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Coexpression of the Simian Immunodeficiency Virus Env and Rev Proteins by a Recombinant Human Adenovirus Host Range Mutant

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Recombinant human adenoviruses (Ads) that replicate in the intestinal tract offer a novel, yet practical, means of immunoprophylaxis against a wide variety of viral and bacterial pathogens. For some infectious agents such as human immunodeficiency virus (HIV), the potential for residual infectious material in vaccine preparations must be eliminated. Therefore, recombinant human Ads that express noninfectious HIV or other microbial proteins are attractive vaccine candidates. To test such an approach for HIV, we chose an experimental model of AIDS based on simian immunodeficiency virus (SIV) infection of macaques. Our data demonstrate that the SIV Env gene products are expressed in cultured cells after infection with a recombinant Ad containing both SIV *env* and *rev* genes. An E3 deletion vector derived from a mutant of human Ad serotype 5 that efficiently replicates in both human and monkey cells was used to bypass the usual host range restriction of Ad infection. In addition, we show that the SIV *rev* gene is properly spliced from a single SIV subgenomic DNA fragment and that the Rev protein is expressed in recombinant Ad-SIV-infected human as well as monkey cells. The expression of SIV gene products in suitable live Ad vectors provides an excellent system for studying the regulation of SIV gene expression in cultured cells and evaluating the immunogenicity and protective efficacy of SIV proteins in macaques.

Major advances made in recent years on the protection of macaques by inactivated whole virus vaccines against simian immunodeficiency virus (SIV) infection suggest that immunoprophylaxis against the human immunodeficiency virus (HIV) is possible (7, 28). However, the use of inactivated virions for HIV vaccines is limited by concerns about residual infectious materials in vaccine preparations. Therefore, safe and effective vaccines composed of noninfectious HIV antigens are desirable. Recently, Hu et al. (18) have shown protection of macaques against a homologous SIV challenge by recombinant subunit vaccines including a live recombinant vaccinia virus expressing SIVmne gp160 for priming and subunit gp160 for boosting. These results provide hope that live viral subunit vaccine approaches to AIDS vaccine development may be feasible.

Replication-competent recombinant DNA viruses that efficiently express foreign gene products are potentially useful as live viral subunit vaccines. For this purpose, human adenoviruses (Ads) are suitable vectors for the expression of foreign (non-Ad) genes because several regions of the 36-kbp Ad DNA genome are dispensable for viral replication (6, 27). In particular, the early region 3 (E3) and its gene products are nonessential for Ad replication in cell culture (19) and animals (12, 23, 27) and can be replaced by foreign DNA. Examples of foreign genes expressed by an Ad vector with an E3 deletion include the hepatitis B surface antigen and HIV type 1 (HIV-1) envelope glycoprotein (2, 8, 23, 27). Importantly, orally administered live wild-type Ad vaccines, used for more than 20 years in military recruits, are well tolerated, safe, and effective (5, 10). Thus, Ad-HIV recombinant viruses are attractive human vaccine candidates.

Experimental infection of macaques with SIV is a relevant and well-characterized animal model of AIDS (7, 28). The SIV model provides an accessible system for testing a wide variety of candidate vaccines, including recombinant Ad vectors expressing SIV gene products. However, there are two potential obstacles for the development and testing of Ad-SIV recombinant viruses. First, Ad replicates in the host cell nucleus, thereby exposing viral RNA transcripts to potential splicing events. Therefore, the efficient expression of SIV structural proteins (e.g., Gag or Env) by Ad may require the presence of Rev, an SIV (and HIV) *trans*-acting nuclear regulatory protein that positively influences the accumulation of unspliced mRNAs for viral structural proteins in the host cell cytoplasm (3, 4, 14, 15, 24, 25). Second, human Ad displays a restricted host range and does not replicate to a high titer in monkey cells in culture (11). Thus, an Ad vector capable of efficient replication in monkey cells is required (20).

In this report, we describe the construction and in vitro characterization of human Ad-SIV recombinant viruses that surmount the obstacles listed above. We demonstrate that (i) the SIV Env and Rev proteins are coexpressed in cells infected with the Ad-SIV recombinant containing a single SIV genomic fragment; (ii) the *rev* gene is properly spliced in these cells; (iii) a human Ad host range mutant that efficiently replicates in monkey cells in culture can serve as a vector for the expression of SIV proteins.

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MATERIALS AND METHODS

Cells and viruses. The human cell lines 293 (ATCC CRL1573) and A549 (ATCC CCL185) and the monkey cell line CV-1 (ATCC CCL70) were maintained in Dulbecco modified Eagle medium supplemented with 10% heat-inactivated fetal calf serum. Ad type 5 (Ad5) containing a host range mutation (Ad5hr404) was kindly supplied by D. Klessig (Rutgers University). Ad5hr404 contains an amino acid substitution (His→Tyr) at map unit 64 in the 72-kDa DNA-binding protein. This mutation results in increased Ad late-phase protein synthesis and more efficient replication in monkey cells. Ad5hr404 and all recombinant Ads described below were grown in 293 cells, and their titers were determined in 293, A549, and CV-1 cells. All Ad infection experiments were done at a multiplicity of infection of 20 PFU per cell.

Recombinant plasmids and Ads. The right end of Ad5hr404 (*Bam*HI fragment B, map units 59.3 to 100) containing an E3-region deletion (from map units 78.8 to 85.7) was cloned into the *Bam*HI site of pBR322 to yield pBRAd5hrΔE3 (2.5)BamB. Ad5hr404-SIV recombinant plasmids were constructed by cloning SIV subgenomic DNA fragments into an *Xba*I cloning site downstream of the E3 promoter in pBR Ad5hrΔE3(2.5)BamB. The characteristics of SIV DNA inserts are described in Fig. 1A. pEXO17/E3 contains an SIVsm subgenomic fragment spanning from nucleotides 6403 to 9470 (numbered according to reference 16). This *Xba*I DNA insert was obtained by modifications of a previously engineered *Bam*HI subgenomic fragment (4). To convert the *Bam*HI ends to *Xba*I ends, the DNA fragment was first digested with *Bam*HI, filled in with deoxynucleoside triphosphates by using DNA polymerase I (Klenow fragment), and then ligated to *Xba*I linkers. pENV9/E3 is almost identical to pEXO17/E3 except that coding exon 1 of *rev* (from nucleotides 6403 to 6549) has been completely deleted. The *Xba*I DNA insert was synthesized by the polymerase chain reaction with pEXO17/E3 as the template. Primers are 5′-GGTCTAGAATGGGATGTCTTGGGAATCA-3′ and 5′-GGTCTAGATCACGGACTCTTTGCAACGT-3′; they contain *Xba*I ends (as underlined) and SIV sequences from nucleotides 6550 to 6569 and 9470 to 9451, respectively. Both pEXO17/E3 and pENV9/E3 were subjected to DNA sequence analyses to confirm the nucleotide sequences of the SIV DNA insert. Plasmid and Ad DNAs (Fig. 1B) were introduced into 293 or A549 cells by cation-mediated transfection (Lipofectin; Bethesda Research Laboratories, Inc., Gaithersburg, Md.) according to protocols supplied by the manufacturer. The resulting recombinant Ads are shown in Fig. 1C.

Protein and RNA analyses. For immunoprecipitation experiments, Ad-infected cells were labeled with [^{35}S]methionine and [^{35}S]cysteine (Amersham) in Dulbecco modified Eagle medium devoid of methionine, cysteine, and fetal calf serum for various periods after infection. Radiolabeled cell lysates were immunoprecipitated with serum from a normal or SIV-infected macaque (3) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography. Details of Western blot (immunoblot) analyses are described in the legends to Fig. 4 and 5A.

For RNA analyses, total cellular RNA was isolated by the guanidinium isothiocyanate method (13) and subjected to Northern (RNA) blot analyses (4) with various radiolabeled DNA probes. Some experiments were done in the presence of the DNA replication inhibitor cytosine arabinonucleoside (Ara-Cyt) (Sigma Chemical Co., St. Louis, Mo.).

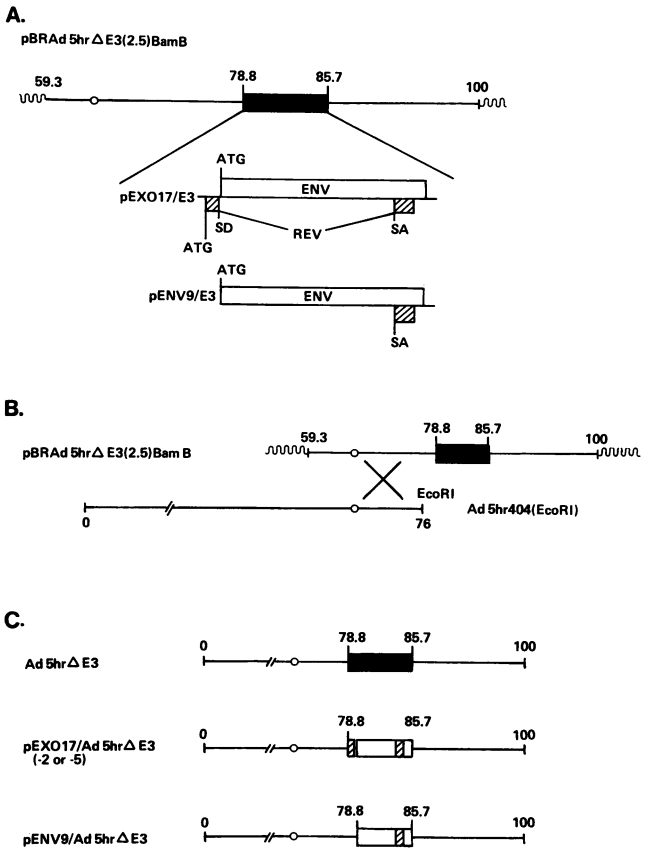

FIG. 1. Construction and description of recombinant Ads. (A) pEXO17/E3 contains an SIV proviral DNA fragment (from nucleotides 6403 to 9470) with the capacity to encode the *env* gene (open box) and both exons of the *rev* gene (shaded boxes). pENV9/E3 is nearly identical to pEXO17/E3 except that coding exon 1 of *rev* (from nucleotides 6403 to 6549) has been completely deleted. The E3 deletion is represented by the black rectangle. A single amino acid substitution responsible for the host range mutation is denoted by a small open circle at map unit 64 (see Materials and Methods). (B) Generation of live Ad-SIV recombinants. The desired Ad-SIV recombinant plasmid (see panel A) was cotransfected with *Eco*RI-digested Ad5hr404 DNA into 293 or A549 cells, and viable recombinant Ads were generated by homologous recombination. Recombinant viruses were analyzed by restriction enzyme mapping of Hirt DNA (17) isolated from infected 293 cells. (C) Depiction of the structure of the recombinant Ads. Ad5hrΔE3 is Ad5hr404 with the described E3 deletion. pEXO17/Ad5hrΔE3 contains the SIV sequences in pEXO17/E3 described in panel A; two separate recombinant viruses were obtained (−2 and −5) that were identical by biochemical and functional analyses. pENV9/Ad5hrΔE3 contains the SIV sequences in pENV9/E3 described in panel A.

Syncytium induction assays. A549 or CV-1 cells were infected with a recombinant Ad; 24 h later, the infected monolayer was overlaid with CEMx174 cells (a human CD4$^+$ T-lymphocyte cell line [21]) at a ratio of 10:1 (CEM to A549 or CV-1). Cocultures were evaluated daily for the presence of multinucleated giant cells (indicating the presence of biologically functional SIV Env protein).

RESULTS

Construction of recombinant Ad containing the SIV *env* gene. Specific SIV DNA sequences were introduced down-

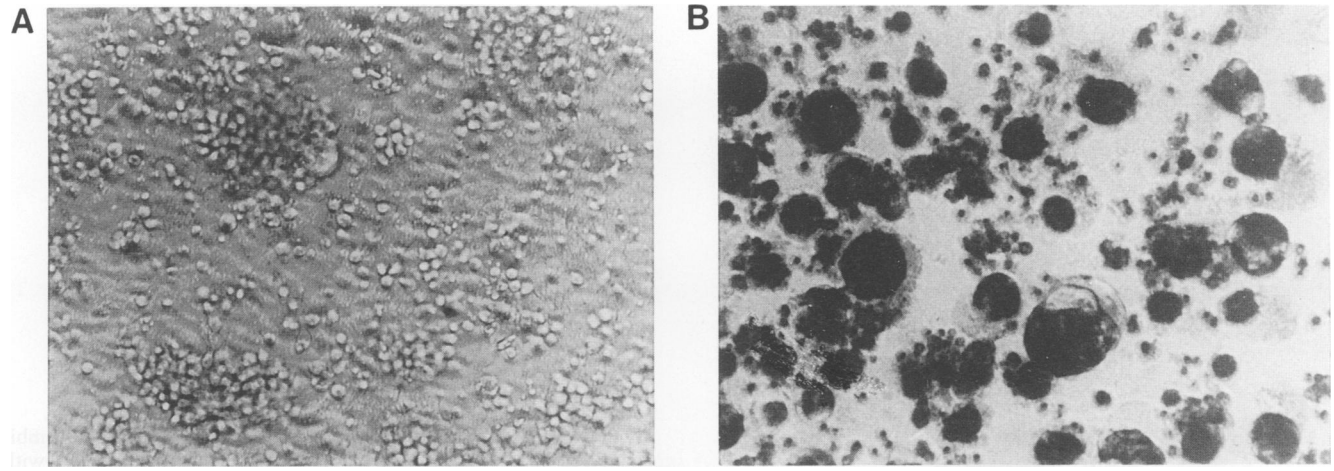

FIG. 2. Syncytium formation in CEMx174 cells after cocultivation with A549 cells infected with recombinant Ads. A549 cells were infected with pENV9/Ad5hrΔE3 (*rev env*$^+$) (A) and pEXO17/Ad5hrΔE3 (*rev*$^+$ *env*$^+$) (B). Twenty-four hours after Ad infection, A549 monolayers were overlaid with CEMx174 cells (see Materials and Methods). Photomicrographs were taken 72 h after cocultivation.

stream of the E3 promoter in the human Ad5hr404 E3-region deletion mutant as described in Fig. 1. Two Ad5hr404-SIV recombinants were constructed. (i) pEXO17/Ad5hrΔE3 contains a subgenomic fragment of SIVsm (a biologically active proviral DNA clone of SIVsm [16]); this fragment contains the *env* gene and both coding exons of the *rev* gene (*rev*$^+$ *env*$^+$). (ii) pENV9/Ad5hrΔE3 contains a nearly identical SIV DNA insert except that coding exon 1 of the *rev* gene (upstream of the *env* gene initiation codon) was removed; thus, this recombinant virus is only capable of expressing the SIV *env* gene (*rev env*$^+$). An Ad5hr404 derivative containing only the E3 deletion (Ad5hrΔE3) was constructed as a control (Fig. 1C). All three Ads described above were plaque purified before further biochemical and functional characterization.

Rev is required for efficient expression of the SIV Env protein in Ad. We previously demonstrated that the SIV *rev* gene is required for the efficient expression of the SIV *env* gene in a simian virus 40-directed transient expression system (3, 4). Because Ad, like the simian virus 40 vector, replicates in the host cell nucleus, we reasoned that the *rev* gene might also be required for SIV *env* gene expression in Ad vectors. To screen quickly for the expression of the SIV Env protein, we cocultured A549 cells infected with Ad5hrΔE3 (negative control; data not shown), pENV9/Ad5hrΔE3 (*rev env*$^+$ construct; Fig. 2A), or pEXO17/Ad5hrΔE3 (*rev*$^+$ *env*$^+$ construct; Fig. 2B) with CEMx174 cells and looked for syncytium formation. Significant syncytium formation was observed in cocultures of CEMx174 cells with A549 cells infected with pEXO17/Ad5hrΔE3 (Fig. 2B). In contrast, only a few giant cells per 10-cm dish were observed in a coculture of CEMx174 cells and A549 cells infected with pENV9/Ad5hrΔE3 (Fig. 2A). The differences in numbers of giant syncytium cells induced by pEXO17/Ad5hrΔE3 versus pENV9/Ad5ΔE3 are greater than 100-fold. The same observation was obtained when CEMx174 cells were cocultured with CV-1 cells infected with Ad-SIV recombinants (data not shown). These data suggested that the SIV *rev* gene was required to enhance SIV Env protein expression in Ad-SIV recombinant virus-infected cells. This is parallel to our previous finding that SIV Env and Rev were expressed from a simian virus 40 system.

SIV-specific RNA transcripts are properly spliced in pEXO17/Ad5hrΔE3-infected cells. To analyze SIV-specific RNA transcripts, we isolated total cellular RNA from A549 cells after infection with pEXO17/Ad5hrΔE3 for 24 h in either the presence (early transcripts) or absence (early plus late transcripts) of Ara-Cyt (which inhibits viral DNA replication and late transcription). For early transcripts (Fig. 3A), three identical RNA blots (described below) were processed in parallel and were hybridized separately with one of three DNA probes. The probe for lane 1 (Fig. 3A) was specific for exon 1 of *rev* (*rev*-only probe). The probe for lane 2 (Fig. 3A) was derived from coding exon 2 of *rev* and therefore would bind to *rev* and *env* transcripts (*rev* and *env* probe). The probe for lane 3 (Fig. 3A) was specific for *env* RNA (*env*-only probe).

Three major early SIV-specific RNA transcripts were observed (Fig. 3A): a 1.3-kb species (detected with probes 1 and 2); a 4-kb species (detected with all three probes); and a 5.5-kb species (detected only with probe 1). The 1.3-kb RNA contained the spliced *rev* transcript and was predicted to initiate from the Ad E3 promoter and terminate in the E3B polyadenylation sequence. Because this small RNA species hybridized only with probes containing *rev* sequences (Fig. 3A, lanes 1 and 2), it was considered to represent the *rev* transcript. The 4-kb RNA was predicted to represent an unspliced RNA that initiated from the E3 promoter and terminated in the E3B polyadenylation sequence. This species contained the *env* mRNA because it hybridized with all three probes and was large enough to encode the Env protein. The 5.5-kb transcript (Fig. 3A, lane 1), although large enough to encode the Env protein, did not hybridize with the *env*-containing probes (lanes 2 and 3) and likely represented an alternate splicing event between the splice donor site of coding exon 1 of *rev* and a downstream splice acceptor site beyond the E3 region (Fig. 1A) (1).

The pattern of late SIV-specific RNA transcripts was more complicated, but indicated that late in infection, larger (*env*-containing) transcripts predominated (data not shown). The ratio of *env*-specific sequences in early plus late versus early RNA transcripts was greater than 30-fold (Fig. 3B). The results of RNA analyses of cells infected with pEXO17/Ad5hrΔE3 can be summarized as follows: (i) the expression

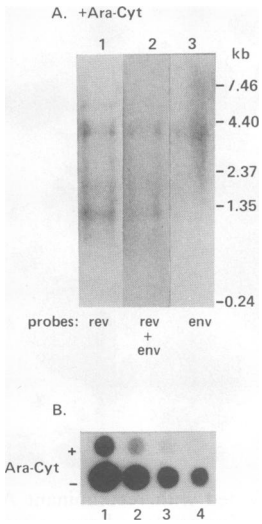

FIG. 3. RNA analyses of SIV-specific RNA transcripts in 293 cells infected with pEXO17/Ad5hrΔE3. Early RNA was isolated in the presence of Ara-Cyt and early plus late RNA was isolated in the absence of Ara-Cyt at 24 h p.i. (A) Northern analysis of early RNA. Total cellular RNA (10 μg) was separated on 1.3% formaldehyde-agarose gels, transferred to nitrocellulose, and hybridized with the following probes: lane 1, *rev* exon 1 probe (nucleotides 6481 to 6539 [16]); lane 2, *rev* exon 2 probe (also hybridizes with *env* RNA) (nucleotides 8766 to 9009 [16]); and lane 3, *env* probe (no *rev* sequences) (nucleotides 8692 to 8751 [16]). All probes were 5'-radiolabeled synthetic oligonucleotides (ranging in size from 60 to 100 nucleotides). Postelectrophoresis staining with acridine orange revealed that each lane had been loaded with an equivalent amount of RNA. (B) RNA dot hybridization. Serial dilutions of total RNA isolated in the presence or absence of Ara-Cyt were hybridized to the *env*-only probe. Lanes: 1, 5 μg; 2, 2.5 μg; 3, 1.25 μg; 4, 0.63 μg of RNA. The amount of *env*-specific RNA was estimated by microdensitometric tracings of the autoradiographs.

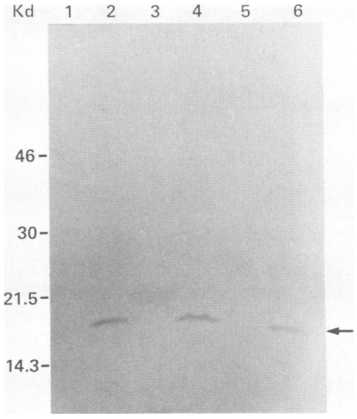

FIG. 4. Immunoblot analyses of SIV Rev protein from recombinant Ad-infected cells. A549 or CV-1 cells were infected with pEXO17/Ad5hrΔE3 (lanes 2 and 6) or pENV9/Ad5hrΔE3 (lanes 1 and 5), harvested at 48 h p.i., and solubilized. Lysates were adsorbed to protein A-Sepharose CL-4B beads conjugated to rabbit anti-3'-*rev* serum (2a). The SIV Rev-specific protein was electrophoresed through an SDS-16% polyacrylamide gel and transferred onto nitrocellulose membranes. Reaction with SIVsm immune serum was performed as previously described (4). COS-1 cells transfected with the plasmid with (lane 4) or without (lane 3) a *rev* cDNA insert were included as controls. Positions of molecular mass markers are shown on the left, and the arrow on the right shows the Rev protein.

of both *env* and *rev* transcripts was detected; (ii) the amount of unspliced mRNAs capable of encoding the Env protein was greatly increased during late infection.

Expression of Rev in pEXO17/Ad5hrΔE3-infected cells. To assess the presence of SIV Rev gene products in pEXO17/Ad5hrΔE3-infected cells, we reacted immunoblots containing the cell lysates with SIVsm immune serum (Fig. 4). An 18-kDa protein corresponding to the expected SIV Rev gene products was detected in infected human A549 (Fig. 4, lane 2) as well as monkey CV-1 (lane 6) cells. This Rev protein has the same mobility on an SDS gel as the Rev protein derived from COS-1 cells transfected with the plasmid containing a *rev* cDNA insert (Fig. 4, lane 4). No Rev production was observed in cells infected with pENV9/Ad5hrΔE3 (Fig. 4, lanes 1 and 5) or transfected with the plasmid lacking a *rev* cDNA insert (lane 3).

Expression and processing of SIV Env glycoprotein in pEXO17/Ad5hrΔE3-infected cells. In SIV-infected $CD4^+$ lymphocytes, the Env glycoprotein is translated as a precursor protein designated gp160. The gp160 precursor is cleaved by a trypsinlike host cell protease to yield the outer surface glycoprotein, gp120, and the transmembrane glycoprotein, gp40. This specific cleavage event is required for biologic activity (fusion) of the Env protein and virus infectivity (26). Because pEXO17/Ad5hrΔE3-infected cells induced syncytium formation (see above), we predicted that the SIV Env protein expressed by pEXO17/Ad5hrΔE3 would be properly processed into gp120 and gp40. Figure 5 shows that pEXO17/Ad5hrΔE3-infected monkey CV-1 (Fig. 5A, lane 2) and human 293 (lanes 4) cells produced expected sizes of SIV Env gp160, gp120, and gp40. Furthermore, gp120 was easily detected in the cell culture medium (also a feature of SIV-infected lymphocyte cultures) (Fig. 5B, lane 2). Therefore, SIV Env expression from pEXO17/Ad5hrΔE3-infected cells closely mimicked Env expression in SIV-infected lymphocytes in culture. Ad5hrΔE3-infected CV-1 (Fig. 5A, lane 1) and 293 (Fig. 5A, lane 3, and B, lanes 3 and 5) cells were included as the negative controls.

Kinetic analyses for production of SIV Env protein in pEXO17/Ad5hrΔE3-infected cells. To examine the early- and late-phase kinetics of SIV Env production in 293 cells infected with pEXO17/Ad5hrΔE3, cells were pulse-radiolabeled at 6 h or 24 h postinfection (p.i.) in the presence or absence of Ara-Cyt (Fig. 6). In the absence of Ara-Cyt, gp160 was more abundant in the late phase (24 h p.i.) than in the early phase (6 h p.i.) of infection (lane 2 versus lane 3), confirming the RNA analyses that demonstrated high levels of late-phase *env* transcripts (described above). In the presence of Ara-Cyt, early-phase gp160 production was virtually unaffected (Fig. 6, lane 1 versus lane 2); however, late-phase gp160 was dramatically reduced by Ara-Cyt (Fig. 6, lane 3 versus lane 4), reflecting inhibition of late transcription.

Ad-SIV recombinant virus retains the host range mutation. One of our goals was to construct a recombinant Ad that would be useful for future studies of immunogenicity in monkeys. The Ad vector (Ad5hr404) used in the construction of the recombinant virus contains a mutation (see Materials and Methods) that confers the ability to replicate efficiently in human and monkey cells in culture (20). To confirm that pEXO17/Ad5hrΔE3 retained this host range mutation, we determined the titers of doubly plaque-purified viruses pEXO17/Ad5hrΔE3 and wild-type Ad5 on A549 cells (human) or CV-1 cells (monkey). The results (Table 1)

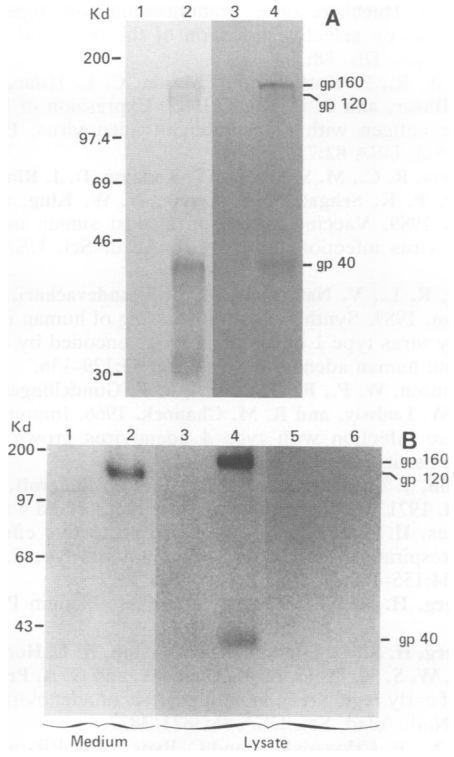

FIG. 5. Analyses of SIV Env proteins from recombinant Ad-infected cells. (A) Immunoblots. CV-1 or A549 cells were infected with pEXO17/Ad5hrΔE3 (lanes 2 and 4) or Ad5hrΔE3 (lanes 1 and 3), harvested at 48 h p.i., and solubilized. Lysates were adsorbed to agarose beads conjugated to lentil-lectin (Sigma). The glycoproteins were electrophoresed through an SDS-12% polyacrylamide gel (see Fig. 4 legend). (B) Radioimmunoprecipitation of SIV Env proteins from 293 cells infected with pEXO17/Ad5hrΔE3. Ad-infected cells were radiolabeled with [^{35}S]methionine and [^{35}S]cysteine for 16 h, lysed, and immunoprecipitated with SIV-infected macaque serum (lanes 1 to 4) or normal macaque serum (lanes 5 and 6). Lane 1 is culture medium, and lanes 3 and 5 are cell lysate from Ad5hrΔE3-infected cells (control). Lane 2 is culture medium, and lanes 4 and 6 are cell lysate from pEXO17/Ad5hrΔE3-infected cells. Immunoprecipitates were analyzed by SDS-PAGE and visualized by fluorography. Positions of molecular mass markers are shown on the left of each panel, and positions of SIV-specific polypeptides are shown on the right of each panel.

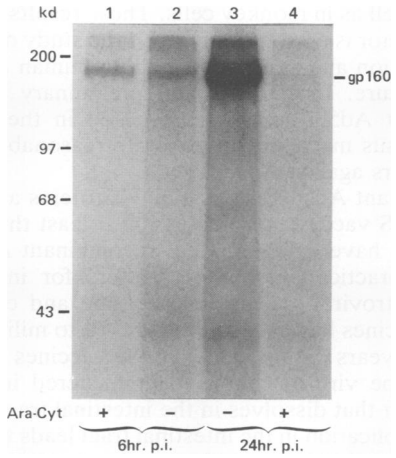

FIG. 6. Effect of Ara-Cyt on synthesis of the SIV Env protein. Two hours after infection with pEXO17/Ad5hrΔE3, Ara-Cyt (20 μg/ml) was added (lanes 1 and 4) or not added (lanes 2 and 3) to the culture medium. At 6 h (lanes 1 and 2) or 24 h (lanes 3 and 4) p.i., the cells were pulse-labeled for 30 min with [^{35}S]methionine and [^{35}S]cysteine (3). After the labeling period, the cells were lysed and immunoprecipitated with SIV-infected macaque serum. Positions of molecular mass markers are shown on the left, and positions of SIV-specific proteins are shown on the right.

suggested that pEXO17/Ad5hrΔE3 replicated equally well in human or in monkey cells, while wild-type Ad5 replicated over 1,000-fold better in human cells than in monkey cells in culture.

DISCUSSION

Our data suggested that expression of the SIV Env protein by an Ad-SIV recombinant virus was regulated by heterologous and autologous *cis*- and *trans*-acting elements. The heterologous regulatory factors were supplied by a single subgenomic fragment of an SIV proviral DNA clone that contained the *env* gene, both coding exons of the *rev* gene, and the *rev*-responsive element (RRE). Rev is a *trans*-acting nuclear regulatory protein that binds to the RRE, an RNA stem-loop structure contained within the *env* and *gag-pol* mRNAs (25). Rev-RRE interactions promote the accumulation and nuclear egress of unspliced mRNAs for structural proteins (14, 15, 24). We demonstrated that the Rev protein was expressed in pEXO17/Ad5hrΔE3-infected cells, which may play a role in enhancing the accumulation of unspliced mRNAs capable of encoding the Env protein.

Autologous *cis* regulation of SIV gene expression was directed by early and late Ad transcriptional promoters. Early SIV-specific transcripts (*rev* and *env*) probably initiated from the immediate upstream E3 promoter since they were detected in the presence of viral DNA replication (and late-phase transcription) inhibitors (Fig. 3A) (8, 27). The abundant late-phase *env* transcripts probably initiated from the further upstream Ad major late promoter and were spliced into the Ad x or y leaders upstream of the E3 promoter and the SIV *env* gene (8, 11, 27). The major late promoter, although active in early-phase transcription (similar to other early promoters), is over 20-fold more active than early promoters during late-phase transcription (22). Similar expression patterns have been observed for the hepatitis B surface antigen or HIV-1 *env* genes inserted downstream of the E3 promoter of an Ad5ΔE3 deletion mutant (8, 27).

Although Ad tightly regulates its own gene expression by complicated RNA splicing schemes, experience with the expression of foreign genes containing introns in Ad vectors is limited (29, 30). Our data are the first to demonstrate that foreign genes inserted in a recombinant human Ad5 host range mutant can be transcribed, spliced, and expressed in

TABLE 1. Growth of Ads on A549 or CV-1 cells[a]

Ad	PFU/10^6 cells	
	A549[b]	CV-1[c]
pEXO17/Ad5hrΔE3	1.5×10^9	6.6×10^8
Wild-type Ad5	7.3×10^9	2.0×10^6

[a] The procedure for titrating is described in reference 20.
[b] Human culture cells.
[c] Monkey culture cells.

human as well as in monkey cells. These results suggest that this Ad5 vector is a useful vehicle for the study of eukaryotic gene regulation and expression in both human and monkey cells in culture. In addition, our preliminary experiments showed that Ad5hr mutants replicated in the respiratory tract of rhesus macaques to generate reasonably high neutralizing titers against Ads.

Recombinant Ads expressing HIV proteins are attractive human AIDS vaccine candidates for at least three reasons. First, as we have demonstrated, recombinant Ads are efficient and practical expression vectors for individual (or multiple) retroviral genes. Second, safe and effective live oral Ad vaccines have been administered to military recruits for over 20 years (5, 10). The live Ad vaccines are unmodified wild-type viruses that are administered in an enteric coated tablet that dissolves in the intestinal tract. Asymptomatic Ad replication in the intestinal tract leads to a vigorous immune response that confers resistance to Ad-induced respiratory tract illness (9). Third, multiple neutralization serotypes of human Ad provide the opportunity to administer booster immunizations with different recombinant Ad serotypes. However, the efficacy of recombinant Ad vaccines in humans remains to be proved, and some important issues must be addressed. Two of these issues include the following. (i) What are the proteins (or epitopes) of HIV that induce a protective immune response? (ii) Can a recombinant Ad vaccine, replicating in the intestine (or respiratory tract), induce a protective immune response against a virus (HIV) that has the potential for sequestration in multiple and varied tissues?

The SIV model of AIDS is an excellent system in which to study these important questions. Macaques can be immunized with various Ad-SIV recombinant viruses via different routes and subsequently evaluated by live virus (SIV) challenge for the induction of protective immune responses. Promising constructs and strategies can then be tested with analogous Ad-HIV recombinant viruses in chimpanzees and, ultimately, in humans.

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