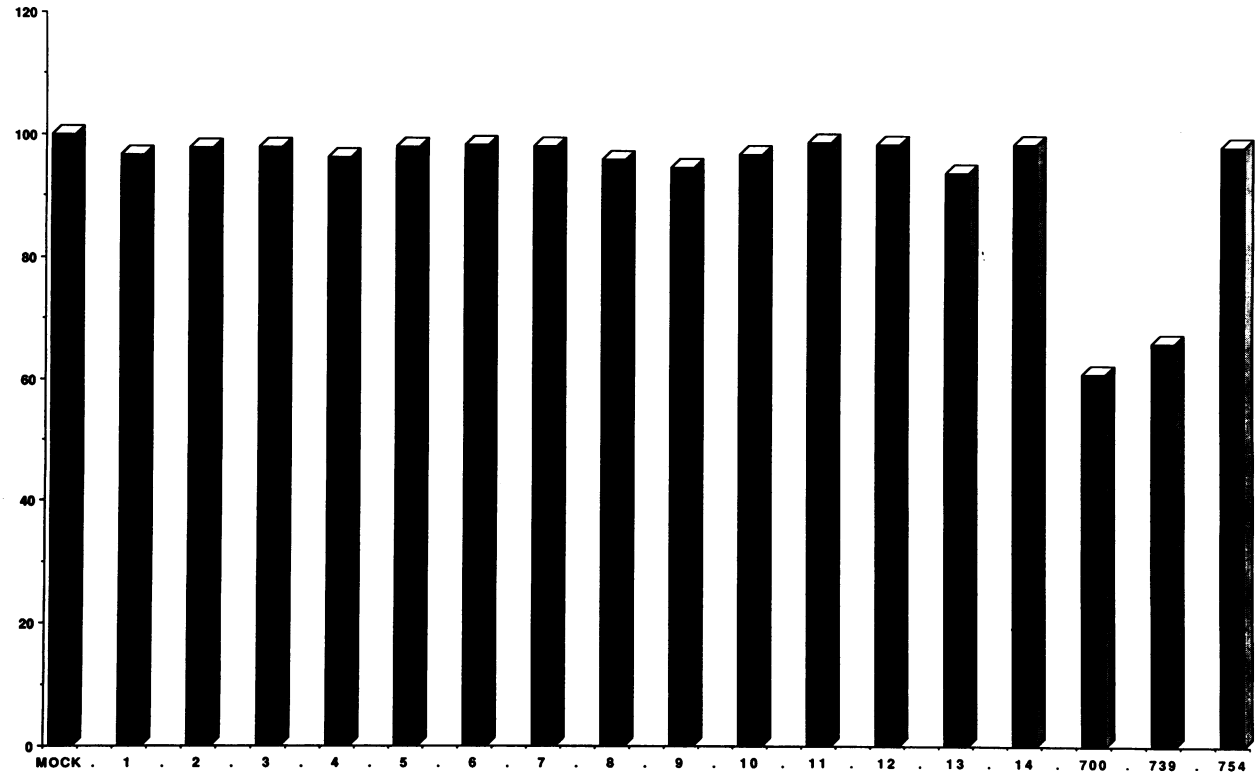

FIG. 5. FACS analysis of MHC class I cell surface expression in mutant- and wild-type-infected human 293 cells. Cells were exposed to monoclonal antibody W6/32 at 18 h p.i. Virus-infected cell surface levels of class I antigens are expressed relative to that of mock-infected cells, which was placed at 100%.

FACS analysis. The cell surface expression of class I molecules can be assayed directly by FACS analysis and serves as another means of testing whether the deletions in gp19K affect class I binding. As depicted in Fig. 5, by 16 h p.i., 293 cells infected with mutants *dl*7019.1 to *dl*7019.14, as well as the gp19K deletion mutant *dl*754, showed cell surface levels of class I expression comparable to the level in mock-infected controls. In contrast, wild-type gp19K protein expressed by *dl*739 and *rec*700 showed levels of cell surface expression lower than that of mock-infected cells. Mutant *dl*7019.13 did not show a lower level of cell surface expression, even though it binds class I molecules (Fig. 4). The inability to detect a shift in the fluorescence intensity in *dl*7019.13 may reflect a lack of ability to detect the reduced level of gp19K binding to class I antigens. (It should be noted that FACS analysis will detect class I molecules that were present on the cell surface prior to virus infection.)

E3-gp19K lumenal domain deletions abrogate suppression of CTL recognition of mutant-infected target cells. Experiments were also conducted to determine whether the gp19K mutant proteins could suppress CTL recognition of virus-infected cells. Polyclonal Ad5-specific CTL were prepared by immunization of either C57BL/10SnJ ($H\text{-}2^b$) or BALB/cJ ($H\text{-}2^d$) mice with Ad5 and then restimulating their splenocytes in vitro with SV40-transformed syngeneic cells infected with the Ad5 E3 deletion mutant *dl*327 (*dl*327 lacks gp19K and all other E3 proteins except 12.5K). In C57BL/10SnJ and BALB/cJ mice, we have shown that CTL activity against Ad-infected target cells is greatly diminished when gp19K is expressed by the virus used for infection of the target cell (55). However, target cells infected with viruses lacking gp19K are readily lysed by both $H\text{-}2^b$- and $H\text{-}2^d$-restricted CTL (55). As shown in Fig. 6, Ad5-specific CTL did not lyse target cells infected with wild-type *rec*700, as expected, but they did lyse target cells infected with any of the gp19K mutants (*dl*7019.1 to *dl*7019.14). Thus, by this assay, all deletions result in a nonfunctional gp19K; this group includes *dl*7019.13, whose gp19K retains some ability to bind class I antigens. These data suggest that the tertiary structure of the ER lumenal domain of gp19K has been altered by deletion such that it is impaired in the ability to associate with class I molecules or retain them in the ER.

Interaction of gp19K with the gp19K-specific monoclonal antibody Tw1.3 is abrogated by deletions within gp19K residues 3 to 82 but not by deletions within residues 84 to 107. To obtain evidence of whether any major tertiary structure had been altered in our mutant proteins, we attempted to immunoprecipitate the mutant proteins with monoclonal antibody Tw1.3, which is specific to gp19K (12). Monoclonal antibodies are often directed against epitopes consisting of noncontiguous amino acids; such monoclonal antibodies can be used to examine the tertiary structure of proteins. Tw1.3 appears to be directed against a noncontiguous epitope, because it did not react with gp19K in immunoblots (in assays in which the protein was denatured with SDS and reduced with β-mercaptoethanol) under conditions in which the P129-143 antipeptide antiserum readily detected gp19K (34). As shown in Fig. 7, Tw1.3 recognized gp19K with deletions within residues 84 to 107 (mutants *dl*7019.11, *dl*7019.12, and *dl*7019.13). In contrast, Tw1.3 did not recognize gp19K from any of the mutants whose deletions span

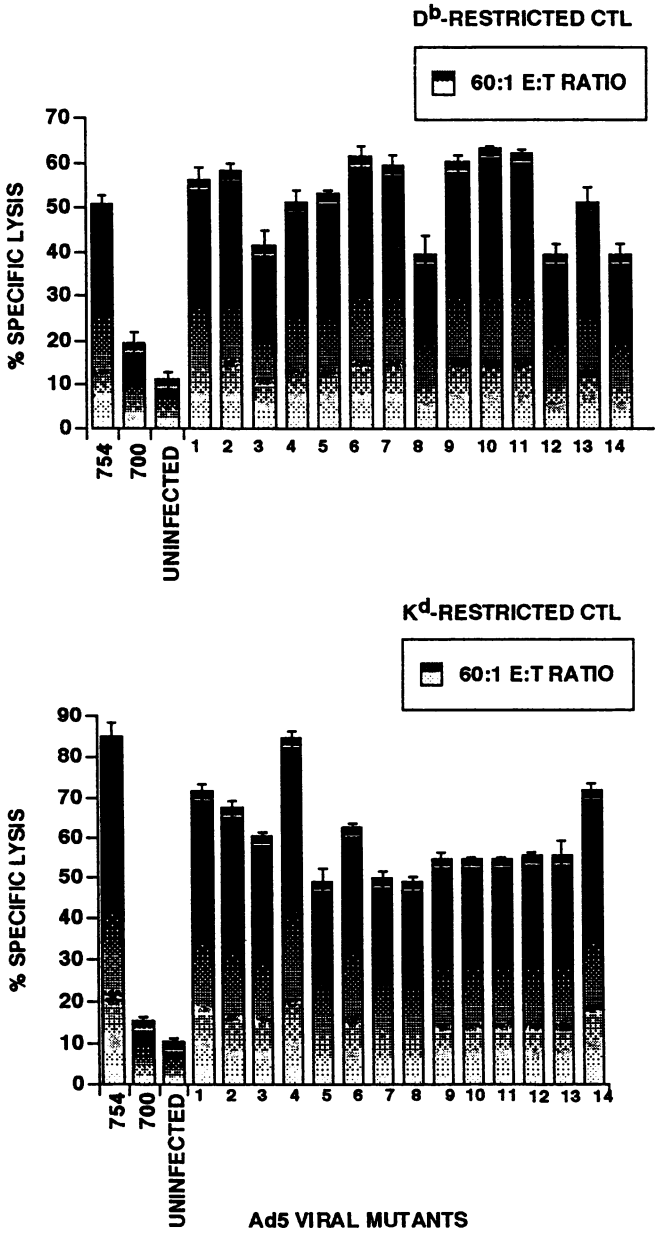

FIG. 6. CTL-mediated killing of virus-infected mouse target cells SV-BALB ($H\text{-}2^d$) and SV-B6KHA ($H\text{-}2^b$). Syngeneic mice were infected, and splenocytes were harvested and boosted for CTL activity as previously described (55). Wild-type-, mutant-, or mock-infected ^{51}Cr-labeled cell targets were assayed 16 to 18 h p.i. for CTL sensitivity. The graphs represent the averages of three experiments. 754, *dl*754; 700, *rec*700; E, effector; T, target.

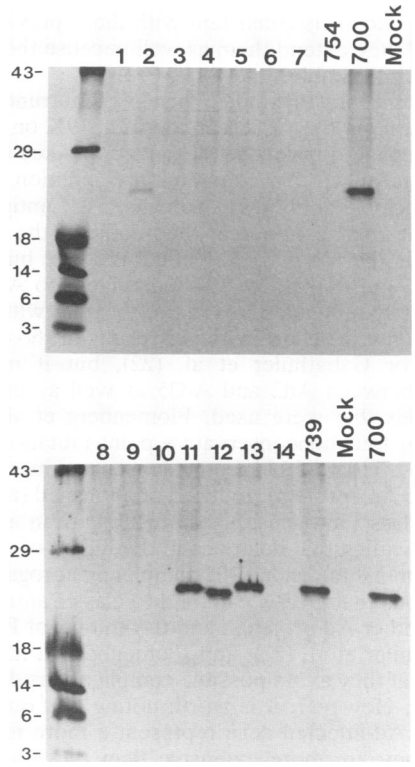

FIG. 7. Immunoprecipitation of wild-type and mutant gp19K proteins from infected A549 cells, using anti-gp19K monoclonal antibody Tw1.3. Conditions and designations are as described for Fig. 3.

the region from residues 3 to 82 (gp19K from *dl*7019.2 reacted very weakly).

These results are consistent with the proposal that the epitope for Tw1.3 consists of noncontiguous amino acids located within residues 3 to 82 of gp19K and that the deletions alter the tertiary structure of gp19K such that the epitope is destroyed and gp19K cannot react with the monoclonal antibody. The results further indicate that residues 84 to 107, deletions of which do not affect binding of gp19K to Tw1.3, are not part of the epitope.

DISCUSSION

The ER lumenal domain of the Ad2 E3-gp19K protein consists of approximately 107 amino acids. We have shown that nearly all deletions examined, ranging from 4 to 12 amino acids in the ER lumenal domain, compromise gp19K's ability to form a complex with MHC class I antigens, as measured by coimmunoprecipitation and endo H sensitivity, by FACS analysis for cell surface expression, and by prevention of cytolysis by Ad-specific CTL. The mutant proteins are retained in the ER and expressed to levels equivalent to that of wild-type gp19K, as determined by immunofluorescence as well as immunoprecipitation. Only gp19K from *dl*7019.13, which deletes amino acids 102 to 107 in gp19K, was able to form a complex with class I molecules, although less efficiently than wild-type gp19K could.

Pääbo et al. (51) showed that an Ad2 gp19K polypeptide truncated at residue 97 was able to bind to human class I antigens immobilized on agarose. Gabathuler et al. (22) reported that an Ad2 gp19K fragment that was also truncated at residue 97 could coimmunoprecipitate with a class I antigen heavy chain if the gp19K and heavy chain were cotranslated in vitro or coexpressed in insect cells from a baculovirus vector. The latter authors could not detect coimmunoprecipitation of the truncated gp19K polypeptide with class I heavy chain from transfected human cells, and they suggested that this may be because the truncated polypeptide is unstable or is secreted from the cell. Our finding that *dl*7019.13 (residues 102 to 107 deleted) is able to bind class I molecules, as determined by coimmunoprecipitation from infected cells as well as sensitivity of class I

antigens to endo H, is consistent with these previous observations, and they extend them as well because the *dl*7019.13 gp19K protein is stable.

Flomenberg et al. (16) studied the effects of truncations and point mutations in the Ad35 version of gp19K on binding to class I antigens in transiently transfected monkey cells; binding was determined by coimmunoprecipitation using the W6/32 monoclonal antibody to human class I antigens. They reported that a truncation near the middle of the transmembrane domain did not markedly affect binding, but a truncation at Ad35 gp19K residue 127 (equivalent to Ad2 residue 106) completely abrogated binding. The result with the latter truncation is not in accord with our results or those of Pääbo et al. (51) or Gabathuler et al. (22), but it may reflect differences between Ad2 and Ad35 as well as the different methodologies that were used. Flomenberg et al. (16) also reported that four nonconservative point mutations, at residues 104, 107, 110, and 114 (equivalent to residues 81, 84, 87, and 91 in the Ad2 protein), reduced or abolished (residue 110) binding to class I antigens. These results are in accord with our data indicating that deletions in the region of Ad2 gp19K that is N terminal to residue 102 completely abrogate binding.

It is obvious that gp19K can bind to class I antigens in the absence of other Ad proteins, and the studies of Pääbo et al. (51), Gabathuler et al. (22), and Flomenberg et al. (16) have the virtue that they avoid possible complications due to other Ad proteins. However, it is worth noting that our deletions analyzed in Ad-infected cells represent a more natural context, that they are more extensive than the other mutants reported, and that they have allowed us to conclude that the deletions in gp19K have abrogated the ability of gp19K to prevent cytolysis by Ad-specific CTL. It also should be noted that we still cannot exclude the possibilities that other Ad-encoded or Ad-induced and cell-encoded proteins could be involved in inhibiting presentation of Ad-specific antigens to T cells and that we simply cannot detect the function of these hypothetical proteins in our analyses.

Although it is clear that the ER lumenal domain is sufficient for binding to class I antigens, there is anecdotal evidence that the transmembrane domain and even the cytoplasmic domain may increase the avidity of binding (22, 48, 51). This would not be surprising considering that class I heavy chain and gp19K are both members of the immunoglobulin superfamily (10) and that they must interact with each other while anchored in the membrane.

Although our results indicate that virtually the entire ER lumenal domain of gp19K is required for maximal binding to class I antigens, for several reasons we propose that the ER lumenal domain actually consists of three subdomains (Fig. 8). We will refer to these subdomains as the ER lumenal variable domain (ERL-VD; residues 1 to ca. 77 through 83), the ER lumenal conserved domain (ERL-CD; residues ca. 84 through 98), and the ER lumenal spacer domain (ERL-SD; residues ca. 99 through 107).

First, as initially noticed by Flomenberg et al. (17) and as indicated in Fig. 1, the ER lumenal domain of gp19K has a region that is conserved (residues 79 through 98 in the Ad2 protein) among Ad serotypes in different subgroups and another region (residues 1 through 78 in the Ad2 protein) that is variable among serotypes in different subgroups. Comparison of Ad2 (subgroup C) with Ad7 (subgroup B), for example, shows that gp19K is 75% identical in the conserved region but only about 22% identical in the variable region.

Second, neither Tw1.3 nor class I heavy chain reacted with gp19K from mutants *dl*7019.1 through *dl*7019.10; this finding is consistent with the idea that the region deleted in these mutants represents a distinct functional and structural unit (the ERL-VD) such that the deletions alter the tertiary structure of this unit and abrogate binding to Tw1.3 (which has a noncontiguous epitope) or class I heavy chain. Stated another way, the binding site in gp19K for class I heavy chain is not a simple linear sequence but rather is embodied in the tertiary structure of the ERL-VD of gp19K. gp19K from mutants *dl*7019.11, *dl*7019.12, and *dl*7019.13 reacted with Tw1.3, which is strong evidence that the region deleted in these mutants (residues 84 through 107) is distinct from the ERL-VD that is deleted in the other mutants. The sequences deleted in *dl*7019.10 (residues 77 through 82) apparently form a hinge region between the ERL-VD and the ERL-CD. The sequences deleted in *dl*7019.11 and *dl*7019.12 (the ERL-CD; residues 84 through 100) appear to be distinct from those deleted in *dl*7019.13 (the ERL-SD; residues 102 through 107). With the former mutants, the deleted sequences are conserved and the mutant proteins do not bind class I antigens. With *dl*7019.13, the deleted sequences are not conserved and the mutated protein binds class I antigens. Recent results with Cys-to-Ser mutations in the ER lumenal domain are also consistent with the existence of three subdomains (34).

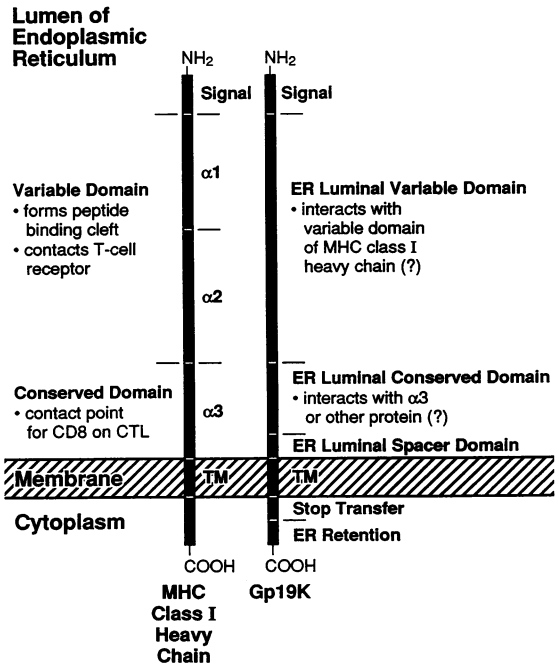

FIG. 8. Schematic depicting the functional domains in gp19K and the class I antigen heavy (α) chain. TM, transmembrane domain.

In summary, gp19K clearly has four very distinct domains: the signal sequence which is cleaved, the ER lumenal domain, the transmembrane domain, and the cytoplasmic domain (Fig. 8). We propose that the ER lumenal domain consists of three subdomains, the ERL-VD, the ERL-CD, and the ERL-SD. The cytoplasmic region also probably has two subdomains, one that consists of about 6 to 10 residues at the extreme C terminus which contains the ER retention signal (12, 38, 48) and another that is the highly positively charged region at residues 128 to 133 which stops translocation of the protein through the membrane and which anchors (together with the transmembrane domain) the protein in the membrane.

What could be the functions of these proposed ER lumenal subdomains? Crystallographic studies conducted on HLA-A2.1 and HLA-Aw68.1 depict a heavy (α) chain that is highly folded and contains noncovalently bound β_2-microglobulin. There are regions that are conserved in the heavy chain (α3 domain, transmembrane domain, and cytoplasmic domain) and regions that are variable (α1 and α2 domains) (reviewed in reference 3). Twenty positions of high variability have been defined among 39 HLA-A, -B, and -C sequences, and the majority of the variable positions map to the α1 and α2 domains, which make up the antigenic peptide binding cleft and also interact with variable T-cell receptors (3–5, 53). The α3 domain and β_2-microglobulin are members of the immunoglobulin superfamily, and gp19K has been proposed to be a member of this family as well (10). We suggest that the ERL-VD of gp19K may interact with the variable α1 and α2 domains of class I heavy chain (Fig. 8). The Ad2 or Ad5 gp19K proteins differ in the ability to form a complex with class I molecules, complexing well with mouse K^d, L^d, and D^b, modestly with D^d, and not at all with D^k, K^k, and K^b (9, 13, 55, 59). Also, Ad2 gp19K binds more than twice as well to HLA-A2 as to HLA-B7 (56). The affinity differences seen between gp19K and different class I molecules may be a measure of the ability of the gp19K ERL-VD to interact with the variable sequences within α1 and α2. We further suggest that the ERL-CD of gp19K may interact with a highly conserved protein such as the α3 domain of class I heavy chain. The ERL-SD of gp19K may play a structural role, allowing the ERL-VD and ERL-CD of gp19K to extend out from the membrane and be presented correctly to class I heavy chain.

Another possible function for gp19K is that it displaces the class I heavy chain from the 88K putative molecular chaperone that interacts with heavy chain before it oligomerizes and interacts with β_2-microglobulin (23). It seems unlikely that gp19K inhibits peptide binding to class I molecules, considering that gp19K lacking only the ER retention signal did not inhibit antigen presentation to CTL (12).

ACKNOWLEDGMENTS

We thank L. Wold for technical assistance, J. Cox for monoclonal antibody Tw1.3, and T. Shenk for *dl*327.

This work was supported by postdoctoral fellowships F32 CA08896 to T.W.H. and F32 AI08232 to R.A.T. and by grant RO1 CA24710 to W.S.M.W., all from the National Institutes of Health, and by grant VM-39 to L.R.G. from the American Cancer Society.

REFERENCES

1. **Andersson, M., A. McMichael, and P. A. Peterson.** 1987. Reduced allorecognition of adenovirus-2 infected cells. J. Immunol. **138**:3960–3966.
2. **Andersson, M., S. Pääbo, T. Nilsson, and P. A. Peterson.** 1985. Impaired intracellular transport of class I MHC antigens as a possible means for adenoviruses to evade immune surveillance. Cell **43**:215–222.
3. **Bjorkman, P. J., and P. Parham.** 1990. Structure, function, and diversity of class I major histocompatibility complex molecules. Annu. Rev. Biochem. **59**:253–288.
4. **Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger, and D. C. Wiley.** 1987. Structure of the human class I histocompatibility antigen, HLA-A2. Nature (London) **329**: 506–512.
5. **Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger, and D. C. Wiley.** 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature (London) **329**:512–518.
6. **Brady, H. A., A. Scaria, and W. S. M. Wold.** 1992. Map of *cis*-acting sequences that determine alternative pre-mRNA processing in the E3 complex transcription unit of adenovirus. J. Virol. **66**:5914–5923.
7. **Burgert, H.-G., and S. Kvist.** 1985. An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. Cell **41**:987–997.
8. **Burgert, H.-G., and S. Kvist.** 1987. The E3/19K protein of adenovirus type 2 binds to the domains of histocompatibility antigens required for CTL recognition. EMBO J. **6**:2019–2026.
9. **Burgert, H.-G., J. L. Maryanski, and S. Kvist.** 1987. "E3/19K" protein of adenovirus type 2 inhibits lysis of cytolytic T lymphocytes by blocking cell-surface expression of histocompatibility class I antigens. Proc. Natl. Acad. Sci. USA **84**:1356–1360.
10. **Chatterjee, D., and J. V. Maizel, Jr.** 1984. Homology of adenoviral E3 glycoprotein with HLA-DR heavy chain. Proc. Natl. Acad. Sci. USA **81**:6039–6043.
11. **Cladaras, C., and W. S. M. Wold.** 1985. DNA sequence of the E3 transcription unit of adenovirus 5. Virology **140**:28–43.
12. **Cox, J. H., J. R. Bennink, and J. W. Yewdell.** 1991. Retention of adenovirus E19 glycoprotein in the endoplasmic reticulum is essential to its ability to block antigen presentation. J. Exp. Med. **174**:1629–1637.
13. **Cox, J. H., J. W. Yewdell, L. C. Eisenlohr, P. R. Johnson, and J. R. Bennink.** 1990. Antigen presentation requires transport of MHC class I molecules from the endoplasmic reticulum. Science **247**:715–718.
14. **Dähloff, B., M. Wallin, and S. Kvist.** 1991. The endoplasmic reticulum retention signal of the E3/19K protein of adenovirus-2 is microtubule binding. J. Biol. Chem. **266**:1804–1808.
15. **Falk, K., O. Rötzschke, S. Stevanović, G. Jung, and H.-G. Rammensee.** 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature (London) **351**:290–296.
16. **Flomenberg, P., J. Szmulewicz, E. Gutierrez, and H. Lupatkin.** 1992. Role of the adenovirus E3-19K conserved region in binding major histocompatibility complex class I molecules. J. Virol. **66**:4778–4783.
17. **Flomenberg, P. R., M. Chen, and M. S. Horwitz.** 1988. Sequence and genetic organization of adenovirus type 35 early region 3. J. Virol. **62**:4431–4437.
18. **Fox, J. P., L. E. Hall, and M. K. Cooney.** 1977. The Seattle virus watch. VII. Observations of adenovirus infections. Am. J. Epidemiol. **105**:362–386.
19. **Fremont, D. H., M. Matsumura, E. A. Stura, P. A. Peterson, and I. A. Wilson.** 1992. Crystal structures of two viral peptides in complex with murine MHC class I H-$2K^b$. Science **257**:919–927.
20. **Friedman, D. J., and R. P. Ricciardi.** 1988. Adenovirus type 12 E1A gene represses accumulation of MHC class I mRNAs at the level of transcription. Virology **165**:303–305.
21. **Gabathuler, R., and S. Kvist.** 1990. The endoplasmic reticulum retention signal of the E3/19K protein of adenovirus type 2 consists of three separate amino acid segments at the carboxy terminus. J. Cell Biol. **111**:1803–1810.
22. **Gabathuler, R., F. Levy, and S. Kvist.** 1990. Requirements for the association of adenovirus type 2 E3/19K wild-type and mutant proteins with HLA antigens. J. Virol. **64**:3679–3685.
23. **Galvin, K., S. Krishna, F. Ponchel, M. Frohlich, D. E. Cummings, R. Carlson, J. R. Wands, K. J. Isselbacher, S. Pillai, and M. Ozturk.** 1992. The major histocompatibility complex class I antigen-binding protein p88 is the product of calnexin gene. Proc. Natl. Acad. Sci. USA **89**:8452–8456.
24. **Ginsberg, H. S., U. Lundholm-Beauchamp, R. L. Horstwood, B. Pernis, W. S. M. Wold, R. M. Chanock, and G. A. Prince.** 1989. Role of early region 3 (E3) in pathogenesis of adenovirus disease. Proc. Natl. Acad. Sci. USA **86**:3823–3827.
25. **Ginsberg, H. S., L. L. Moldawer, P. B. Sehgal, M. Redington, P. L. Kilian, R. M. Chanock, and G. A. Prince.** 1991. A mouse model for investigating the molecular pathogenesis of adenovirus pneumonia. Proc. Natl. Acad. Sci. USA **88**:1651–1655.
26. **Gooding, L. R.** 1992. Virus proteins that counteract host immune defenses. Cell **71**:5–7.
27. **Gooding, L. R., L. W. Elmore, A. E. Tollefson, H. A. Brady, and W. S. M. Wold.** 1988. A 14,700 MW protein from the E3 region of adenovirus inhibits cytolysis by tumor necrosis factor.

Cell **53**:341–346.
28. **Gooding, L. R., T. S. Ranheim, A. E. Tollefson, L. Aquino, P. Duerksen-Hughes, T. M. Horton, and W. S. M. Wold.** 1991. The 10,400- and 14,500-dalton proteins encoded by region E3 of adenovirus function together to protect many but not all mouse cell lines against lysis by tumor necrosis factor. J. Virol. **65**:4114–4123.
29. **Gooding, L. R., I. O. Sofola, A. E. Tollefson, P. Duerksen-Hughes, and W. S. M. Wold.** 1990. The adenovirus E3-14.7K protein is a general inhibitor of tumor necrosis factor-mediated cytolysis. J. Immunol. **145**:3080–3086.
30. **Gooding, L. R., and W. S. M. Wold.** 1990. Molecular mechanisms by which adenoviruses counteract antiviral immune defenses. Crit. Rev. Immunol. **10**:53–71.
31. **Graham, F. L., J. Smiley, W. C. Russell, and R. Nairn.** 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. **36**:59–72.
32. **Green, M., and W. S. M. Wold.** 1979. Preparation of human adenoviruses. Methods Enzymol. **1979**:425–435.
33. **Hérissé, J., G. Courtois, and F. Galibert.** 1980. Nucleotide sequence of the EcoRI D fragment of adenovirus 2 genome. Nucleic Acids Res. **8**:2173–2192.
34. **Hermiston, T. W., and W. S. M. Wold.** Unpublished results.
35. **Horton, T. M., T. S. Ranheim, L. Aquino, D. I. Kusher, S. K. Saha, C. F. Ware, W. S. M. Wold, and L. R. Gooding.** 1991. Adenovirus E3 14.7K protein functions in the absence of other adenovirus proteins to protect transfected cells from tumor necrosis factor cytolysis. J. Virol. **65**:2629–2639.
36. **Horton, T. M., A. E. Tollefson, W. S. M. Wold, and L. R. Gooding.** 1990. A protein serologically and functionally related to group C E3 14,700-kilodalton protein is found in multiple adenovirus serotypes. J. Virol. **64**:1250–1255.
37. **Hunt, D. F., R. A. Henderson, J. Shabanowitz, K. Sakaguchi, H. Michel, N. Sevilir, A. L. Cox, E. Apella, and V. H. Engelhard.** 1992. Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. Science **255**:1261–1263.
38. **Jackson, M. R., T. Nilsson, and P. A. Peterson.** 1990. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. EMBO J. **9**:3153–3162.
39. **Jardetzky, T. S., W. S. Lane, R. A. Robinson, D. R. Madden, and D. C. Wiley.** 1991. Identification of self peptides bound to purified HLA-B27. Nature (London) **353**:326–329.
40. **Jefferies, W. A., and H.-G. Burgert.** 1990. E3/19K from adenovirus 2 is an immunosubversive protein that binds to a structural motif regulating the intracellular transport of major histocompatibility complex class I proteins. J. Exp. Med. **172**:1653–1664.
41. **Kämpe, O., D. Bellgrau, U. Hammerling, P. Lind, S. Pääbo, L. Severinsson, and P. A. Peterson.** 1983. Complex formation of class I transplantation antigens and a viral glycoprotein. J. Biol. Chem. **258**:10594–10598.
42. **Kelly, T. J., Jr., and A. M. Lewis, Jr.** 1973. Use of nondefective adenovirus-simian virus 40 hybrids for mapping the simian virus 40 genome. J. Virol. **12**:643–652.
43. **Kornfeld, R., and S. Kornfeld.** 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. **54**:631–664.
44. **Kornfeld, R., and W. S. M. Wold.** 1981. Structures of the oligosaccharides of the glycoprotein coded by early region E3 of adenovirus 2. J. Virol. **40**:440–449.
45. **Matsumura, M., D. H. Fremont, P. A. Peterson, and I. A. Wilson.** 1992. Emerging principles for the recognition of peptide antigens by MHC class I molecules. Science **257**:927–934.
46. **Mei, Y.-F., and G. Wadell.** 1992. The nucleotide sequence of adenovirus type 11 early 3 region: comparison of genome type Ad11p and Ad11a. Virology **191**:125–133.
47. **Morin, J. E., M. D. Lubeck, J. E. Barton, A. J. Conley, A. R. Davis, and P. P. Hung.** 1987. Recombinant adenovirus induces antibody response to hepatitis B virus surface antigen in hamsters. Proc. Natl. Acad. Sci. USA **84**:4626–4630.
48. **Pääbo, S., B. M. Bhat, W. S. M. Wold, and P. A. Peterson.** 1987. A short sequence in the COOH-terminus makes an adenovirus membrane glycoprotein a resident of the endoplasmic reticulum. Cell **50**:311–317.
49. **Pääbo, S., T. Nilsson, and P. A. Peterson.** 1986. Adenoviruses of subgenera B, C, D, and E modulate cell-surface expression of major histocompatibility complex class I antigens. Proc. Natl. Acad. Sci. USA **83**:9665–9669.
50. **Pääbo, S., L. Severinsson, M. Andersson, I. Martens, T. Nilsson, and P. A. Peterson.** 1989. Adenovirus proteins and MHC expression. Adv. Cancer Res. **52**:151–163.
51. **Pääbo, S., F. Weber, T. Nilsson, W. Schaffner, and P. A. Peterson.** 1986. Structural and functional dissection of an MHC class I antigen-binding adenovirus glycoprotein. EMBO J. **5**:1921–1927.
52. **Pacini, D. L., E. J. Dubovi, and W. A. Clyde, Jr.** 1984. A new animal model for human respiratory tract disease due to adenovirus. J. Infect. Dis. **150**:92–97.
53. **Parham, P., L. E. Lomen, D. A. Lawlor, J. P. Way, N. Holmes, H. L. Coppin, R. D. Salter, A. M. Wan, and P. D. Ennis.** 1988. Nature of polymorphism in HLA-A, -B, and -C molecules. Proc. Natl. Acad. Sci. USA **85**:4005–4009.
54. **Ranheim, T. S., J. Shisler, T. M. Horton, L. J. Wold, L. R. Gooding, and W. S. M. Wold.** 1993. Characterization of mutants within the gene for the adenovirus E3 14.7-kilodalton protein which prevents cytolysis by tumor necrosis factor. J. Virol. **67**:2159–2167.
55. **Rawle, F. C., A. E. Tollefson, W. S. M. Wold, and L. R. Gooding.** 1989. Mouse anti-adenovirus cytotoxic T lymphocytes. Inhibition of lysis by E3 gp19K but not E3 14.7K. J. Immunol. **143**:2031–2037.
56. **Severinsson, L., I. Martens, and P. A. Peterson.** 1986. Differential association between two human MHC class I antigens and an adenoviral glycoprotein. J. Immunol. **137**:1003–1009.
57. **Shemesh, J., R. Rotem-Yehudar, and R. Ehrlich.** 1991. Transcriptional and posttranscriptional regulation of class I major histocompatibility complex genes following transformation with human adenoviruses. J. Virol. **65**:5544–5548.
58. **Signäs, C., G. Akusjärvi, and U. Pettersson.** 1986. Region E3 of human adenoviruses; differences between the oncogenic adenovirus-3 and the non-oncogenic adenovirus-2. Gene **50**:173–184.
59. **Tanaka, Y., and S. S. Tevethia.** 1988. Differential effect of adenovirus 2 E3/19K glycoprotein on the expression of H-2Kb and H-2Db class I antigens and H-2Kb- and H-2Db-restricted SV40-specific CTL-mediated lysis. Virology **165**:357–366.
60. **Thimmappaya, B., C. Weinberger, R. J. Schneider, and T. Shenk.** 1982. Adenovirus VAI RNA is required for efficient translation of viral mRNAs at late times after infection. Cell **31**:543–551.
61. **Vaessen, R. T. M. J., A. Houweling, and A. S. van der Eb.** 1987. Post transcriptional control of class I MHC mRNA expression in adenovirus 12-transformed cells. Science **235**:1486–1488.
62. **Van Bleck, G. M., and S. G. Nathenson.** 1990. Isolation of an endogenously processed immunodominant viral peptide for the class I H-2K^b molecule. Nature (London) **348**:213–216.
63. **Wilson-Rawls, J., S. K. Saha, P. Krajcsi, A. E. Tollefson, L. R. Gooding, and W. S. M. Wold.** 1990. A 6700 MW membrane protein is encoded by region E3 of adenovirus type 2. Virology **178**:204–212.
64. **Wold, W. S. M., C. Cladaras, S. L. Deutscher, and Q. S. Kapoor.** 1985. The 19-kDa glycoprotein coded by region E3 of adenovirus: purification, characterization, and structural analysis. J. Biol. Chem. **260**:2424–2431.
65. **Wold, W. S. M., S. L. Deutscher, N. Takemori, B. M. Bhat, and S. C. Magie.** 1986. Evidence that AGUAUAUGA and CCAA GAUGA initiate translation in the same mRNA in region E3 of adenovirus. Virology **148**:168–180.
66. **Wold, W. S. M., and L. R. Gooding.** 1989. Adenovirus region E3 proteins that prevent cytolysis by cytotoxic T cells and tumor necrosis factor. Mol. Biol. Med. **6**:433–452.
67. **Wold, W. S. M., and L. R. Gooding.** 1991. Unique adenovirus proteins that may allow the virus to evade the cytotoxic T-lymphocyte and macrophage/tumor necrosis factor branches of the immune system. Life Sci. Adv. **10**:89–99.
68. **Wold, W. S. M., and L. R. Gooding.** 1991. Region E3 of adenovirus: a cassette of genes involved in host immunosurveillance and virus-cell interactions. Virology **184**:1–8.