JOURNAL OF VIROLOGY, May 1996, p. 2832–2841 Vol. 70, No. 5
0022-538X/96/$04.00+0

Early Region 3 of Adenovirus Type 19 (Subgroup D) Encodes an HLA-Binding Protein Distinct from That of Subgroups B and C

FRANÇOIS DERYCKERE AND HANS-GERHARD BURGERT*

Hans-Spemann-Laboratorium, Max-Planck-Institut für Immunbiologie, D-79108 Freiburg, Germany

Received 23 October 1995/Accepted 31 January 1996

Early region 3 (E3) of human adenoviruses (Ads) codes for proteins that appear to control viral interactions with the host. For example, the most abundant E3 protein, E3/19K, inhibits the transport of newly synthesized class I major histocompatibility molecules to the cell surface, thereby interfering with antigen presentation. So far, the E3 regions of Ad subgroups A, B, C, and F have been characterized. We have cloned the E3A region of Ad type 19a (Ad19a), which belongs to the largest subgroup, D, and causes epidemic keratoconjunctivitis in humans. The sequence reveals five open reading frames (ORFs) with the potential to encode the Ad19 equivalent of pVIII, as well as proteins 12.2K, 16.2K, and 18.6K. The last ORF predicts a novel 49K protein which has no counterpart in other subgroups. Both the sequence and the overall organization of the E3 region from Ad19a shows a closer relationship to group B than to group C Ads. The 18.6K ORF represents the Ad19 homolog of the Ad2 E3/19K protein. By using 293 cells stably transfected with the Ad19a E3A region, we showed by immunoprecipitation, pulse-chase experiments, and fluorescence-activated cell sorter analysis that the Ad19 E3/19K protein binds to and prevents the transport of major histocompatibility complex molecules to the cell surface. The similar but distinct functional activity of the Ad19 E3/19K protein, combined with the new sequence which differs from those of subgroup B and C proteins, allows a more precise definition of amino acids essential for HLA binding.

The 47 different serotypes of human adenoviruses (Ads) cause a variety of diseases and are classified in six different subgroups, A to F. Subgroup F typically causes acute gastroenteritis in infants and young children, while the most prevalent subtypes of subgroups B and C cause mild to severe upper and lower respiratory tract diseases (24, 43). Some members of the largest subgroup, D (Ad type 8 [Ad8], Ad19, and Ad37), which contains more than half of all known subtypes, are associated with a severe eye disease called epidemic keratoconjunctivitis (24, 45).

Following the acute disease phase, a proportion of people develop persistent infections which may last months or even years (17, 24, 43). This phenomenon may be related to early transcription unit 3 (E3) of Ads. Although this region is dispensable for virus replication in tissue culture cells, it is present in all human Ad subtypes (9, 11, 16, 22, 32, 33, 40, 42). This suggests an important role during pathogenesis in vivo. In support of this idea, E3 proteins 14.7K and/or 10.4K and 14.5K protect infected cells from lysis by tumor necrosis factor alpha (TNF-α; reviewed in reference 48), while the E3/19K protein inhibits the cytolytic attack by cytotoxic T cells (1, 8, 10, 37).

The organization of the E3 region is distinct for each subgroup. Common to all E3 regions analyzed so far (subgroups A to C and F) are open reading frames (ORFs) for E3 proteins 10.4K, 14.5K, and 14.7K (9, 11, 40, 42), while an E3/19K-like protein was identified in subgroups B and C but not in subgroups A and F. Conversely, unique ORFs exist for each subgroup. Subgroup B has two unique 20.1K and 20.5K ORFs downstream of the E3/19K gene, as well as the capacity to encode a 16-kDa protein (16, 40), while subgroup C uniquely encodes 6.7- and 11.6-kDa proteins (9, 22; see Fig. 2). Thus, the composition of genes within the E3 region differs among subgroups; this may relate to the different disease patterns of the viruses.

The E3/19K protein interacts specifically with class I major histocompatibility (MHC) antigens in the rough endoplasmic reticulum (ER) and interferes with transport of newly synthesized MHC molecules to the cell surface (2, 6; reviewed in reference 5). Consequently, T-cell recognition of E3/19K$^+$ target cells is markedly reduced (1, 8, 10, 37). The E3/19K-like proteins are type I transmembrane glycoproteins with a 12- to 15-amino-acid-long cytoplasmic tail and a luminal portion comprising ~106 (±4) amino acids which is N glycosylated at variable positions (23, 39). Functionally, the E3/19K-like proteins are composed of two modules (5): an MHC antigen-binding module within the luminal portion (14, 19, 23, 34, 39) is combined with a structurally separated ER retrieval signal at the C terminus of the cytoplasmic tail (KKXX or KXKXX; 18, 25, 26).

Surprisingly, the overall amino acid sequence homology of E3/19K-like proteins is poor (identity of only ~25%; 23, 39). Nevertheless, the ability to inhibit the transport of MHC antigens is conserved (33). Therefore, the question of which amino acids of the protein are essential for HLA binding arises. Complex formation with HLA appears to require a certain tertiary structure (14, 23, 39) which depends in the Ad2 protein upon two intramolecular disulfide bonds formed between conserved cysteines (39). Other conserved amino acids are found dispersed throughout the luminal domain and are enriched in a stretch in front of the transmembrane segment.

The present study was performed to investigate the organization of the E3 region of subgroup D Ads and to determine the structure of the protein analogous to E3/19K. Cloning and sequencing of the E3A region of Ad19a revealed an E3/19K protein distinct from those of subgroups B and C. This allowed better identification of conserved residues critical for binding

* Corresponding author. Mailing address: Hans-Spemann-Laboratorium, Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-79108 Freiburg, Germany. Phone: (49)-761-5108487. Fax: (49)-761-5108468.

of MHC molecules. In addition, a unique ORF was identified that predicts a large and highly glycosylated transmembrane protein which may be unique for Ads causing epidemic keratoconjunctivitis.

MATERIALS AND METHODS

Cloning of the E3 region of Ad19. Ad19a (46), a kind gift of G. Wadell (University of Umea, Umea, Sweden), was grown in HeLa cells and CsCl purified by standard procedures (36). The virus contained in the CsCl band was diluted with TE buffer (10 mM Tris [pH 7.6], 1 mM EDTA) and pelleted by ultracentrifugation at 90,000 × *g* for 1 h; the virus pellet was resuspended in 500 μl of proteinase K buffer (100 mM Tris [pH 7.6], 150 mM NaCl, 12.5 mM EDTA, 1% sodium dodecyl sulfate [SDS]) and digested with 0.5 mg of proteinase K per ml for 1 h at 37°C; the viral DNA was extracted once with an equal volume of phenol-chloroform and ethanol precipitated. All molecular biology techniques were performed as described by Sambrook et al. (38). The 5′ part of the Ad19 E3 region was PCR amplified by using the κ2 primer (CTGAGTGTACCAG GAAAATCCC) and the C5SP primer (GAAAAGGGGTTGAAGTAAAC). The PCR product from the Ad19 E3 region was labeled by random priming with the Prime-a-Gene kit (Promega, Heidelberg, Germany) and used as a probe to identify, by Southern blotting analysis, restriction fragments containing the Ad19 E3 region. This allowed the subcloning of a 2.4-kb-long *Sst*II-*Xba*I fragment (see Fig. 2) into pBluescript KSII$^+$ (Stratagene, Heidelberg, Germany). Preliminary sequence data showed that the 3′ portion of the E3 region was missing. After further restriction analysis, a 1.6-kb-long *Eco*RV fragment overlapping the *Sst*II-*Xba*I fragment and containing the remaining part of the E3A unit was subcloned. Since the two subclones had the same orientation within pBluescript KS, the *Xba*I site shared by the two subclones was used in combination with the vector *Hin*dIII site to assemble the missing part (*Xba*I-*Hin*dIII) from the *Eco*RV subclone into the *Sst*II-*Xba*I subclone (see Fig. 2). Both strands of the resulting 3.4-kb *Sst*II-*Eco*RV fragment were sequenced after generation of serial deletions obtained with the Erase-a-Base kit (Promega); the segments not well covered with deletion clones were sequenced with oligonucleotides complementary to the already determined sequence. ORFs were sought with software from DNAstar, London, United Kingdom. Each polypeptide potentially encoded by an Ad19 E3 region greater than 8 kDa was compared with previously described E3 protein sequences. Alignments were done with the DNAstar software package by using the Clustal method with the PAM250 residue weight table. Subsequently, some alignments were refined manually.

Monoclonal antibodies (MAbs) and antisera. The following MAbs were used in this study: W6/32, anti-HLA-A, -B, and -C (4); BB7.2, anti-HLA-A2 and -Aw69 (35); MA2.1, anti-HLA-A2 and -B17 (31); ME.1, anti-HLA-B7, -B27, and -Bw22 (13); 4E, anti-HLA-B and -C (49). The polyclonal antiserum which recognizes the C terminus of the Ad2 E3/19K protein has been described previously (39). Rabbit serum specific for the Ad19 E3/19K protein was raised against the synthetic peptide CKRKPRYGNEEKEKLL, corresponding to the C terminus of Ad19 E3/19K. The N-terminal cysteine was added for directed coupling to keyhole limpet hemocyanin. Coupling and immunization were done essentially as previously described (39).

Transfection of cells and flow cytometry. The Ad19 E3A region contained within the 3.4-kb *Sst*II-*Eco*RV fragment was transfected into 293 cells together with the pSV2-neo^r plasmid conferring G418 resistance (41). The Ad19 and pSV2-neo^r plasmids were linearized with *Hin*dIII and *Pvu*I, respectively. 293 cells were transfected by electroporation as previously described (39). G418-resistant clones were initially screened for downregulation of cell surface expression of HLA molecules by flow cytometry with antibody W6/32. Fluorescence-activated cell sorter (FACS) analysis was carried out essentially as previously described (39).

Cell labeling, immunoprecipitation, endoglycosidase H (endo H) treatment, and SDS-PAGE. Labeling of cells with [^{35}S]methionine, tunicamycin treatment, immunoprecipitation, and SDS-polyacrylamide gel electrophoresis have already been described in detail (6, 7). Immunoprecipitation with digitonin was performed as with Nonidet P-40 (NP-40), except that the lysis buffer contained 1% digitonin (Sigma, Deisenhofen, Germany), 10 mM Tris (pH 7.4), and 150 mM NaCl. The same buffer containing 0.1% digitonin was used to wash the precipitated material four times. Endo H treatment was carried out as previously described (39). Infection of cells for immunoprecipitation was performed with a multiplicity of infection of about 30. The end of the 1-h adsorption period was considered the start of infection (time zero).

Nucleotide sequence accession number. The nucleotide sequence data reported in here will appear in the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession number X95259.

RESULTS

Isolation of the Ad19 E3A transcription unit. To investigate the organization of the E3 region of subgroup D Ads and to determine the structure of the E3/19K analogous protein, we chose Ad19, a serotype that causes epidemic keratoconjunctivitis. As the Ad19 prototype has not been isolated in Europe and the United States, we used for our study the genome type Ad19a, which has contributed substantially since 1973 to Ad-associated eye disease (27, 46). Because of the low degree of homology within the E3 region of Ads from different subgroups, cross hybridization did not seem feasible for identification of the Ad19 E3 region. Therefore, a PCR approach was used to generate an Ad19-specific E3 probe. Two primers, κ2 and C5SP, were employed to amplify a 373-bp segment from the 5′ part of the E3 region (see Fig. 2). κ2 is identical to the high-affinity NF-κB-binding site present in the E3 promoters of all of the Ads analyzed so far (12, 47), while C5SP is complementary to a short, conserved sequence in the 5′ untranslated region of the E3 mRNA (nucleotides +193 to +213 of the Ad2 mRNA). The amplified Ad19 PCR fragment was used as a probe to identify and subclone an *Sst*II-*Xba*I restriction fragment. This fragment was recombined with an overlapping *Eco*RV fragment to obtain a 3.4-kb *Sst*II-*Eco*RV fragment containing a large part of the Ad19 E3 transcription unit (see Fig. 2; for details, see Materials and Methods).

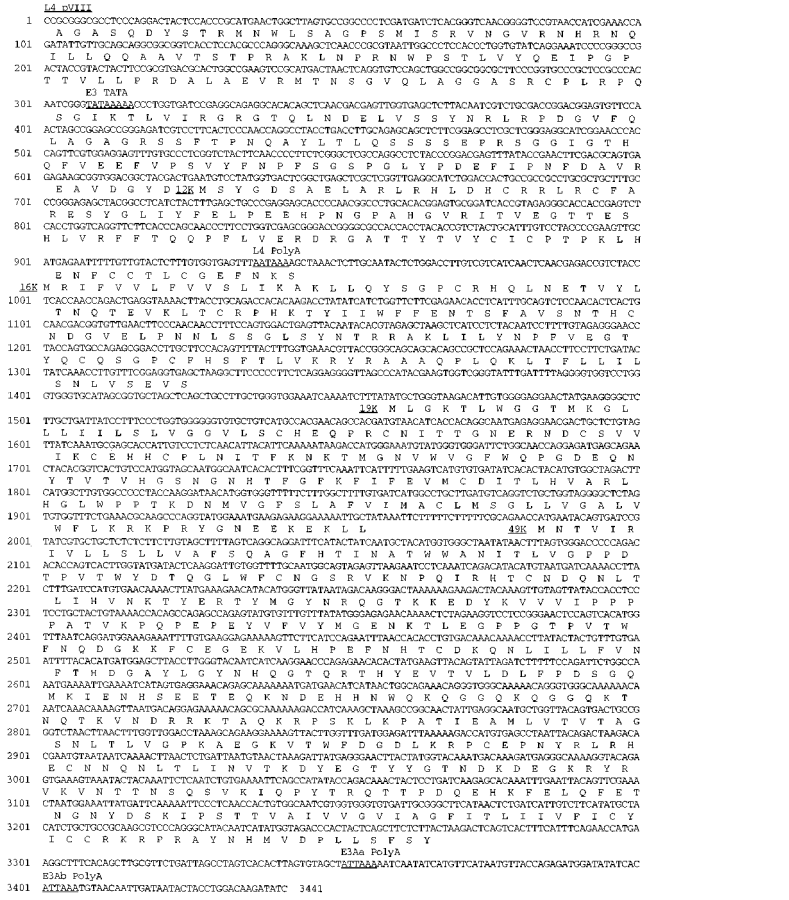

FIG. 1. Nucleotide sequence of the *Sst*II-*Eco*RV fragment containing the E3A unit from Ad19. The L4 pVIII, 12.2K, 16.2K, 18.6K, and 49K protein sequences are in one-letter code below the nucleotide sequence. The TATA box, potential polyadenylation sites, and ORF designations are underlined. Two ATTAAA motifs, E3Aa and E3Ab, were found close to the 3′ end of the isolated fragment.

General features of the sequence and ORFs. The DNA sequence revealed a TATA box at positions 308 to 313 and

three putative signals for polyadenylation (Fig. 1). The first, located at bp 936 to 941, a location equivalent to that of the L4 poly(A) site in Ad2, displays the consensus sequence AATAAA. The other two consist of an ATTAAA hexanucleotide, which is characteristic for the poly(A) signal of the E3A unit (9, 40). They are located within a 50-bp stretch at the 3′ end of the cloned DNA. These features, together with the presence of ORFs characteristic for the E3 region (see below), suggest that the cloned DNA encompasses the entire E3A region. Translation of the L strand of the DNA sequence in all three reading frames revealed five ORFs with the capacity to encode proteins larger than 8 kDa. The predicted molecular masses of the proteins were 22.5, 12.2, 16.2, 18.6, and 49 kDa (Fig. 2). On the basis of previous analysis of other Ads, the 22.5K ORF represents a portion of the Ad19 pVIII protein. The complete ORF predicts a protein of 24.8 kDa (data not shown) whose structure is remarkably well conserved among Ads. The Ad19 pVIII protein has the highest homology to the Ad35 equivalent (79.7%) and is least similar to the Ad12 protein (73.6% homology). A somewhat lower level of conservation was found for the 12.2K ORF, ranging from 54.7% similarity to the Ad2 homolog to 67% homology to the Ad3 12.1K protein (data not shown). Like those of Ads in subgroup B (e.g., Ad3), the E3A region of Ad19 also contains an ORF for an ~16-kDa protein (Fig. 1 and 2). Its homology to the group B proteins, however, is very limited (23.3 to 26.8%; data not shown).

The 18.6K ORF encodes a structurally distinct Ad19 homolog of the E3/19K protein. The 16.2K ORF is followed by an ORF encoding a protein of 166 amino acids (Fig. 1 and 2). The presence of two hydrophobic sequences, one at the N terminus, presumably corresponding to the signal sequence, and a second one close to the C terminus, suggests a transmembrane protein of 18.6 kDa (Fig. 3). Its predicted molecular weight, its hydrophilicity profile, the sequence similarity to known E3/19K proteins, and the location between the 16.2K ORF and the ORF(s) for a unique protein(s) suggest that the 18.6K ORF encodes the Ad19 equivalent of the E3/19K protein. It is not immediately obvious where the mature protein starts. According to the −3, −1 rule for predicting signal sequence cleavage sites (44), cleavage is likely to occur after Gly-23 or Ser-20 (Fig. 3, upper part). The situation is further complicated by the presence of a second ATG codon (corresponding to the second Met) in the proposed signal peptide (as in the Ad2 protein) which may also be used for initiation of translation. In this case, the signal sequence cleavage site would be shifted further C terminally (e.g., to Ser-4 or Cys-5 in the proposed mature protein). According to the Kozak rules for translation initiation (30), the second ATG may be favored (A and G at −3 and −6, respectively). However, examination of other start sites for known E3 products of Ad2 shows that the rules do not strictly apply to Ad E3 protein synthesis. Frequently, only one of the three consensus nucleotides in −3, −6, and +4 is present. For the alignment (Fig. 3), we assumed that Gly-24 is the start of the mature protein, since the size of the mature deglycosylated protein is nearly identical to that of Ad2 E3/19K (see Fig. 6B). The proposed mature Ad19 protein reveals almost equal levels of homology to E3/19K proteins of subgroups C (36.6% for Ad2 and 37.3% for Ad5) and B (35.2% for Ad11 and 32.7% for Ad3). Sixteen amino acid positions exclusively match those found in the corresponding proteins of subgroup C (Fig. 3, upper two sequences), while 16 other amino acids are identical only to those of subgroup B (Fig. 3, lower two sequences). The amino acid identities for either subgroup are not clustered but alternate. Some amino acids of Ad19 E3/19K are shared with only one member of a particular subgroup, whereas others are found in all of the E3/19K proteins examined. We predict that these conserved residues are crucial for the function of the E3/19K protein, in particular, for its ability to bind MHC class I molecules.

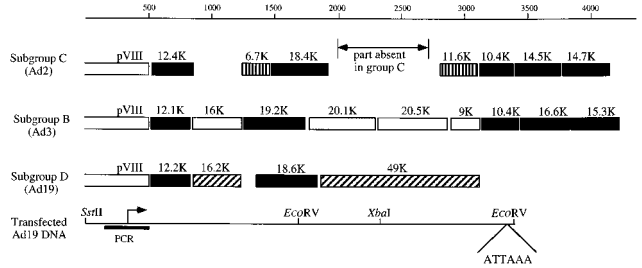

FIG. 2. Organization of Ad2 (subgroup C), Ad3 (subgroup B), and Ad19 (subgroup D) E3 regions. The different ORFs greater than 8 kDa encoded (except for ORF 6.7K of Ad2) are represented by boxes with the predicted molecular weights (10^3) on top. The graph is drawn to scale (lengths indicated in base pairs). Transfected Ad19 DNA is shown at the bottom of the graph, and the relevant restriction sites and the PCR probe are indicated. □, non-E3 ORFs; ■, E3 ORFs common to at least three subgroups; ▥ subgroup C-specific E3 ORFs; ▨, subgroup B-specific E3 ORFs; ▨, Ad19-specific E3 ORFs.

The ER retention signal, consisting of a C-terminal dilysine motif, has the same KXKXX configuration as in E3/19K proteins of subgroup B (16, 32, 40). However, there are some unique features that distinguish the Ad19 protein from all other E3/19K proteins. For instance, the last two amino acids are leucines rather than the methionine and proline found in all others. Furthermore, six amino acids previously thought to be conserved within the conserved region (amino acids 77 to 98 in the Ad2 sequence) located adjacent to the membrane domain are replaced, although predominantly by homologous amino acids (Fig. 3). Furthermore, the amino acid sequence predicts five sites for N-linked glycosylation, one and three more than are present in E3/19K proteins of Ads from subgroups B and C, respectively.

The E3A unit of Ad19a contains a large ORF predicted to encode a unique 49-kDa protein. An unusually large ORF with the capacity to encode a 49-kDa protein was found downstream of the 18.6K ORF (Fig. 2). Such a large ORF was not observed within E3 regions of Ads from other subgroups. Thus, this may be a unique feature of subgroup D Ads. Close inspection of the sequence revealed a stretch of ~80 amino acids which is repeated three times (Fig. 4A), comprising amino acids 24 to 102 (R1), 119 to 197 (R2), and 268 to 342 (R3). The highest homology (Fig. 4C) occurs between R1 and R2 (46.8%), while R3 is somewhat more diverged (36.0% similarity between R1 and R3 and 29.3% similarity between R2 and R3). Interestingly, sequence comparison revealed some homology of the R1 and R3 repeats to the 20.1- and 20.5-kDa proteins (Fig. 2) from subgroup B Ads, respectively (34.2 and 29.3% for the respective Ad3 proteins; Fig. 4C). Another region of homology (CR2) was found within the C-terminal parts of the predicted proteins, presumably encoding transmembrane segments. The 49K-CR2 region is 34.4 and 28.1% identical to corresponding regions in the 20.1- and 20.5-kDa proteins (Fig. 4D). Overall, the 49K ORF predicts a protein of 428 amino acids which has all of the characteristics of a type I transmembrane protein. It contains two longer hydrophobic regions, one at the N terminus, possibly representing the signal sequence, and a second one that may correspond to the transmembrane segment (Fig. 4B). The presence of 14 potential N-glycosylation sites in the sequence suggests that protein 49K will be highly glycosylated.

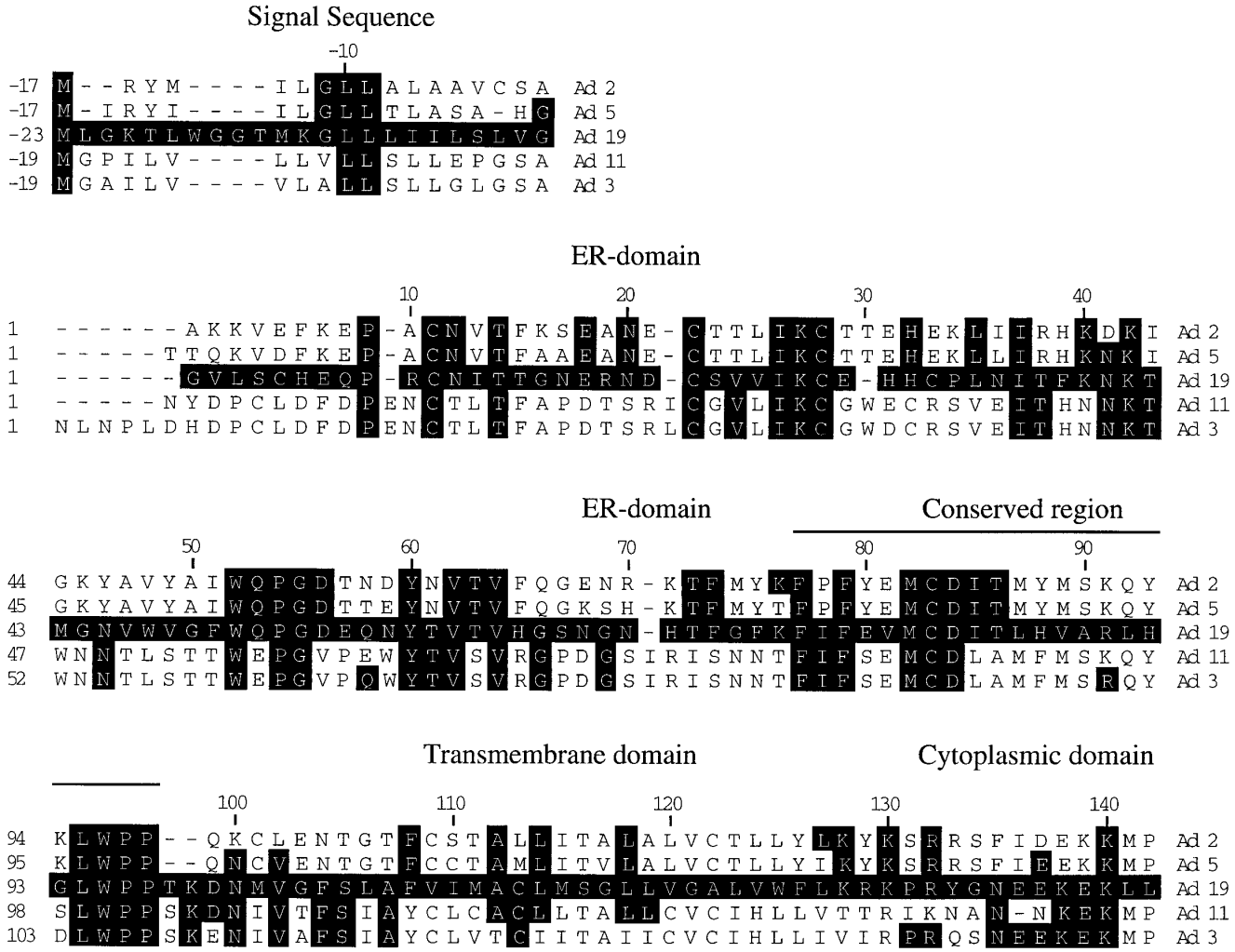

FIG. 3. Alignment of E3/19K amino acid sequences from Ad2 (22) and Ad5 (9) (subgroup C), Ad19 (subgroup D), and Ad3 (40) and Ad11 (32) (subgroup B). Proposed or proven signal sequences are shown at the top, and the black shading corresponds to amino acids which match the Ad19 sequence. For clarity, the Ad7 and Ad35 E3/19K sequences were omitted since after corrections of the Ad35 sequence, it was identical to that of Ad11 (23a) and Ad7 displays only three exchanges to Ad3 which do not influence the alignment. The ER luminal domain (ER-domain), conserved region, transmembrane domain, and cytoplasmic region are indicated above the sequences (15, 23, 39).

The 18.6K ORF codes for an HLA-binding protein with an approximate molecular mass of 34 kDa. Our further work concentrated on the putative E3/19K homolog of Ad19 encoded by the 18.6K ORF. To test whether this ORF is expressed, we generated antibodies against a C-terminal peptide. In addition, the cloned *Sst*II-*Eco*RV fragment was transfected into 293 cells (20), assuming that the Ad5 E1A product synthesized in these cells also promotes expression of Ad19 E3 genes. We predicted that the expression of a functional Ad19 HLA-binding protein would result in a reduced level of HLA on the cell surface. Therefore, G418^r clones were initially screened for this phenotype by flow cytometry. Subsequently, individual clones were analyzed by immunoprecipitation with the rabbit antiserum raised against a peptide of the Ad19 18.6K ORF. Figure 5A shows a typical result obtained with cell clone C5. In these cells, the Ad19 serum specifically recognized a protein migrating as a broad band with a molecular mass of 32 to 36 kDa (Fig. 5A, lane 4). This protein was precipitated neither from lysates of untransfected 293 cells (Fig. 5A, lanes 3) nor by preimmune serum (Fig. 5A, lanes 1 and 2). The 32- to 36-kDa protein band disappeared when the peptide used for immunization was added to the lysates, thus confirming the specificity of the antiserum (Fig. 5A, lanes 5 and 6).

We next examined whether this protein can be detected during infection with Ad19. HeLa cells were labeled for 1 h at different times postinfection, and radiolabeled proteins were precipitated with the antiserum specific for the E3/19K protein of Ad19. The protein was detected first at around 6 h postinfection. Its expression increased, reaching a peak at about 14 h postinfection (Fig. 5B), and remained at a high level over the 24-h observation period. Several protein bands were distinguished which differed by approximately 3,500 Da. The regular pattern suggests that they represent different glycosylated versions of the Ad19 E3/19K protein, the 34-kDa form, presumably representing the fully glycosylated protein with five oligosaccharides (marked 5), being the most prominent one (>65%). Quantification of the individual bands showed that the relative intensities did not change during the infection

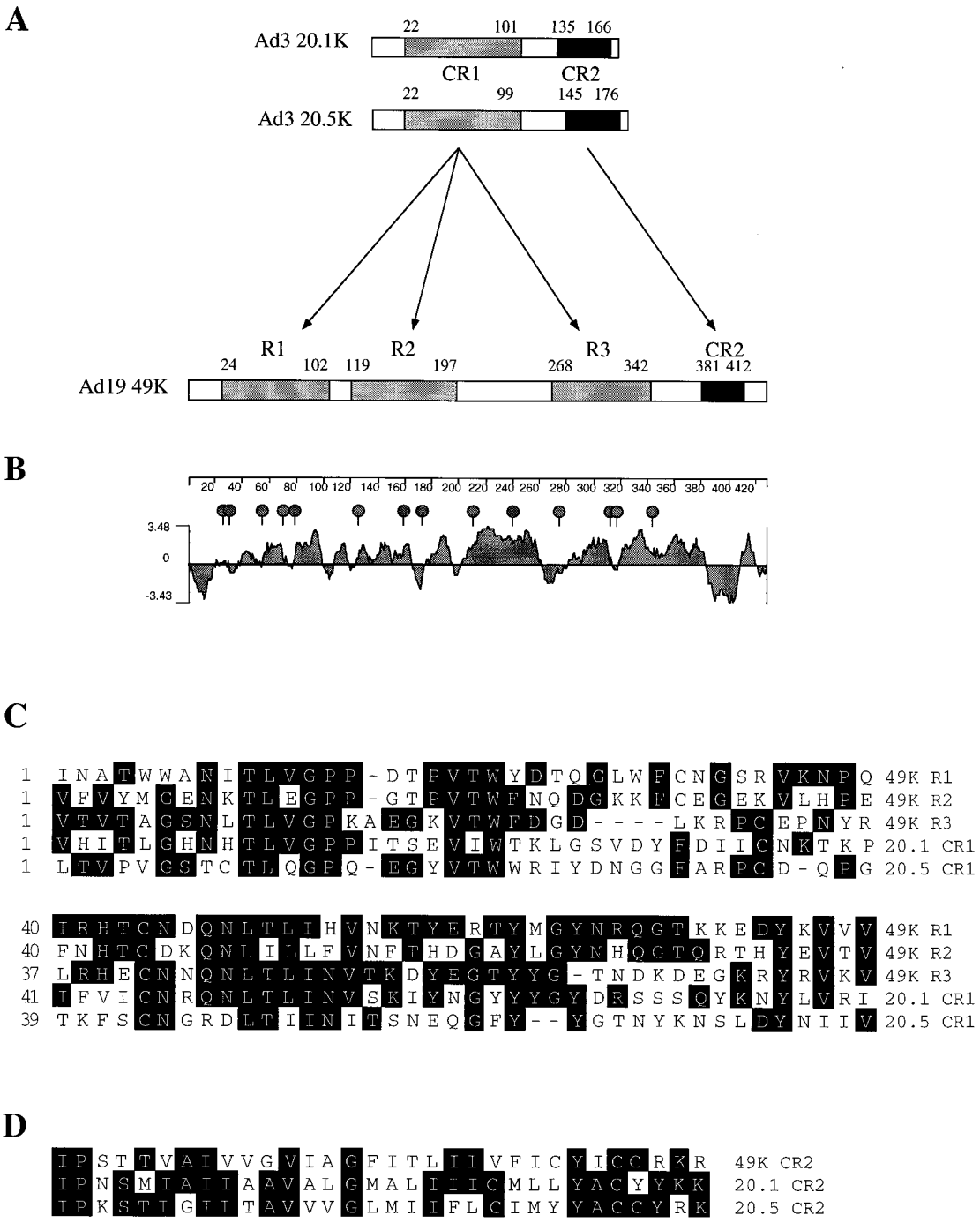

FIG. 4. Ad19 protein 49K is related to Ad3 proteins 20.1K and 20.5K. (A) Two conserved region (CR1 and CR2) are present in both Ad3 proteins 20.1K and 20.5K. CR1 corresponds to a segment in Ad19 protein 49K repeated three times (R1, R2, and R3), and CR2, also conserved in protein 49K, is a hydrophobic region which represents the putative transmembrane domain. (B) Hydrophilicity plot of E3/49K from Ad19. The potential sites for N-linked glycosylation are indicated by the racket-like symbols. (C) Multiple alignment of repeats R1, R2, and R3 from Ad19 protein 49K and CR1 from Ad3 proteins 20.1K and 20.5K (40). Black shading corresponds to amino acid residues identical to the consensus. (D) Multiple alignment of the CR2 repeats of proteins 49K, 20.1K, and 20.5K.

cycle. Furthermore, all forms were also visualized in transfected cells (Fig. 6A, lane 7). Taken together, our data show that the Ad19 E3 region expresses a novel HLA-binding protein which can be conveniently analyzed in transfected 293 cells.

The affinity of Ad19 E3/19K for HLA molecules in vitro is weaker than that of Ad2 E3/19K. We next examined whether the Ad19 protein coprecipitates with HLA molecules in 293 cells transfected with the E3A region of Ad19a. Conventional immunoprecipitation of HLA molecules from NP-40 lysates with a MAb directed to HLA-A, -B, and -C alleles (W6/32) did not reveal any coprecipitating protein, whereas the same antibody readily coprecipitated the Ad2 E3/19K protein (Fig. 6A, compare lanes 2 and 3). However, complex formation between

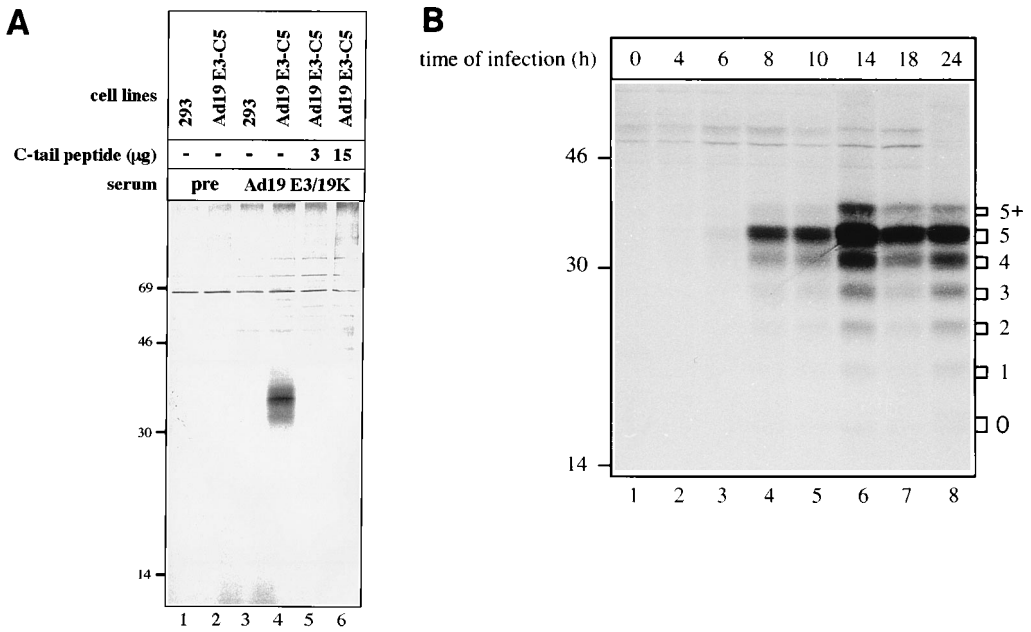

FIG. 5. Ad19 protein E3/19K is expressed during infection and in cells transfected with the E3A region of Ad19. (A) Immunoprecipitation of Ad19 E3/19K with a serum directed against its cytoplasmic tail. The first two lanes are control reactions with preimmune serum (pre). The serum precipitated a major band of about 34 kDa only from 293 cells transfected with the Ad19 E3A region (lane 4), which was not visualized upon addition of 3 or 15 μg of the peptide used to raise the antibody (lanes 5 and 6). The numbers on the left are molecular masses in kilodaltons. (B) Time course of expression of Ad19 E3/19K after infection. Ad19 E3/19K was precipitated at different times after infection, as indicated at the top. The start of infection was defined as the end of the 1-h period of adsorption with the virus (see Materials and Methods). The indicated time corresponds to the start of metabolic labeling of the cells. The different protein species seemed to migrate as doublets, which are marked with brackets and numbered 0 to 5, indicating the putative number of oligosaccharides within each Ad19 E3/19K species. The corresponding calculated molecular masses are the following: 16.6 to 17.5 kDa for band 0, 20.3 to 20.7 kDa for band 1, 23.6 to 24.2 kDa for band 2, 27.2 to 27.9 kDa for band 3, 30.8 to 31.5 kDa for band 4 and 34.1 to 35 kDa for band 5. The protein species marked 5+ is proposed to contain the untrimmed core high-mannose oligosaccharide chains, including the three glucoses initially transferred. Please note that the band intensities at 18 h postinfection do not appropriately reflect the actual abundance of the products because of a lower efficiency of metabolic labeling (70%). The numbers on the left are molecular masses in kilodaltons.

HLA and Ad19 E3/19K was visualized when the detergent digitonin was used instead of NP-40. In this case, coprecipitated proteins comigrated with the protein species detected by the Ad19 E3/19K-specific antiserum in the Ad19E3-C5 transfectant (Fig. 6A, compare lanes 6 and 7). Obviously, the interaction is preserved only in a very mild detergent. Interestingly, the pattern of protein species migrating faster than the predominant Ad19 E3/19K product confirms our interpretation with respect to the glycosylation in that the three smallest species comigrate with Ad2 E3/19K forms presumably representing proteins with two, one, and no carbohydrates (Fig. 6A, lanes 7 and 8).

Complex formation does not require carbohydrates, since the E3/19K protein of Ad19, like the Ad2 homolog, was also coprecipitated when cells were treated with tunicamycin, which interferes with the transfer of N-linked carbohydrates (Fig. 6B, lane 3). Interestingly, the apparent molecular mass of the nonglycosylated Ad19 protein (16.8 kDa) is almost identical to that of the Ad2 protein (17.2 kDa). The calculated molecular weight of mature Ad2 E3/19K is 16,660. After subtraction of a signal peptide of 23 or 20 amino acids, the estimated molecular weight of the mature Ad19 protein is 16,213 or 16,482, respectively. As mentioned above, there is also the possibility of a shorter protein (15,856 Da) if the second ATG codon is used for translation initiation. Indeed, three protein species were visible in the Ad19 E3/19K-expressing cell line treated with tunicamycin (Fig. 6B, lane 4, E3/19K and *). Their apparent molecular masses of 16.8, 16.3, and 15.6 kDa are compatible with those estimated. Thus, leaky scanning and different signal sequence cleavage sites may create different proteins, with the most slowly migrating form being by far the most strongly expressed.

The Ad19 E3/19K protein interferes with transport of HLA antigens to the cell surface. To investigate whether Ad19 E3/19K also inhibits transport of HLA molecules, two types of experiments were performed. First, pulse-chase experiments were carried out. The Ad19 E3/19K expressing 293 cell line C5 and, for comparison, untransfected cells and Ad2 E3/19K-expressing transfected cells were pulse-labeled and lysed or further incubated with an excess of unlabeled methionine for various times (Fig. 7). To increase E3 expression (12, 29), cells were treated with TNF-α overnight. For simplicity, only one HLA allele, the HLA-A2 molecule, was monitored. Upon immunoprecipitation with the HLA-A2-specific antibody, the precipitated material was analyzed by SDS-polyacrylamide gel electrophoresis. While in 293 cells the apparent molecular mass of HLA-A2 molecules increased as a result of carbohydrate modifications in the Golgi (Fig. 7, lanes 1 to 3), this shift was not observed in both Ad2 and Ad19 E3/19K-expressing cell lines (Fig. 7, lanes 4 to 9). These carbohydrate modifications in the Golgi were assessed more directly by treating the precipitated glycoproteins with endo H (Fig. 7, lanes 10 to 18). Oligosaccharides in the high-mannose form are sensitive to this enzyme, while modified oligosaccharide moieties containing, e.g., galactose and sialic acids attached in the medium/trans Golgi acquire endo H resistance. After a 150-min chase, HLA-A2 molecules in 293 cells were almost exclusively resistant (Fig. 7, lane 12, HLA-A2^r), reflecting transit through the medium/trans Golgi compartments. In contrast, in both E3$^+$ cells HLA-A2 molecules remained largely sensitive (Fig. 7,

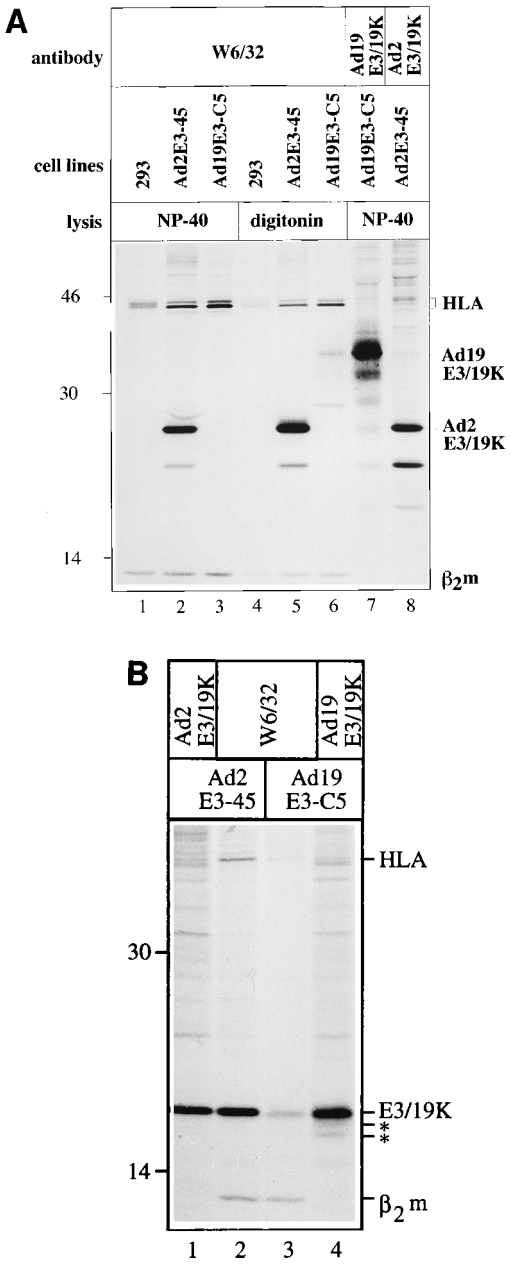

FIG. 6. (A) Coprecipitation of Ad19 E3/19K with HLA molecules when digitonin is used to lyse the cells. Cell lines designated Ad2E3-45 and Ad19E3-C5 are both derivatives of 293 cells (20). Ad2E3-45 is identical to the previously described 293E3-45 cell line, which was established by transfection of 293 cells with the *Eco*RV fragment encompassing the Ad2 E3 region (29). HLA antigens were precipitated from 293, Ad2E3-45, and Ad19E3-C5 cells with either NP-40 or digitonin, as indicated above the autoradiograph (see Materials and Methods). The proteins coprecipitated with HLA comigrated with the respective E3/19K proteins from Ad19 (compare lanes 6 and 7) and Ad2 (compare lanes 2, 5, and 8). All cells were treated with TNF-α (320 U/ml) 15 h before metabolic labeling to increase the expression of E3 proteins (12, 29). The numbers on the left are molecular masses in kilodaltons. (B) Binding of Ad19 E3/19K to HLA independent of its glycosylation. Cells were treated with tunicamycin (7 μg/ml) 3 h before and during metabolic labeling to prevent N-glycosylation. HLA molecules were precipitated with MAb W6/32 (lanes 2 and 3) and with antisera specific for Ad2 E3/19K (lane 1) and Ad19 E3/19K (lane 4). Cells were lysed with digitonin, allowing coprecipitation of Ad19 E3/19K with HLA. The Ad19 E3/19K serum precipitated a major protein migrating at a position similar to that of unglycosylated Ad2 E3/19K, as well as two less abundant, smaller products marked on the right with asterisks. The apparent molecular masses of the E3/19K proteins are 17.2 kDa for Ad2, 16.8 kDa for the predominant Ad19 product, and 16.3 and 15.6 kDa for the minor species. The numbers on the left are molecular masses in kilodaltons. β_2m, β_2-microglobulin.

lanes 15 and 18, HLA-A2*). This demonstrates that, similar to the Ad2 E3/19K protein, Ad19 E3/19K is capable of retaining the HLA-A2 molecule in the ER.

Without TNF-α treatment, the amounts of endo H-resistant HLA-A2 molecules were 1.7% in cells expressing the Ad2 E3/19K protein and 8.9% in Ad19 E3/19K$^+$ cells (Fig. 8A). HLA-B/C antigens seem to be less susceptible to the E3/19K effect, because 7.5 and 25.8% became endo H resistant in Ad2E3-45 and Ad19E3-C5 cells, respectively (Fig. 8A). Thus, Ad19 E3/19K does not as effectively downregulate HLA molecules in 293 cells as does the Ad2 counterpart.

To substantiate this conclusion and to examine the effect of Ad19 E3/19K on other HLA alleles, FACS analysis was performed with MAbs directed to different HLA alleles. To reduce the effect of clonal variation, the cell surface expression of two E3-negative cell lines was compared with that of the same number of cell lines expressing the Ad19 E3/19K molecules and three expressing the Ad2 equivalent. In Ad19 E3/19K$^+$ cells, expression of HLA-A, -B, and -C was reduced by 69% (Fig. 8B, HLA-ABC, hatched bar), compared with 85.9% in cells expressing the E3/19K protein of Ad2 (Fig. 8B, HLA-ABC, solid bar). We do not think that this was due to lower expression of the Ad19 protein, since quantification of E3/19K proteins in Fig. 6A and B indicated a similar expression level. Consistent with the pulse-chase data, the allele most strongly affected was HLA-A2, with residual expression levels of 20% in cells containing the Ad19 protein and 6.1% in Ad2 E3/19K-expressing cells (Fig. 8B, calculated mean of BB7.2 and MA2.1 results). For HLA-B7 (ME.1), the expression levels were 42.6 and 16.1%, respectively, while expression of HLA-B/C alleles was the least affected. Thus, the relative susceptibility to E3/19K-mediated transport inhibition by Ad2 and Ad19 is highest for HLA-A2, followed by B7 and B/C antigens. Relating the differential suppression of the HLA-A2 allele by Ad19 and Ad2 E3/19K proteins to the different overall reduction as detected by W6/32, it becomes apparent that the affinity of the Ad19 protein for HLA-A2 is about 66% lower than that of the Ad2 E3/19K protein. Thus, E3/19K proteins from different virus subgroups seem to exhibit different affinities for individual HLA alleles.

DISCUSSION

In this study, we have characterized the E3 region of an Ad subtype associated with epidemic keratoconjunctivitis. Ad19a, the subtype used here, belongs to subgroup D, which comprises the largest subgroup of Ads. The previous characterization of E3 regions of other Ads indicated that this nonessential transcription unit contains both genes common to all Ads and unique genes typical for each subgroup. For instance, Ads of subgroup A lack an E3/19K equivalent but do contain homologs for proteins 12.4K, 10.4K, 14.5K, and 14.7K of Ads in subgroup C (42). Subgroup B Ads have two novel ORFs encoding 20.1K and 20.5K proteins which are apparently related to each other. The unique 20.5K ORF was recently shown to be expressed in Ad3- and Ad7-infected cells (21). Thus, it was interesting to know whether this combination of common and unique genes is also preserved in Ads of subgroup D.

The organization of the E3A region of Ad19a resembles that of subgroup B in that it contains a 16K ORF between the 12.2K and 18.6K ORFs, which are homologs of the respective E3/12K and E3/19K ORFs of subgroups B and C (Fig. 2). Moreover, the 18.6K ORF is followed by a unique 49K ORF which is unrelated to the 11.6K protein of subgroup C but shares some similarity with proteins 20.1K and 20.5K of subgroup B (Fig. 4A, C, and D). With the exception of the E3/19K

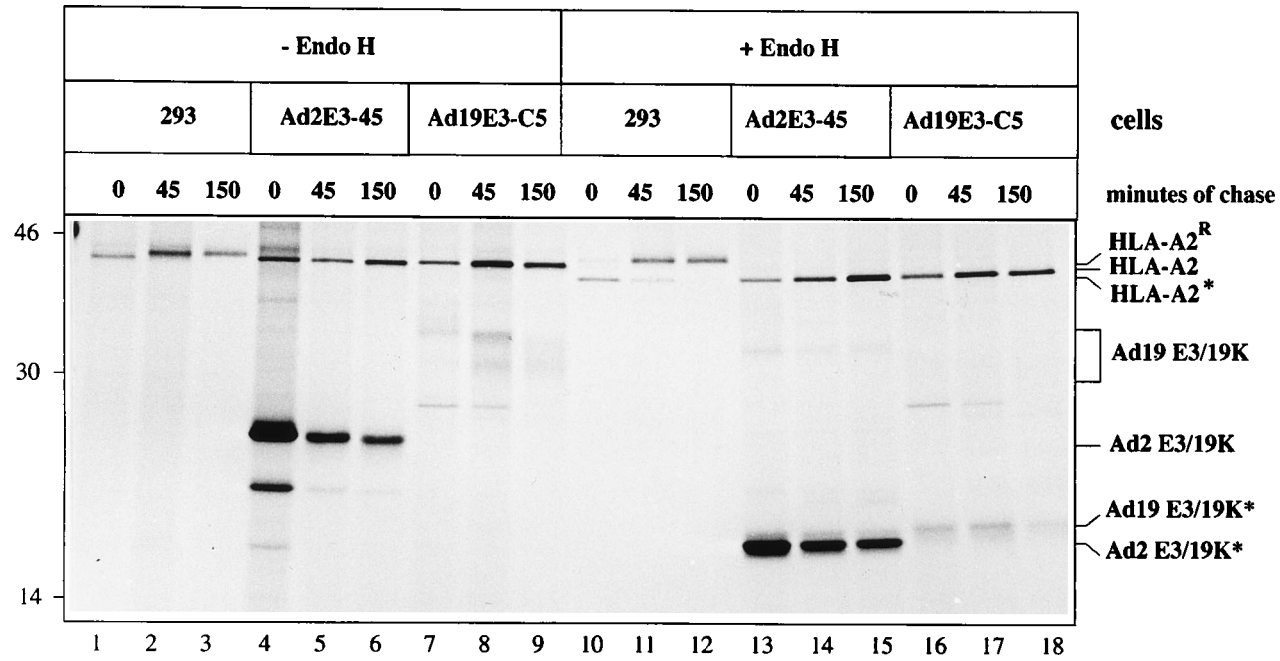

FIG. 7. Transport of HLA-A2 antigens in cells expressing E3/19K molecules from either Ad2 (Ad2E3-45) or Ad19 (Ad19E3-C5). Cells were pulse-labeled for 25 min with 200 μCi of [^{35}S]methionine per ml, chased for 0, 45, and 150 min in an excess of cold methionine, and lysed. HLA-A2 antigens were immunoprecipitated with MAb BB7.2. The precipitated material was split, and one half was treated with endo H (lanes 10 to 18) while the other was mock treated (lanes 1 to 9). Positions of HLA-A2 (heavy chain) and E3/19K are marked on the right, and the numbers on the left are molecular masses in kilodaltons. Asterisks denote endo H-cleaved products; a superscript R indicates endo H resistance. All cells were treated with TNF-α 14 h before immunoprecipitation to increase the expression of E3 proteins (12, 29). 293 cells were lysed with NP-40, whereas E3-positive 293 cells were lysed with digitonin-containing buffer to preserve HLA-E3/19K complexes. The amounts of endo H-resistant forms after a 150-min chase are: 96.2, 3.9, and 4.5% for 293, Ad2E3-45, and Ad19E3-C5 cells, respectively. The data (percent) are mean results of two experiments.

proteins, the sequence similarity between individual E3 products is more pronounced for subgroup B than for subgroup C. This is consistent with a recent report by Bailey and Mautner (3), who suggested that Ads of subgroup D originated from a lineage intermediate between subgroups C and B/E. Whether it is justified to place subgroup D viruses in one monophyletic cluster with subgroup C awaits further sequencing of Ad products from subgroup D.

On the basis of the existence of unique genes in the E3 regions of different subgroups, it was proposed that these products may correlate with clinical manifestations (3, 21). The presence of the unique 49K ORF in Ad19a (subgroup D) corroborates the concept of unique proteins in each subgroup. However, subgroup D forms the largest subgroup of Ads and causes a broad spectrum of diseases. Thus, in the absence of sequence data on other members of subgroup D, we also consider the possibility that the putative 49K protein is unique to viruses causing epidemic keratoconjunctivitis, such as Ad8, Ad19, and Ad37. With the Ad19 sequence in hand, this can be easily tested. If there is a strong correlation between the presence of protein 49K and the ability to cause keratoconjunctivitis, the 49K protein may be involved in that particular pathogenesis of the eye. By using antibodies specific for the 49K protein, we demonstrated that the 49K ORF is indeed expressed as a high-molecular-mass ~90-kDa protein, consistent with our prediction that the protein is highly glycosylated (data not shown).

We are particularly interested in the structure and function of the E3/19K protein. The drastic variability in the primary amino acid sequences of E3/19K proteins of subgroups B and C contrasts with their common function, namely, to bind to and inhibit the transport of MHC class I molecules (5, 33, 39). Thus, it seems that only a few conserved amino acids (together with variable spacer amino acids) are required for binding to MHC class I molecules. One major aim was, therefore, by determining the structure of the E3/19K equivalent of subgroup D, to obtain a more complete picture of which amino acids are crucial for HLA binding. Before this analysis, sequence alignment of the group B and C proteins revealed 43 conserved residues. By including the Ad19 sequence, we reduced the number of invariant residues from 43 to 25. Frequently, a particular amino acid thought to be conserved is replaced with a homologous amino acid in Ad19. For example, the C-terminal residues (Met and Pro) previously thought to be conserved are replaced by two leucines. Most strikingly, six amino acids of the so-called conserved region (amino acids 77 to 98 in Ad2, adjacent to the membrane segment; 15) are replaced and two such exchanges exhibit a quite different character (Val instead of Glu in position 81 and His in Ad19 for Tyr-93). Apparently, many positions require only a certain hydrophobicity or hydrophilicity but not the identical amino acid. The remaining 25 conserved amino acids (Fig. 3) are almost exclusively (24 of 25) located in the luminal domain (amino acids 1 to 100; 24% identity) previously shown to contain the HLA-binding module (5, 7, 19, 23, 34, 39). The reduced number of conserved residues allows us to make better predictions of the importance of individual amino acids for HLA binding and to use scanning mutagenesis to test them.

The degree of similarity of Ad19 E3/19K to the homologous proteins of either subgroup B or C is essentially equal. Sixteen residues of Ad19 are identical to those found in all members of subgroup B only, while 16 other positions are shared exclusively with those of subgroup C. Residues identical to those of group C are not clustered in one particular region but alternate

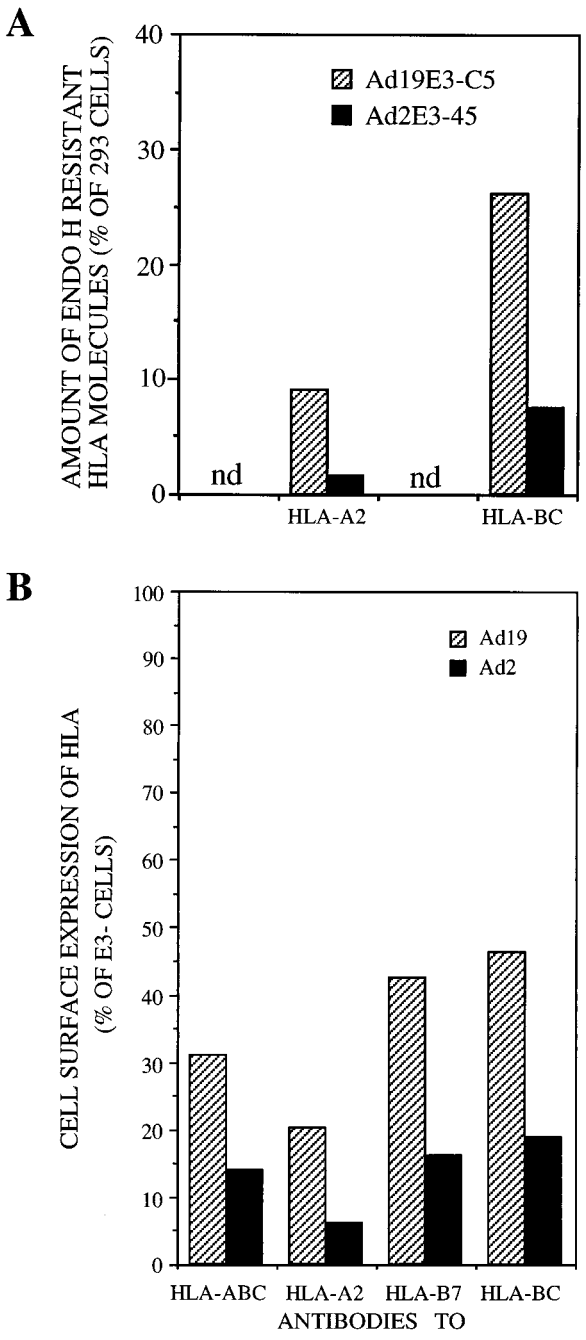

FIG. 8. (A) Transport of HLA-A2 and HLA-B/C antigens in Ad2E3-45 and Ad19E3-C5 cells. A pulse-chase experiment was carried out exactly as described in the legend to Fig. 7, except that TNF-α treatment was omitted. The amount of endo H-resistant HLA species was determined after a 150-min chase with a PhosphorImager (Fujix Bas 1000; Fuji Photo Film Co., Ltd., Tokyo, Japan). The values given are relative to those obtained with 293 cells, which were set to 100%. nd, not done. (B) Cell surface expression of different HLA alleles in 293 cells negative and positive for the E3 region from either Ad2 or Ad19. The bars represent percentage of expression compared with $E3^-$ cells. The mean fluorescence value for each antibody with 293 cells and a $G418^r$ $E3^-$ transfectant was set to 100%. The cell surface expression, relative to that of the $E3^-$ cells, of two clones expressing the Ad19 E3/19K protein and three cell lines containing the Ad2 E3/19K protein is indicated. The latter cell lines, 293E22.7, 293E3-45, and 293.12, have been described previously (6, 29). For FACS analysis, the MAbs used were W6/32, (HLA-ABC), BB7.2, MA2.1 (both HLA-A2), ME.1 (HLA-B7), and 4E (HLA-BC). The HLA-A2 value represents the mean between BB7.2 and MA2.1 staining. Each bar is the average value of at least two different clones and two independent experiments.

with those found in subgroup B. This patchwork homology suggests that the Ad19 E3/19K protein diverged before the separation of subgroups B and C (data not shown).

The Ad19 E3/19K protein is expressed in 293 cells transfected with a genomic fragment, as well as during infection with Ad19 (Fig. 5A and B). Coprecipitation of Ad19 E3/19K with HLA was not observed in NP-40 buffers, while the Ad2 E3/19K protein was readily detected. However, the HLA-E3/19K complex could be recovered when cells were lysed in digitonin. This indicates a lower affinity of Ad19 E3/19K for HLA, at least in vitro. However, lack of coprecipitation was also observed for Ad2 E3/19K when HLA-B/C-specific antibodies were used, although the transport inhibition was similar to that observed for HLA-A2 (data not shown). Thus, the coprecipitation assay is useful but is not a reliable indicator of complex formation in vivo. Therefore, in the absence of E3/19K coprecipitation, additional assays such as pulse-chase and FACS analyses are necessary to assess the function of E3/19K. Flow cytometry showed that residual expression of HLA-A2 and HLA-B/C molecules in Ad19 E3/19K-expressing cells was 3.3- and 2.5-fold higher, respectively, than in cells expressing the Ad2 protein (Fig. 8B). This parallels the higher proportion of HLA molecules becoming endo H resistant in cells expressing the Ad19 protein than in cells expressing the Ad2 E3/19K protein (Fig. 8A; 5.2-fold for HLA-A2 and 3.4-fold for HLA-B/C). Together, these data suggest that the Ad19 protein exhibits a lower affinity for HLA molecules in 293 cells than does the Ad2 protein.

However, the presence of TNF-α improved the inhibitory activity of the Ad19 E3/19K protein on HLA-A2 transport, almost reaching that of the Ad2 E3/19K protein (Fig. 7). This was confirmed by flow cytometry in the presence of TNF-α (data not shown). We previously showed that TNF-α enhances the action of Ad2 E3/19K (12, 29) by activating the E3 promoter (11a). Thus, we conclude that TNF-α also stimulates the E3 promoter of Ad19, allowing higher expression of Ad19 E3/19K and thus more complete inhibition of HLA transport. The slight differences between the FACS and pulse-chase data might be due to (i) the different cells examined (only one cell line from each group was analyzed in the pulse-chase experiments, compared with two or three in the FACS analysis) and (ii) the different techniques employed. While the FACS technique analyzes a steady-state situation that depends on the transport rate, the turnover on the cell surface, and/or cell cycle control of HLA and E3/19K expression, pulse-chase experiments analyze the kinetics of transport only.

We have not completely ruled out the possibility that the relatively higher level of HLA expression on the cell surface of Ad19 $E3/19K^+$ cells is due to lower expression of the Ad19 protein than the Ad2 protein, because different antibodies were used to detect the E3/19K proteins. However, irrespective of the cause of the lower efficiency of Ad19 in this system, HLA molecules exhibit a differential sensitivity for the viral proteins: HLA-A2 is affected most, followed by B7 and HLA-B/C molecules. Similar findings were made for the Ad2 and Ad5 proteins (5). In a previous study, we found that HLA-A3 is more susceptible to the Ad2 E3/19K protein than is the HLA-B35 allele (28). Together with our new data, this may indicate that HLA-A alleles, in general, are more strongly affected than HLA-B alleles.

Apart from different affinities of HLA alleles for one particular E3/19K molecule, E3/19K proteins produced by viruses from different subgroups may exhibit differential binding to HLA alleles. As first evidence, we found, by comparing the down-regulation of individual alleles with that obtained for all HLA alleles (as detected by MAb W6/32), that the relative

sensitivity of the HLA-A2 molecule for the transport-inhibitory effect of E3/19K is stronger for Ad2 than for Ad19. Further studies including more HLA alleles and E3/19K proteins of subgroup B Ads are necessary to investigate this more comprehensively.

ACKNOWLEDGMENTS

We are grateful to G. Wadell (Umea, Sweden) for kindly providing the Ad genome type Ad19a. We thank Susanne Etteldorf for expert technical help and Heike Pahl, Peter Shaw, and all members of the group for comments and for critical reading of the manuscript. The photographic work of Lore Lay and Edith Ruscher is appreciated.

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. **Bailey, A., and V. Mautner.** 1994. Phylogenetic relationships among adenovirus serotypes. Virology **205:**438–452.
4. **Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler.** 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—new tools for genetic analysis. Cell **14:**9–20.
5. **Burgert, H.-G.** Subversion of the MHC class I antigen presentation pathway by adenoviruses and herpes simplex viruses. Trends Microbiol, in the press.
6. **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.
7. **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.
8. **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.
9. **Cladaras, C., and W. S. Wold.** 1985. DNA sequence of the early E3 transcription unit of adenovirus 5. Virology **140:**28–43.
10. **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.
11. **Davison, A. J., E. A. Telford, M. S. Watson, K. McBride, and V. Mautner.** 1993. The DNA sequence of adenovirus type 40. J. Mol. Biol. **234:**1308–1316.

11a. **Deryckere, F., and H.-G. Burgert.** Unpublished data.

12. **Deryckere, F., C. Ebenau-Jehle, W. S. M. Wold, and H.-G. Burgert.** 1995. Tumor necrosis factor α increases expression of adenovirus E3 proteins. Immunobiology **193:**186–192.
13. **Ellis, S. A.** 1982. Recognition of HLA-B27 and related antigens by a monoclonal antibody. Hum. Immunol. **5:**49–59.
14. **Feuerbach, D., S. Etteldorf, C. Ebenau-Jehle, J. P. Abastado, D. Madden, and H.-G. Burgert.** 1994. Identification of amino acids within the MHC molecule important for the interaction with the adenovirus protein E3/19K. J. Immunol. **153:**1626–1636.
15. **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.
16. **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.
17. **Fox, J. P., C. E. Hall, and M. K. Cooney.** 1977. The Seattle virus watch. VII. Observations of adenovirus infections. Am. J. Epidemiol. **105:**362–386.
18. **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.
19. **Gabathuler, R., F. Lévy, 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.
20. **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.
21. **Hawkins, L., and W. S. M. Wold.** 1995. A 20,500-dalton protein is coded by region E3 of subgroup B but not subgroup C human adenoviruses. Virology **208:**226–233.
22. **Herisse, J., G. Courtois, and F. Galibert.** 1980. Nucleotide sequence of the EcoRI D fragment of adenovirus 2 genome. Nucleic Acids Res. **8:**2173–2192.
23. **Hermiston, T. W., R. A. Tripp, T. Sparer, L. R. Gooding, and W. S. Wold.** 1993. 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. J. Virol. **67:**5289–5298.

23a. **Horwitz, M.** Personal communication.

24. **Horwitz, M. S.** 1990. Adenoviruses, p. 1723–1740. *In* B. N. Fields and D. M. Knipe (ed.), Virology. Raven Press, New York.
25. **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.
26. **Jackson, M. R., T. Nilsson, and P. A. Peterson.** 1993. Retrieval of transmembrane proteins to the endoplasmic reticulum. J. Cell Biol. **121:**317–333.
27. **Kemp, M. C., J. C. Hierholzer, C. P. Cabradilla, and J. F. Obijeski.** 1983. The changing etiology of epidemic keratoconjunctivitis: antigenic and restriction enzyme analyses of adenovirus types 19 and 37 isolated over a 10-year period. J. Infect. Dis. **148:**24–33.
28. **Körner, H., and H.-G. Burgert.** 1994. Down-regulation of HLA antigens by the adenovirus type 2 E3/19K protein in a T-lymphoma cell line. J. Virol. **68:**1442–1448.
29. **Körner, H., U. Fritzsche, and H.-G. Burgert.** 1992. Tumor necrosis factor alpha stimulates expression of adenovirus early region 3 proteins: implications for viral persistence. Proc. Natl. Acad. Sci. USA **89:**11857–11861.
30. **Kozak, M.** 1991. An analysis of vertebrate mRNA sequences: intimations of translational control. J. Cell Biol. **115:**887–903.
31. **McMichael, A. J.** 1980. A monoclonal antibody that recognizes an antigenic determinant shared by HLA-A2 and B17. Hum. Immunol. **1:**121–129.
32. **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.
33. **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.
34. **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.
35. **Parham, P., and F. M. Brodsky.** 1981. Partial purification and some properties of BB7.2: a cytotoxic monoclonal antibody with specificity for HLA-A2 and a variant of HLA-A28. Hum. Immunol. **3:**277–299.
36. **Precious, B., and W. C. Russel.** 1985. Growth, purification and titration of adenoviruses, p. 193–205. *In* W. J. Mahy (ed.), Virology, a practical approach. IRL Press, Oxford.
37. **Rawle, F. C., E. Tollefson, W. S. M. Wold, and L. R. Gooding.** 1989. Mouse anti-adenovirus T lymphocytes. Inhibition of lysis by E3 gp19K but not E3 14.7K. J. Immunol. **143:**2031–2037.
38. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
39. **Sester, M., and H.-G. Burgert.** 1994. Conserved cysteine residues within the E3/19K protein of adenovirus type 2 are essential for binding to major histocompatibility complex antigens. J. Virol. **68:**5423–5432.
40. **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.
41. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1:**327–341.
42. **Sprengel, J., B. Schmitz, D. Heuss-Neitzel, C. Zock, and W. Doerfler.** 1994. Nucleotide sequence of human adenovirus type 12 DNA: comparative functional analysis. J. Virol. **68:**379–389.
43. **Straus, S. E.** 1984. Adenovirus infections in humans, p. 451–496. *In* H. S. Ginsberg (ed.), The adenoviruses. Plenum Press, New York.
44. **von Heijne, G.** 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. **14:**4683–4690.
45. **Wadell, G.** 1984. Molecular epidemiology of human adenoviruses. Curr. Top. Microbiol. Immunol. **110:**191–220.
46. **Wadell, G., and J. C. de Jong.** 1980. Restriction endonucleases in identification of a genome type of adenovirus 19 associated with keratoconjunctivitis. Infect. Immun. **27:**292–296.
47. **Williams, J. L., J. Garcia, D. Harrich, L. Pearson, F. Wu, and R. Gaynor.** 1990. Lymphoid specific gene expression of the adenovirus early region 3 promoter is mediated by NF-kappa B binding motifs. EMBO J. **9:**4435–4442.
48. **Wold, W. S. M.** 1993. Adenovirus genes that modulate the sensitivity of virus-infected cells to lysis by TNF. J. Cell. Biochem. **53:**329–335.
49. **Yang, S. Y.** 1984. Comparison of one-dimensional IEF patterns for serological detectable HLA-A and B allotypes. Immunogenetics **19:**217–231.