Effect of CD4 Gene Expression on Adenovirus Replication

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Received 23 June 1993/Accepted 29 July 1994

The gene encoding the CD4 receptor was introduced into KB cells to establish the KBT4 cell line, a cell line susceptible to infection with human immunodeficiency virus type 1. Adenovirus replication was found to be significantly less in these cells than in the parental KB cells. Similar decreased adenovirus type 5 (Ad5) replication occurred in HeLaT4 cells compared with the original HeLa cells. The presence of CD4 did not alter the cell surface population of KB cell adenovirus receptors, since viral adsorption was similar in the two cell lines. Moreover, addition of soluble CD4 did not reduce viral replication in either KB or KBT4 infected cells. Uncoating of viral DNA was also unchanged in KBT4 cells compared with the parental KB cells. In contrast, migration to or entrance of viral DNA into nuclei and synthesis of early viral RNAs was delayed and reduced in KBT4 cells. These effects were more pronounced for Ad7 than for Ad5. The yields of infectious viruses were the same in both cell lines, however, after transfection of naked viral DNAs to initiate infection. These results imply that the expression of the CD4 gene in KBT4 cells interfered with passage of uncoated virus across endosomal vesicles and/or transfer of uncoated core viral DNA into the nucleus.

Latent and persistent infections are one of the hallmarks of adenovirus infections in humans, but the mechanism(s) by which the virus effects this process is not understood. Adenoviruses were originally isolated from cultured explants of tonsils and adenoids (28) and from army recruits with acute respiratory disease (16). Although adenoids are primarily composed of lymphoid cells, isolation of virus or even replication of exogenous adenovirus in cultured lymphocytes purified from adenoids has been difficult (20, 31, 36). Several papers reported the presence of adenovirus DNA sequences in human peripheral blood lymphocytes (12, 14, 18, 25). It appears, however, that even though peripheral blood lymphocytes frequently carry adenovirus DNA, only rare cells are infected (12) and many may be refractory to viral replication.

Adenoviruses are conventionally propagated in epithelial cell lines, and despite extensive studies of the virus in these cells, relatively little is known of the virus in a lymphoid cell background. During development of a quantitative assay for human immunodeficiency virus (HIV) (unpublished data), the gene encoding the CD4 receptor (23) was introduced into KB cells (8) to produce a line termed KBT4. The yields of adenovirus replication were much lower in KBT4 cells than in the parental KB cell line.

The goal of this investigation was to determine the mechanism by which adenovirus replication was adversely affected in the CD4-bearing KB cells. Early adenovirus-cell interactions that lead to productive infection can be summarized as follows: (i) recognition and attachment of virions to specific cell surface receptors (33); (ii) clustering of receptors followed by invagination of receptors and virus into clathrin-coated pits (27, 32); (iii) dissociation of the clathrin coats to generate endosomal vesicles (15); (iv) acidification within the endosomes, resulting in the translocation of virus across the endosomal membranes (29, 30); and (v) transfer of the viral genome into the nucleus (2, 3, 7, 26). The results of experiments to be described, which compare adenovirus type 5 (Ad5) and Ad7 replication in KB and KBT4 cells, suggest that CD4 may interfere with passage

of DNase-sensitive adenovirus DNA particles across endosomal vesicles and/or transfer of its genome into the nucleus.

MATERIALS AND METHODS

Cells and viruses. KB, KBT4, HeLa, HeLaT4, and A549 cells were maintained as monolayer cultures in Dulbecco minimum essential medium supplemented with 8% bovine calf serum and L-glutamine (9). Adenovirus stocks were propagated in KB cells (9) and assayed by an indirect immunofluorescence assay (fluorescent focus assay), using a polyclonal rabbit antiserum prepared against intact purified virus (34). All cell lines and viral stocks were routinely tested and found to be free of mycoplasma contamination (Gen Probe II; Gen-Probe Inc., San Diego, Calif.).

Flow cytometric analysis to detect cell surface T4 expression. Cells were removed from their growth surfaces by treatment with 50 mM EDTA in phosphate-buffered saline (PBS) and incubated at 37°C for 45 min with saturating amounts of a monoclonal anti-CD4 antibody, OKT4. The cells were washed three times with PBS to remove unbound OKT4, incubated with a fluorescein isothiocyanate-conjugated sheep anti-mouse antibody (Cappel, Cochranville, Pa.), and fixed with 1% paraformaldehyde in PBS. Fluorescence intensity was measured on a FACScan cytofluorograph with the Consort-30 software (Becton Dickinson, Mountain View, Calif.).

Preparation and blot analysis of RNA. Cytoplasmic RNAs were extracted by hypotonic lysis with Nonidet P-40 (10). The RNA was denatured and electrophoresed on a 1% agarose gel containing formaldehyde (1). After electrophoresis, the RNA was transferred to a nitrocellulose filter, immobilized, and hybridized to nick-translated DNA probes (35).

Purification of virus. Confluent monolayers of KB cells were infected with adenovirus at a multiplicity of infection (MOI) of 5 to 10 fluorescent focus-forming units (FFU) per cell and incubated until complete cytopathic effect was observed. Adenovirus was purified from infected KB cells by centrifugation through cesium chloride gradients as previously described (4).

Radioactive labeling. Ten hours after infection, virus-infected cells were labeled for 18 h with 5 μ Ci of [35 S]methionine (Amersham Corp.) per ml in medium containing low methio-

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nine or with 5 μ Ci of [methyl- 3 H]thymidine (Amersham) per ml as previously described (11). Infected cells and media were harvested after complete cytopathic effect appeared, and virus was purified (4).

Assay of DNase sensitivity of viral DNA. Uncoating of adenovirus particles was determined by measuring the conversion of viral DNA from a state of DNase resistance (intact virions) to DNase sensitivity (22). Purified 3 H-labeled adenovirus (MOI = 200 to 500 FFU per cell) was adsorbed to KB or KBT4 monolayers for 15 min at 4°C. Under these conditions, adsorption and very little penetration occur (27). Cells were washed three times with cold medium and overlaid with medium that had been prewarmed to 37°C. Time zero was the time at which incubation of infected cells at 37°C was initiated. At various times postinfection, 5×10^6 cells were harvested, washed three times with cold PBS, and resuspended in 0.3 ml of Tris-EDTA buffer containing 0.5% deoxycholate. After cell lysis, $MgCl_2$ and DNase were added to final concentrations of 20 mM and 200 μ g/ml, respectively, and the suspension was incubated at 37°C for 1 h. An equal volume of cold 10% trichloroacetic acid (TCA) was added, and after 30 min on ice, the tubes were centrifuged at $2,000 \times g$ for 30 min. The supernatants were removed, and the pellets were washed two times with 0.1 ml of cold ethanol. The DNase-sensitive, TCA-soluble fractions (supernatant and ethanol washes) were dried onto glass fiber filters, overlaid with Ecosint (National Diagnostics, Manville, N.J.), and counted in a Packard liquid scintillation counter. The DNase-resistant fractions (TCA-precipitated pellets) were resuspended in 0.1 ml of Tris-EDTA buffer and processed for counting as described above. To determine the percentage of uncoated virus, the TCA-soluble counts were divided by the total amount of 3 H radioactivity (TCA-soluble plus TCA-precipitated pellet counts per minute) and multiplied by 100.

Cell fractionation and viral compartmentalization. The method used was previously described (7). Briefly, purified 3 H-labeled virus (MOI = 200 to 500 FFU per cell) was adsorbed to cells at 4°C, washed with cold medium, and incubated at 37°C as described above. At various times postinfection, the amount of virus penetrating the nucleus was assayed by comparing the radioactivity found in the nuclear fraction with the counts in the cytoplasmic fraction. The cytoplasmic fraction was recovered as the supernatants of cells lysed with alkaline hypotonic buffer (ATE buffer; 10 mM Tris-HCl [pH 8.9], 1 mM EDTA) and then with TKMT buffer (50 mM Tris-HCl [pH 7.4], 25 mM KCl, 5 mM $MgCl_2$, 0.5% Triton X-100) containing 0.5% Sarkosyl. The residual pellet contained nuclei, insoluble cell organelles, and matrix. Equal volumes of cold 10% TCA were added to the cellular fractions, and the TCA precipitates were washed with cold ethanol, dried onto glass fiber filters, and measured for 3 H radioactivity. The percent nuclear penetration was determined by dividing the counts in the nuclear fraction by the total number of counts (nuclear plus cytoplasmic) and multiplying by 100.

Transfection of KB and KBT4 cells with Hirt DNA. Adenovirus DNA was isolated from infected KB cells as Hirt DNA (17). The viral DNA was digested with proteinase K, extracted with phenol and chloroform, precipitated with cold ethanol, and then gel purified. KB or KBT4 cells (2.5×10^6) were seeded in 60-mm-diameter dishes. The culture medium was removed after 18 h, and the subconfluent monolayers were transfected with 20 μ g of Ad7 DNA or with 15 μ g of Ad5 DNA and 5 μ g of carrier DNA, using the calcium phosphate method (13).

A.

B.

FIG. 1. Cytofluorometric analysis to detect cell surface T4 expression. KB (A) and KBT4 (B) cells were removed from their growth surfaces by treatment with EDTA and reacted with monoclonal anti-CD4 antibody OKT4 and then with a fluorescein-conjugated sheep anti-mouse antibody (Cappel). The cells were fixed in 1% paraformaldehyde before analysis in a fluorescence-activated cell sorter (FACS IV; Becton Dickinson).

RESULTS

Transformation of KB cells with CD4 gene. To obtain a monolayer cell line that can be readily infected with HIV, KB cells were transfected with plasmid DNA containing the CD4 and neomycin resistance genes (23). The transfected cells were cultured in a medium containing G418 (500 μ g/ml) to select and clone the CD4-transformed cells. Immunofluorescence flow cytometry demonstrated the expression of CD4 on the surfaces of KBT4 cells (Fig. 1). The ability to infect KBT4 cells with HIV and their production of infectious virus (measured by reverse transcriptase, p24 antigen, syncytium formation, and infection of other KBT4 cells) further indicated that the KBT4 cells expressed CD4, the HIV receptor, on their surfaces (unpublished data).

Replication of adenovirus in KBT4 cells. The decreased ability of KBT4 cells to support adenovirus production was noted when yields from Ad5- and Ad7-infected KB cells were compared with those from virus-infected KBT4 cells. KB and

FIG. 2. Adenovirus multiplication in KB and KBT4 cells. Monolayer cultures were infected with 5, 25, and 125 FFU of Ad5 (A) or Ad7 (B) per cell. Samples were withdrawn at the times indicated, freeze-thawed six times to release intracellular virus, and titrated by fluorescent focus assay on A549 cells. Symbols represent MOIs of 5 in KB (○) and KBT4 (●) cells, 25 in KB (□) and KBT4 (■) cells, and 125 in KB (△) and KBT4 (▲) cells.

KBT4 cells were each infected with 5 to 125 FFU of Ad5 and Ad7 per cell, and the viral yields were assayed on KB cells. Replication of Ad5 and Ad7 in permissive KB cells for 3 days resulted in maximum yields of 9×10^3 FFU per cell in 48 h with an inoculum of 125 FFU per cell of Ad5 in KB cells and a yield of 2.5×10^2 FFU per cell in KBT4 cells with the same MOI. These differences in yields were consistently of the same order of magnitude when the MOI was 5, 25, or 125 FFU per cell (Fig. 2). The suppression of Ad7 replication in KBT4 cells was even greater than that of Ad5, and the viral yield was more suppressed with the higher viral MOIs (Fig. 2B). The reasons for this latter phenomenon are still not understood. Thus, regardless of the quantity of virus used to infect the two different cell lines, the differences in viral yields could not be overcome. Also, the decreased viral yields in KBT4 cells could

FIG. 3. Effect of rsCD4 on Ad5 absorption. KB cell monolayers were treated with rsCD4 or bovine serum albumin (BSA) for 1 h before Ad5 infection (5 FFU per cell). rsCD4 or BSA was maintained in the culture medium (final concentration = 50 μ g/ml) throughout the infection. KB and KBT4 cells infected in the absence of CD4 or BSA were included as controls. At 24 h postinfection, the infected cells were harvested and virus yields were determined.

not be attributed to general metabolic differences in the two cell lines, since the growth rates of KB and KBT4 cells were similar. Moreover, neither cell line was contaminated with mycoplasmas. The only apparent difference was the expression of CD4 molecules in KBT4 cells. Furthermore, when replication of Ad5 was compared in HeLa and HeLaT4 cells, the decreased viral yield in HeLaT4 cells was of the order of 90%, which was similar to that in KBT4 cells (data not presented).

Effect of CD4 on initial stages of adenovirus infections in KB and KBT4 cells. Since KBT4 cells express CD4 on their surfaces, it was possible that the CD4 molecules interfered with adenovirus-receptor interactions. To test this possibility, the efficiency of Ad5 infection in the presence of 50 μ g of soluble CD4 per ml was examined. Fifty micrograms of recombinant soluble CD4 (rsCD4) per ml, a quantity sufficient to block adsorption of HIV-1 to KBT4 cells (unpublished data), was added to KB and KBT4 cells before infection with 5 FFU of Ad5 per ml, and rsCD4 was maintained in the culture medium throughout infection. KB and KBT4 cells were similarly infected in the absence of rsCD4. The cells were harvested 24 h after infection, and viral yields were determined. The presence of rsCD4 did not affect viral production (Fig. 3). In the presence or absence of rsCD4, Ad5 viral yields from KB cells averaged 500 FFU per cell, whereas the yield from KBT4 cells was decreased approximately 99%. These data imply that the cell surface-active region of the CD4 receptor is not responsible for the reduction in Ad5 and Ad7 viral yields.

Earlier studies showed that adenoviruses of different subgroups probably have different receptors, since there is no

FIG. 4. Adsorption of Ad5 and Ad7 to KB and KBT4 cell monolayers. Cells were infected with equal amounts of purified, radiolabeled adenovirus, and samples were removed and washed three times with PBS at the indicated times. The cell pellet was dissolved in Ecoscint, and the amount of adsorbed virus was determined by measuring radioactivity. (A) ^3H -Ad5 adsorption to KB (●) and KBT4 (○) cells and ^{35}S -Ad5 adsorption to KB (■) and KBT4 (□) cells; (B) ^3H -Ad7 adsorption to KB (●) and KBT4 (○) cells.

heterotypic competition for cell membrane receptors (7). The decreased viral production in KBT4 cells could be due to a decreased number of these receptors on their surfaces, which would result in a reduction in the adsorption efficiency of virus. Rates of viral adsorption to KB and KBT4 cells were compared by using purified radiolabeled viruses. The results of adsorption experiments (Fig. 4) indicated that both ^3H - and ^{35}S -Ad5, as well as ^3H -Ad7, adsorbed to both cell lines at similar rates and to the same extent. These results support previous data showing that subgroup B and subgroup C viruses have similar association constants for the binding reactions with their receptor sites (7). These data further indicate that the reduced

adenovirus production in KBT4 cells was not due to an impairment in this initial step in viral infection.

Transcription of early viral RNAs in KB and KBT4 cells. The difference in viral yields in the two cell lines could be due to differences in transcription of early RNAs, the earliest biosynthetic events in the viral replication cycle. The presence of early adenovirus RNA in KB and KBT4 cells during the course of infections was examined by Northern (RNA) blot analysis using a cDNA clone for the Ad5 E1a RNAs as a probe. KB and KBT4 cells were infected with 1 FFU of Ad5 or 5 FFU of Ad7 per cell, and total cellular RNAs were isolated 8, 18, and 24 h postinfection. Ad5 E1a RNAs were detected at the same times in both cell lines, but fewer copies were present in KBT4 cells (Fig. 5A), and therefore a long exposure time was required to detect the E1a transcripts in the Ad5-infected KBT4 cells. The inability to detect the E1a RNAs earlier in infection was undoubtedly due to the small infectious dose of virus used.

An Ad5 E1a cDNA probe permitted a comparison of the relative levels of Ad7 E1a RNA in infected KB and KBT4 cells, since the E1a sequences of Ad5 and Ad7 are about 50% homologous (37). Ad7 E1a RNAs were first detected 18 h postinfection in KB cells (Fig. 5B) but could not be detected in KBT4 cells even 24 h after infection. Since analysis with a nick-translated actin cDNA insert demonstrated that equal amounts of RNA had been transferred, it can be concluded that the total amount of virus-specific E1a RNA transcribed in KBT4 cells was significantly lower than in KB cells.

Uncoating of viral DNA in KB and KBT4 cells. To identify the block in adenovirus production in KBT4 cells, the stages of viral replication between viral adsorption and transcription of early viral RNAs were examined.

The capsid of an intact viral particle forms a protective shell around the viral genome; "uncoating" refers to alterations of the capsid to release the viral genome, which renders the genome sensitive to nuclease hydrolysis. After adenoviruses attach to specific receptors on the plasma membrane, the receptors and viruses are internalized via coated pits and vesicles and are temporarily housed in endosomes, which then become acidified (15, 27, 30, 32). This process results in the release of pentons, which renders the virion DNA sensitive to DNase (27, 29, 32). Thus, partial uncoating occurs shortly after adsorption, probably in the endocytic vesicles, which the penton then punctures to permit the partially uncoated virion to enter the cytoplasm (29).

The initial viral uncoating step was compared in KB and KBT4 cells by determining the time at which viral DNA became sensitive to DNase. Patterns of uncoating of cell-associated Ad5 and Ad7 DNAs were similar in KB and KBT4 cells (Fig. 6), but Ad5 was uncoated slightly faster than Ad7. At 1 h postinfection, 70 to 80% of cell-associated Ad5 radioactivity and 50 to 60% cell-associated Ad7 radioactivity was DNase sensitive. These data indicate that the decreased replication of Ad5 and Ad7 in KBT4 cells was not the result of defective viral uncoating in these cells.

Nuclear penetration by adenovirus into KB and KBT4 cells. Previous studies showed that although the viral capsid is altered to expose the viral DNA to DNase, viral proteins are still associated with the DNA (22). Final uncoating occurs within the vicinity of the nuclear envelope, and viral DNA extracted from the nuclei sediments with the buoyant density of free adenovirus DNA (2). Measurement of the amount of viral DNA within the nuclei permitted determining whether the CD4 molecules affected transport of viral DNA into the nucleus and, hence, the quantity of DNA available for synthesis of early viral RNA.

FIG. 5. Transcription of Ad5 or Ad7 in KB and KBT4 cells. KB and KBT4 cells were infected, and total cellular RNA was isolated at the indicated times. After electrophoresis in formaldehyde denaturing agarose gels, the RNAs were transferred to a nitrocellulose filter, immobilized, and then hybridized to an Ad5 ^{32}P -labeled, nick-translated probe. Actin RNA served as an internal control between different samples. (A) Ad5-infected cells (MOI = 1 FFU per cell); (B) Ad7-infected cells (MOI = 5 FFU per cell).

Ad5- and Ad7-infected cells were fractionated into nuclear and cytoplasmic compartments, and the amount of ^3H -labeled viral DNA (intravirion and uncoated) in each fraction (TCA-precipitable counts) was determined at increasing times after infection (Fig. 7). By 1 to 2 h postinfection, 80% of the adsorbed Ad5 DNA and 70% of the adsorbed Ad7 DNA had reached the nuclei of KB cells. Previous studies attribute differences in viral recovery in the nuclear fraction to differences in their modes of travel to the nucleus. While members of subgroup C (Ad2 and Ad5) mediate lysis of endosomal membranes and are released into the cytoplasm, members of subgroup B (Ad3 and Ad7) were reported to be sequestered within phagosomes during passage to the nucleus (2, 3, 7, 26).

The amount of Ad5 reaching the nucleus of KBT4 cells 1 h after infection was slightly less than in KB cells, but this difference was reproducible and compatible with the smaller amounts of Ad5 early RNAs transcribed in KBT4 cells at 8 and 18 h postinfection and with production of lower levels of infectious virus 24 h after infection. The differences between KB and KBT4 cells were much greater when the cells were infected with Ad7. At 2 h postinfection, only 24% of the adsorbed ^3H -Ad7 was found in the nuclear fraction of KBT4 cells. These differences were not due to loss of counts during the fractionation procedures, since the total number of TCA-precipitable counts remained fairly constant for each cell line (data not shown).

Transfection of viral DNA into KB and KBT4 cells. If the differences in viral yields between KB and KBT4 cells were due to differences in transport of the uncoated viral DNA-protein complex to the nucleus and nuclear entry of the viral DNA, transfection of naked viral DNA into the cells should yield comparable amounts of virus in both cell lines. This possibility was tested: 35 h after transfection of Ad5 and Ad7 DNA into

KB and KBT4 cells, cells were harvested and viral yields were determined. Although the viral titers were low, which is consistent with yields from transfected viral DNA, the yields were similar for each virus in the two cell lines (Fig. 8).

DISCUSSION

An earlier study indicated that adenovirus replication in human monocyte lines was hampered because of a block in a late stage of synthesis, probably during production of late viral mRNAs (5). It was also found that viral uncoating was slower and less efficient in human peripheral blood lymphocytes than in HEP-2 cells (19). In contrast, Lavery and colleagues (21) reported that the kinetics of Ad5 infection in a B-cell myeloma line and a T-cell line were similar to those in HeLa cells. Thus, there may be many different levels of restriction to adenovirus production in human lymphocytes, resulting in permissive, semipermissive, or nonpermissive infection. These differences could be the result of the presence or absence of CD4-expressing cells or the differences in the number of cells expressing CD4 in the various cells used.

The data obtained in this study demonstrate that adenovirus production is less in KBT4 cells than in the parental KB cells and that the earliest block to viral production occurs after early stages of viral uncoating, at the stage of cytoplasmic transport or completion of viral DNA uncoating and nuclear penetration. Since the only known difference between the two cell lines is the presence of CD4 in the KBT4 cells, the results of this study lead to the conclusion that CD4 interferes with viral production at an early step in viral infection and that the CD4-virus interaction results in a semipermissive infection.

The T4 receptor consists of several structural domains: an N-terminal extracellular region containing a leader sequence

A.

B.

FIG. 6. Uncoating of ^3H -labeled adenovirus DNAs. Purified ^3H -labeled adenovirus (MOI = 250 FFU per cell) was adsorbed to KB and KBT4 cells at 8°C for 15 min. The cells were washed three times, overlaid with warm medium, and incubated at 37°C . At indicated times, samples were removed, the cells were lysed and treated with DNase (200 $\mu\text{g}/\text{ml}$) as described in Materials and Methods. The percent uncoated virus equaled the amount of ^3H radioactivity (TCA-soluble DNA plus TCA-precipitated pellet) times 100. (A) ^3H -labeled Ad5-infected cells; (B) ^3H -labeled Ad7-infected cells.

and four immunoglobulin-like domains; a membrane-spanning region; and a highly charged cytoplasmic domain (24). It is noteworthy that infection of KB cells in the presence of rsCD4, which lacks the membrane-spanning and cytoplasmic domains, did not interfere with adenovirus adsorption and that viral DNA in the cytoplasmic fraction of infected cells is still associated with viral proteins. Therefore, in KBT4 cells, it is possible that the membrane-spanning and/or cytoplasmic domains, which are not present in rsCD4, may interact with the viral core proteins to restrict transport of the partially uncoated DNA-protein complex and entry of viral DNA into the nucleus. This event(s) would result in the reduction of early

A.

B.

FIG. 7. Nuclear penetration by ^3H -labeled Ad5 and Ad7 DNAs. Purified ^3H -labeled adenovirus (MOI = 250 FFU per cell) was adsorbed to KB and KBT4 cell monolayers at 8°C for 15 min. The cells were washed three times, overlaid with warm medium, and incubated at 37°C . At the indicated times, samples were removed and fractionated into nuclear and cytoplasmic compartments, and the ^3H -labeled viral DNA (virion or uncoated) in each fraction was precipitated with TCA as described in Materials and Methods. The percent nuclear penetration was determined by dividing the amount of ^3H radioactivity in the nuclear fraction by the total amount of ^3H radioactivity and multiplying by 100. (A) ^3H -labeled Ad5-infected cells; (B) ^3H -labeled Ad7-infected cells.

viral RNA transcription noted. Alternatively, the uncoating process could reveal hidden viral epitopes that react with the intracellular domains of the CD4 receptor. In support of these possibilities, removal of the viral proteins and transfection of KBT4 cells with naked viral DNA eliminated these putative interfering protein-protein interactions and resulted in comparable levels of viral production in KB and KBT4 cells (Fig. 8). Moreover, the fact that transfected viral DNAs produce similar Ad5 and Ad7 yields in KB and KBT4 cells implies that the decreased viral replication in KBT4 cells is not a feature of the

FIG. 8. Yields of Ad5 and Ad7 after transfection of viral DNAs into KB and KBT4 cells. Hirt DNA was isolated from virus-infected KB cells, treated with proteinase K, extracted with phenol and chloroform, precipitated with ethanol, and gel purified as described in Materials and Methods. KB and KBT4 cells were transfected with 15 μ g of purified Hirt Ad5 DNA or 20 μ g of purified Hirt Ad7 DNA. At 35 h postinfection, the cells were harvested and viral yields were determined by fluorescent focus assay on KB cells.

particular KBT4 cell line used in this study but rather is a reaction of the CD4 molecule on an early event in adenovirus multiplication. Moreover, the fact that Ad5 replication in HeLaT4 cells is also less than in parental HeLa cells strengthens the conclusion that the expressed CD4 molecules, not a particular cell line, is responsible for the suppressed adenovirus replication.

The decrease in nuclear penetration was much more pronounced for Ad7 than Ad5, which suggests that the restriction of adenovirus replication in the CD4-bearing cells may be related to subtle differences in the virion structures of these viruses or molecular events in their replication cycles. Viruses of subgroups B and C differ in their modes of travel to the nucleus. While Ad5 is engulfed by and penetrates through the endosomal vesicles very quickly and travels to the nucleus in the cytoplasm, Ad7 is reported to pass to the nucleus in large phagosomes (2, 3, 7, 26). How intralysosomal Ad7 DNA is actively transferred into the nucleus is not known. The interfering CD4-viral protein interactions could occur while the viral particles are housed in the endosomes. A delay and reduction in the translocation of viral DNA across the endosomal membranes into the cytoplasm and/or transfer to the nucleus would lead to the observed delay and reduction of early viral RNA transcription and viral replication in KBT4 cells.

CD4 interference with viral replication, however, may be only fortuitous. After lysis of the endocytic vacuoles, viral particles that penetrate into the cytoplasm appear to bind

specifically to microtubules (6, 38), after which they are rapidly transported to the nuclear membrane. Expression of a foreign protein, CD4, in the cell may alter its cytoskeleton to effect interactions between viral proteins and microtubules and thereby inhibit transport of viral particles to the nucleus. However, this hypothesis may only partially explain the findings that adenoviruses have limited replication in peripheral blood lymphocytes (19) even though viral DNAs are present (12, 18).

The results presented here occurred in an artificial environment, in nonlymphoid cells transformed with and expressing the CD4 T-cell surface receptor gene. Therefore, the relevance of these findings to viral pathogenesis is ambiguous. Greater understanding of the molecular biology of adenovirus-human lymphoid cell interactions that result in nonpermissive or semipermissive infections and of the role of CD4 expression should help clarify the mechanism(s) by which adenoviruses are dormant in lymphoid organs and the potential role of adenovirus persistent infections in human diseases.

ACKNOWLEDGMENTS

We thank L. Chess for rsCD4 and OKT4, W. Wong for technical assistance, and G. Liu for helpful discussions throughout the course of this work.

This research was supported by Public Health Service grants AI-26886 and AI-12052 from the National Institute of Allergy and Infectious Diseases.

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