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Ovine Adenovirus Vectors Overcome Preexisting Humoral Immunity against Human Adenoviruses In Vivo

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Recombinant human adenoviruses (hAd) have become widely used as tools to achieve efficient gene transfer. However, successful application of hAd-derived vectors in clinical trials is limited due to immunological and potential safety problems inherent in their human origin. In this study, we describe a recombinant ovine adenovirus (OAV) as an alternative vector for gene transfer in vivo. In contrast to an hAd vector, the OAV vector was not neutralized by human sera. An OAV vector which contained the cDNA of the human α_1-antitrypsin (hAAT) gene linked to the Rous sarcoma virus promoter was generated and administered systemically to mice. The level and duration of hAAT gene expression was similar to that achieved with an hAd counterpart in both immunocompetent and immunodeficient mice. However, the tissue distribution of the OAV vector differed from that observed for hAd vectors in that the liver was not the dominant target. Significantly, we demonstrated efficient gene transfer with the OAV vector into mice immunized with hAd vectors and vice versa. We also confirm that the immune response to a transgene product can prevent its functional expression following sequential application of a vector. Our results suggest a possible solution to endemic humoral immunity against currently used hAd vectors and should therefore have an impact on the design of improved gene therapy protocols utilizing adenovirus vectors.

Recombinant human adenoviruses (hAd) are excellent tools for efficient gene transfer into a variety of cell types in vitro and in vivo (24). Despite the obvious potential for treatment of inherited and acquired disorders and their application in numerous animal studies, the use of hAd vectors is still limited to a small percentage of current clinical gene therapy trials (3). This situation is principally due to limitations caused by the host immune response (9). The obstacles to application of hAd vectors are due to the humoral response against the vector per se and to the immune response against residual expression of viral genes in transduced cells (55). Endogenous expression of viral genes leads to transient transgene expression, necessitating repetitive administration of the vector. However, successful readministration of hAd vectors to animals (23, 39, 46) is prevented by neutralizing antibodies induced after their first application. Since currently used hAd vectors are derived from types 2 and 5, which are endemic in the majority of the population (13–15), preexisting neutralizing antibodies may preclude even a single successful administration of hAd in humans. With respect to overcoming the immune response to adenovirus, strategies involving immunomodulation (11, 18, 19, 29, 40, 56), induction of immunotolerance (16, 17), or switching (8, 26, 28) of the hAd serotype have been employed. More recently, "gutless" hAd vectors, in which all coding regions of the virus are removed, have been generated to avoid the immune response to endogenous adenovirus gene expression and improve expression (38). However, none of these strategies can exclude the possibility that replication of a recombinant hAd may be complemented by a wild-type hAd during opportunistic coinfection. Thus, issues concerning safety require further consideration.

As a solution to these concerns, we describe the use of a non-human adenovirus vector for human gene therapy. Adenoviruses are divided into the mastadenoviruses, the aviadenoviruses, and a third group which contains the Australian ovine adenovirus (OAV) isolate OAV287 (12). We selected OAV because it has been engineered to produce recombinants (50, 54) and because of its unique features and degree of biological characterization, including the demonstration that it replicates abortively in nonovine cell lines (4, 20, 21, 45, 47–49, 53). Importantly, OAV was not neutralized by a polyclonal serum raised against hAd type 5 (53).

In this study, we describe the generation of an OAV-based vector expressing the human α_1-antitrypsin (hAAT) gene. We show that this OAV vector was not neutralized by a significant number of human sera in vitro, whereas preexisting immunity against hAd was strikingly apparent. OAV-mediated hAAT gene expression was similar to that mediated by an analogous, replication-deficient hAd in either immunocompetent or immunodeficient mice. Analysis of vector DNA distribution and hAAT gene transcription showed that after systemic application in mice, the OAV vector infected various tissues with no particular preference for liver. Furthermore, in a series of cross-administration experiments, efficient gene transfer with OAV vectors was clearly demonstrated in vivo despite the presence of neutralizing antibodies against hAd.

MATERIALS AND METHODS

Plasmids. Plasmid pOAV204 contains the full-length OAV genome with an expression cassette (MLP/TLS-VP7sc) inserted in *Bsp*120I/*Not*I sites at a nonessential position (21, 50). An *Nhe*I/*Bfr*I fragment (3,617 bp) of pOAV204, flanking the insert, was subcloned into *Nhe*I and *Bfr*I sites of a truncated pSL1190 (Invitrogen) that lacked the polylinker sites between *Eco*RV and *Sma*I. The MLP/TLS-VP7sc expression cassette was replaced by a *Bsp*120I/*Not*I polylinker

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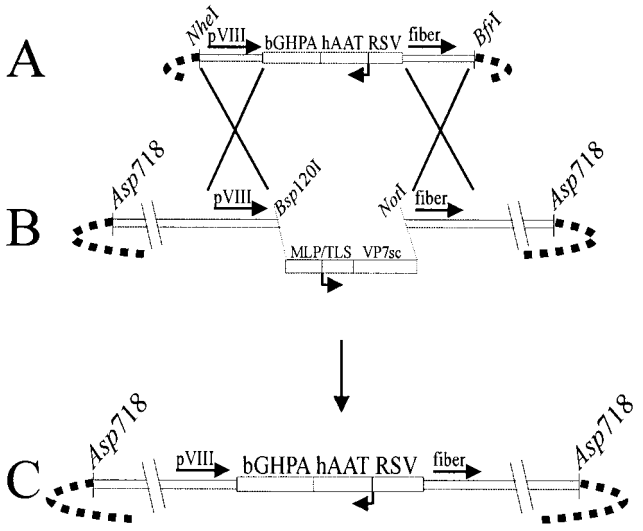

FIG. 1. Construction of recombinant OAVhAAT. The shuttle plasmid pOAV-sh-hAAT (A) was assembled by insertion of an RSV-hAAT-bGHPA expression cassette into the *Xho*I-site of pOAVrec. (B) Plasmid pOAV204 was cut with *Bsp*120I/*Not*I to release the MLP/TLS-VP7sc cassette (21). Cotransformation of *E. coli* BJ5183 with the *Nhe*I/*Bfr*I fragment from panel A and the linearized plasmid from panel B generated the 34.8-kb circular plasmid pOAVhAAT by homologous recombination. (C) pOAVhAAT cut with *Asp*718 was transfected into CSL503 cells for virus rescue.

fragment of pBSKS$^-$ (Stratagene) using the same restriction sites. The resulting shuttle plasmid (pOAVrec) contained a multiple cloning site inserted between the pVIII and fiber genes. pOAV-sh-hAAT (Fig. 1A) was constructed by insertion of an *Xho*I fragment of pRSVhAAT, which contains the hAAT cDNA under control of the Rous sarcoma virus (RSV) long terminal repeat (LTR) and the bovine growth hormone polyadenylation signal (bGHPA), into the *Xho*I site of pOAVrec. The plasmid (pOAVhAAT [Fig. 1C]) for rescue of OAVhAAT virus was generated by homologous recombination in *Escherichia coli* BJ5183 after cotransformation of *Bsp*120I/*Not*I-linearized vector backbone (Fig. 1B) of pOAV204 (20 ng) with 100 ng of an *Nhe*I/*Bfr*I fragment from pOAV-sh-hAAT containing the hAAT expression cassette. pOAVhAAT was amplified by transformation into *E. coli* JM109 (Stratagene).

Viruses and cells. Fetal ovine lung cells (33), CSL503, for rescue and propagation of OAV were cultured in Dulbecco modified Eagle medium plus 10% fetal calf serum. Rescue of OAVhAAT was achieved after Lipofectamine transfection (Gibco) of CSL503 cells (3×10^5/6-cm dish) with linear recombinant genome (6 μg) released from pOAVhAAT by digestion with *Asp*718. OAVhAAT was propagated by incubation of CSL503 cells at a multiplicity of infection (MOI) of 0.1 for 4 days. Preparation of recombinant replication-deficient hAd Ad-lacZ and Ad-hAAT was described previously (7). Ad-hAAT contains the same expression cassette as OAVhAAT. Purification of OAVhAAT was equivalent to hAd vectors. The total numbers of particles in OAV and hAd vector preparations were determined spectrophotometrically (27), and infectious units (PFU) were determined by end-point dilution assays on the corresponding cell line (293 or CSL503, respectively). The amounts of vector used in each experiment refer to PFU levels. Particle-to-PFU ratios for individual virus preparations were 25:1 (OAVhAAT), 34:1 (Ad-hAAT), and 17:1 (Ad-lacZ).

In vivo application of adenovirus vectors. Experiments were performed with female, 6-week-old (18 to 20 g) BALB/c mice (Bomholtgard, Bommice, Denmark) and scid mice (C.B-17/Icr/BlnA-scid/scid [authors' own breeding]). Adenovirus vectors (5×10^9 PFU) were injected via the tail vein in a volume of about 100 μl in all experiments. Blood was collected from the external jugular vein at the indicated times to assess neutralizing antibodies and to measure α_1-antitrypsin levels.

Determination of neutralizing antibody titers. Human sera were collected from healthy volunteers and mouse sera were isolated from animal experiments at the indicated times. The titer of neutralizing antibodies was determined by the ability of the sera to prevent infection of CSL503 cells by OAV-hAAT or of 293 cells by Ad-hAAT. Heat-inactivated (30 min at 56°C) serum samples were twofold serially diluted and incubated for 30 min at 37°C with OAV or hAd. A serum-treated virus sample (equivalent of an MOI of 1) was added to the cells, previously seeded in 96-well plates at a density of 3×10^3 cells/well. The neutralizing antibody titer was calculated from the reciprocal serum dilution in the last well showing no significant cytopathic effect, in comparison to control cells that were infected with untreated virus.

Detection of adenovirus-mediated gene transfer. The enzyme-linked immunosorbent assay to quantify hAAT in mouse serum was described recently (7). Expression of *E. coli* β-galactosidase in liver sections was detected histochemically by X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining.

Southern blotting and RNase protection assays. Mice were sacrificed at the times indicated and liver, spleen, kidneys, lung, and heart were frozen and homogenized in liquid nitrogen immediately. DNA and RNA were isolated separately from the same tissue sections by standard procedures. For Southern blotting of OAV DNA, genomic DNA (20 μg) was digested with *Eco*RI, which releases a 2,399-bp fragment from the OAV genome. After separation on a 1% agarose gel and transfer to a nylon membrane, hybridization was performed with a probe spanning bp 1968 to 3408 of the OAV genome. A specific OAV *Eco*RI fragment (250 fg to 12.5 pg, equivalent to 0.2 to 5 copies per cell) was used as a standard. Detection of hAd DNA by Southern blotting has been described previously (36).

RNase protection assays were carried out with total RNA (20 μg) by standard procedures. A radiolabelled RNA fragment of 362 bases comprising the *Eco*NI fragment of the hAAT gene was used as a probe. In vitro-transcribed hAAT RNA (10 to 50 pg) was used as a standard.

RESULTS

Generation of recombinant OAV vectors. A recombinant plasmid carrying an RSV 3′ LTR-driven hAAT cDNA inserted between the pVIII and fiber genes of OAV was generated by homologous recombination in *recBC sbcBC E. coli* (Fig. 1) by a procedure described previously for hAd-derived vectors (6). The recombinant virus, OAVhAAT, was rescued following transfection of the linear recombinant genome into CSL503 cells (50). The hAd-derived vector, Ad-hAAT (7), carried the identical expression cassette in place of the E1 region.

Neutralizing antibodies in human sera. The presence of preexisting neutralizing antibodies against hAd in most of the population is a major limitation to the use of hAd-derived vectors in gene therapy. Indeed, an analysis of random samples of human sera and serum pools revealed the presence of neutralizing antibodies against hAd (type 5) at titers of 1:10 to 1:5,120. In contrast, no neutralizing antibodies against OAV were detected in any sample (Fig. 2).

hAd- and OAV-mediated gene expression in vivo. OAV-mediated gene transfer was assessed relative to hAd by comparing the levels of serum hAAT after intravenous injection of equal amounts of the respective vectors (5×10^9 PFU) into BALB/c mice. Application of either vector resulted in hAAT gene expression with comparable peak levels and no detectable hAAT protein after 20 days (Fig. 3A). Since at this time no vector DNA was detectable in the livers of mice from either group (data not shown), mice were injected with a second dose of the same vector at day 31 after the first injection. However, second vector administration did not result in hAAT gene expression in either case (Fig. 3A). To exclude the possibility that antibodies raised against the transgene product prevented detection of the hAAT protein in mouse sera, we performed an RNase protection assay specific for hAAT RNA. No hAAT-specific transcript was detected in mouse liver 4 days after the second administration of OAVhAAT (Fig. 3B) or Ad-hAAT (see Fig. 5B, lanes 4 and 5). Therefore, after second administration, both vectors failed to infect the liver, a result which was most likely caused by neutralizing antibodies against OAV or hAd vectors. The titer of neutralizing antibodies in mouse sera against the respective vector types (OAVhAAT and Ad-hAAT) ranged equally high, from 1:320 to 1:1,280, at the time of the second administration, and no cross-neutralizing antibodies were detected in either case (data not shown).

In a second series of experiments, we compared the duration of OAVhAAT- and Ad-hAAT-mediated gene expression in a congenic scid mouse strain under the conditions described for immunocompetent mice. The peak levels of serum hAAT expression were similar to that observed in immunocompetent

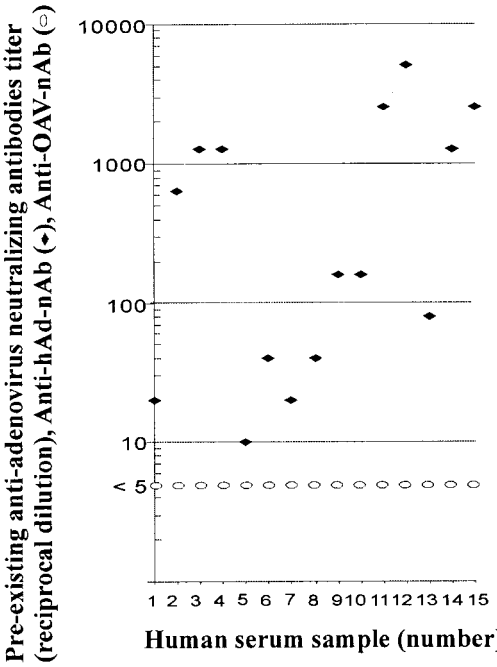

FIG. 2. Neutralizing antibodies against hAd and OAV in human sera. Neutralizing antibodies against hAd (filled rhombi) and OAV (open ovals) were determined in individual human sera (samples 1 to 13) or human serum pools (samples 14 and 15) by the ability to prevent infection of the respective producer cell lines (293 and CSL503) by the corresponding adenovirus. The neutralizing antibody titer was determined from the reciprocal serum dilution, which showed no significant cytopathic effect compared to unimpeded infection.

mice, but gene expression did not decline (Ad-hAAT) or declined by ~50% (OAVhAAT) over the duration of the experiment (56 days, Fig. 3C).

Tissue distribution of OAV vectors after intravenous delivery. Since the OAV fiber and penton proteins are significantly different from their homologues in other adenovirus vectors (47, 49), we determined the tissue distribution of systemically administered OAVhAAT after intravenous i.v. injection, as shown in Fig. 3A. We found almost equal amounts of OAV-specific DNA in spleen, kidney, heart, and liver at day 4 after vector administration, whereas the lung was infected to a significantly lower degree (Fig. 4A). The distribution of vector DNA corresponded well with hAAT gene expression in several organs, as determined by RNase protection assays (Fig. 4C). Thus, total serum hAAT was recruited from several organs which expressed the transgene to similar extents. In contrast, systemic application of hAd-hAAT to mice resulted in the accumulation of viral DNA and transgene expression mainly in the liver, with apparently lower infection of other organs (Fig. 4B).

Cross-administration of hAd and OAV vectors. The presence of neutralizing antibodies against hAd in human sera may limit the effectiveness of hAd vectors. Mice immunized against hAd represent a relevant model in which to show the potential advantages of OAV vectors for human gene therapy. Thus, cross-administration experiments were performed. Mice were systemically infected with either Ad-hAAT or OAVhAAT; at day 31, animals received a second administration of the other virus (OAVhAAT or Ad-hAAT, respectively). In neither case did administration of the second virus cause an elevation of serum hAAT levels (Fig. 5A). Analysis of serum samples showed that after the first infection, type-specific neutralizing

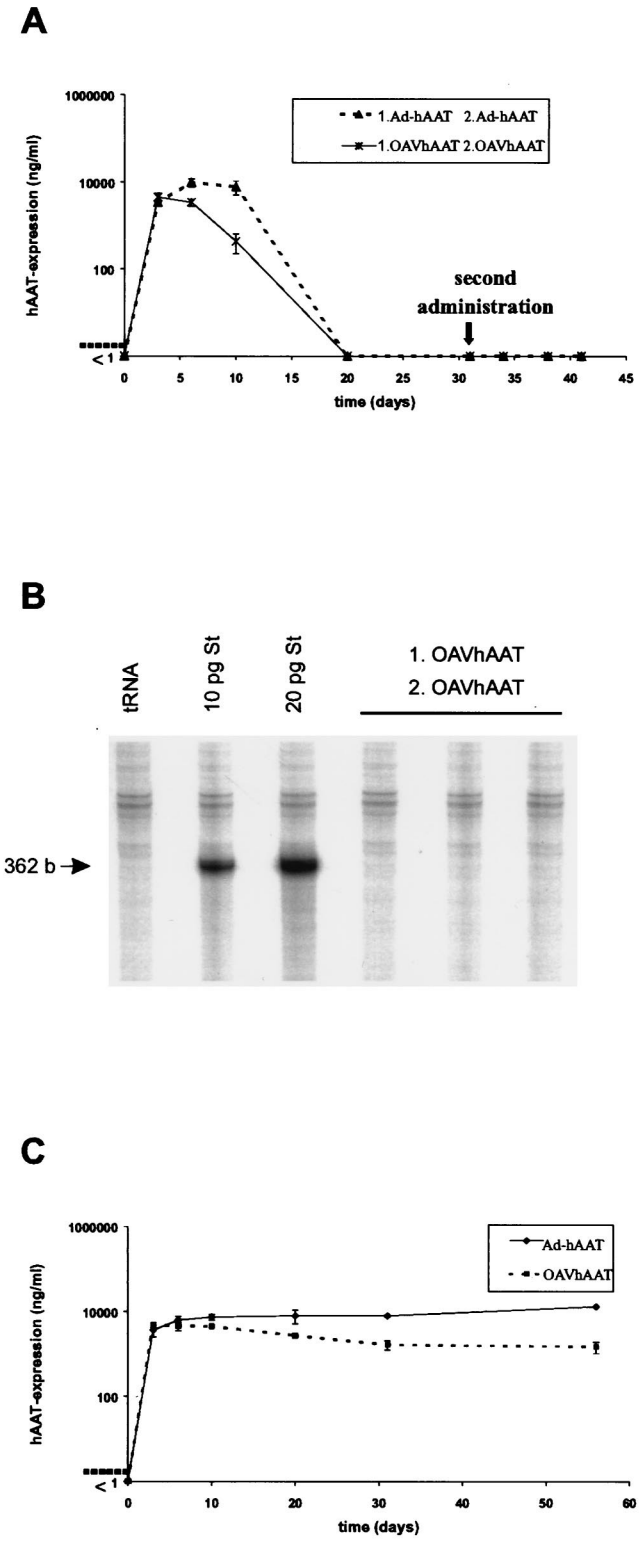

FIG. 3. hAd- and OAV-mediated hAAT expression in vivo. Equal amounts of either OAVhAAT or Ad-hAAT (5×10^9 PFU) were intravenously injected into BALB/c mice ($n = 4$) on days 0 and 31 (A) or into congenic scid mice ($n = 4$) on day 0 (C). Levels of serum hAAT were determined for each animal in duplicate at the indicated times. (B) RNase protection assay for hAAT-specific RNA (from 20 μg of total mouse liver RNA) performed 4 days after readministration (day 31) of OAVhAAT into BALB/c mice initially injected with OAVhAAT. Ten- and 20-pg in vitro-transcribed hAAT RNA samples were used as standards (St).

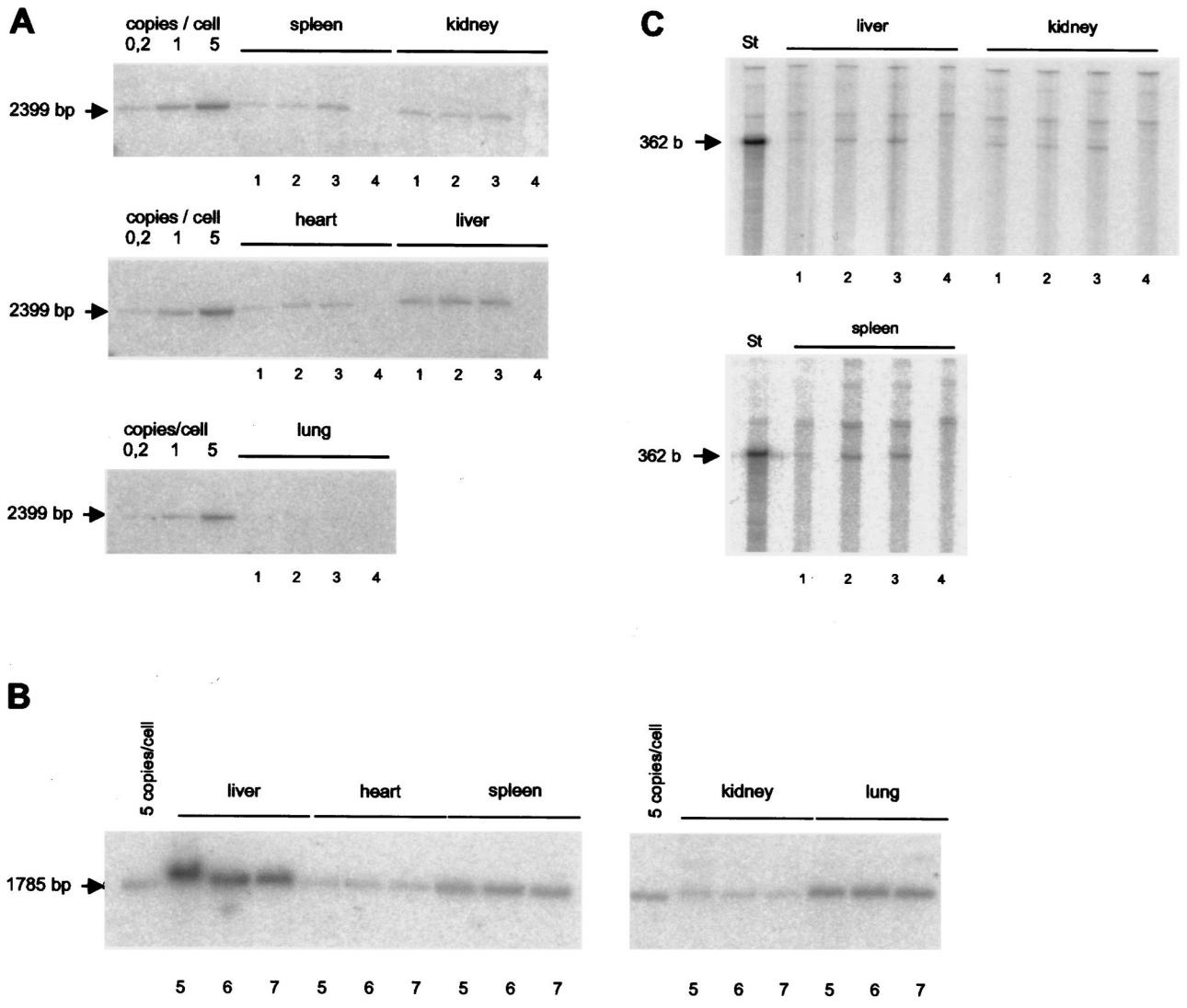

FIG. 4. Tissue distribution of hAd and OAV vector DNA and site of hAAT RNA synthesis. (A) Southern blot analysis of spleen, kidney, heart, liver, and lung DNA from BALB/c mice infected with OAVhAAT (lanes 1 to 3) and a control mouse (lanes 4) at day 4 after infection. The position of the expected 2,399-bp *Eco*RI fragment from OAV is indicated by an arrow. *Eco*RI-cut OAV DNA equivalent to 0.2, 1, and 5 OAV genome copies per cell served as standards. Identical numbers refer to identical animals. (B) Southern blot analysis of DNA from indicated organs from mice infected with Ad-hAAT (lanes 5 to 7) at day 4 after infection. The position of the expected 1,785-bp *Nco*I-fragment from hAd is indicated by an arrow. *Nco*I-cut hAd DNA equivalent to 5 hAd genome copies per cell served as standards. (C) RNase protection assays for hAAT-specific transcripts in total RNA (20 μg) from liver, kidney, and spleen of mice from panel A. The position of the protected 362-base fragment is indicated. In vitro-transcribed hAAT RNA (25 pg) was used as a standard (St).

antibodies against the respective adenovirus vectors were detected at titers ranging from 1:320 to 1:1,280. In addition, antibodies against hAAT were detected in mouse sera prior to application of the second vector, showing that the human transgene product was immunogenic in mice. However, despite the lack of detectable hAAT protein, analysis of hAAT-specific RNA 4 days after the second vector administration clearly revealed expression of the transgene in livers of mice that were reinjected with Ad-hAAT after an initial application of OAVhAAT, whereas no hAAT-specific transcripts were detected when the initial application was performed with Ad-hAAT (Fig. 5B). Specific hAAT-transcripts were also detected in several organs of mice injected with OAVhAAT after initial infection with Ad-hAAT (data not shown).

Since immunity against the transgene was a confounding factor in the previous set of experiments, we injected mice with Ad-lacZ (containing an RSV promoter-driven *E. coli* β-galactosidase gene) followed by a second administration of either Ad-hAAT or OAVhAAT and vice versa (Fig. 6). Although high levels of β-galactosidase were detected histochemically in mouse livers at day 3 after Ad-lacZ injection (Fig. 6B), gene expression had completely disappeared after 31 days (Fig. 6C). At that time point, the hAd-specific antibody titer ranged from 1:320 to 1:1,280 (not shown). OAVhAAT and Ad-hAAT were then applied. Injection with OAVhAAT resulted in hAAT levels in serum comparable to those obtained with a single administration in untreated mice, whereas injection of Ad-hAAT did not produce detectable hAAT expression (Fig. 6A). In addition, hAAT-specific transcripts were not detectable in the liver (not shown). In the reciprocal experiment, mice were immunized with OAVhAAT, which produced OAV-neutralizing antibody titers of 1:1,280. In spite of this immunity, appli-

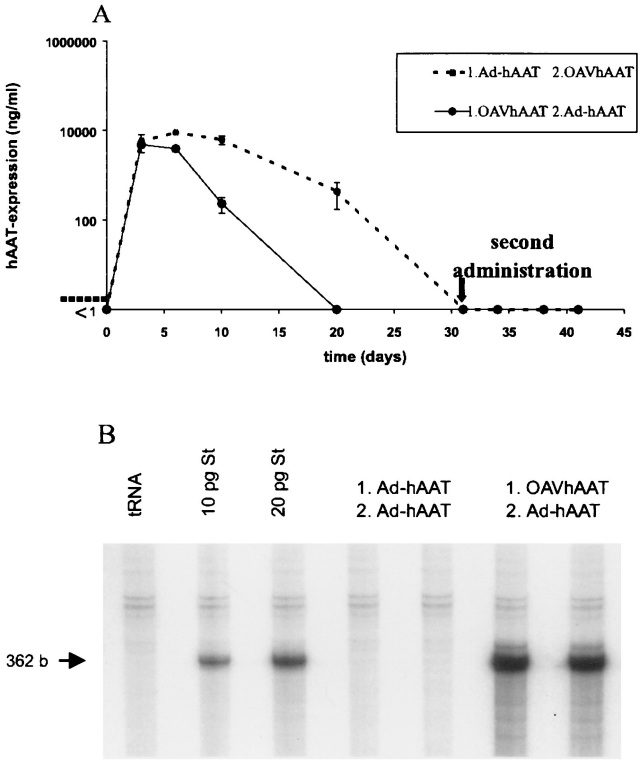

FIG. 5. Cross-administration of hAd and OAV vectors expressing the same transgene. BALB/c mice ($n = 3$ [each group]) received systemically an initial dose (5×10^9 PFU) of either Ad-hAAT or OAVhAAT, followed by a second administration (day 31) of the other virus (OAVhAAT or Ad-hAAT, respectively). (A) Serum hAAT levels were determined from each animal in duplicate at the indicated times. (B) hAAT-specific RNA in mouse livers (from 20 μg of total RNA) was analyzed 4 days after readministration (day 31) of Ad-hAAT to either Ad-hAAT- or OAVhAAT-immunized BALB/c mice. Ten- and 20-pg in vitro-transcribed hAAT RNA samples were used as standards (St). The gel is the same as in Fig. 3B.

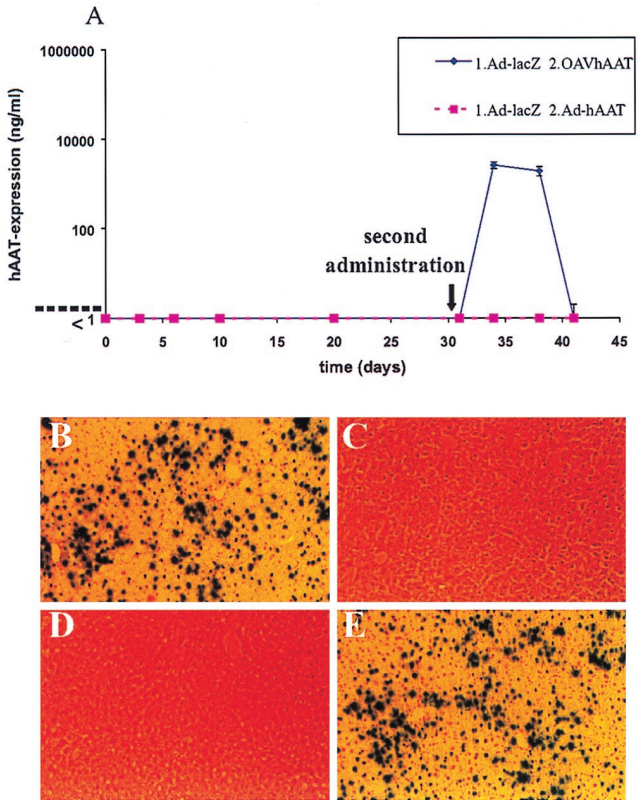

FIG. 6. Cross-administration of hAd and OAV vectors expressing different transgenes. BALB/c mice ($n = 3$ [each group]) received an initial systemic dose (5×10^9 PFU) of Ad-lacZ followed by a second administration (day 31) of either OAVhAAT or Ad-hAAT (5×10^9 PFU each). (A) Serum hAAT levels were determined in duplicate for each animal at the indicated times. Ad-lacZ-mediated gene expression was examined in mouse livers on day 3 (B) and day 31 (C) by histochemically staining for β-galactosidase. OAVhAAT-immunized BALB/c mice ($n = 3$) exhibited a neutralizing antibody titer of 1:1,280 and were injected on day 31 with Ad-lacZ (5×10^9 PFU). Livers were stained for β-galactosidase before (day 31) (D) and 3 days after (E) injection.

cation of Ad-lacZ virus at day 31 resulted in efficient β-galactosidase expression 3 days later (Fig. 6D and E).

DISCUSSION

Recombinant hAd vectors have proven to be potent gene delivery agents in numerous preclinical gene therapy studies. However, immunological problems and potential safety aspects inherent in the human origin of the vector may limit their utility and efficacy in clinical trials. The development of adenovirus vectors of non-human origin may circumvent these problems (32). In this study, we demonstrate the ability of an OAV vector to overcome preexisting humoral immunity to hAd in vivo. In addition, given that OAV has unique, short ITRs (46 bp) and at least one structural protein not found in hAd (49), it is most unlikely that productive recombination or complementation could occur between OAV vectors and an opportunistic, coinfecting hAd from the mastadenovirus genus. However, it is unknown whether human adenoviruses that may be classified within the third adenovirus group also exist.

The recombinant OAV vectors used in these experiments carried an additional expression cassette (RSV-hAAT) without a compensating deletion elsewhere in the genome. Recombinant vectors are stable up to a size of at least 114% of the wild-type OAV genome, and an initial internal deletion has enlarged the potential vector capacity to 6.3 kbp of foreign DNA (54). The method used to generate hAd plasmids by homologous recombination in *E. coli* (6) has been successfully adapted here for the generation of OAV plasmids, which will further facilitate the construction and rescue of OAV vectors.

Preexisting neutralizing antibodies against hAd vectors in a majority of the human population represent a major hurdle for efficient hAd administration. In an attempt to avoid this problem, construction of an hAd vector with an altered hexon protein was recently described (35). We tested 15 random samples of human sera and found type 5 anti-hAd antibody titers ranging between 1:10 and 1:5,120. Because it has been shown that as few as 1.4 antihexon antibodies can neutralize one virus particle (52), it is important to emphasize that even in sera exhibiting the highest anti-hAd titer, no cross-reactive antibodies against OAV were detected. This result contrasts with observations made with canine adenovirus vectors (22, 32) and justifies further investigation of OAV vectors in vivo.

We compared the duration of transgene expression by OAV and hAd vectors after intravenous application to immunocompetent BALB/c mice and the corresponding immunodeficient scid mouse counterpart. We chose BALB/c mice for these experiments because transgene expression declines very rapidly in this mouse strain (1), which could, among other reasons, be explained by a strong cytotoxic T-cell response against endogenous hAd gene expression (1, 34). We confirmed transient

expression of the transgene in this mouse strain by using the hAd vector and observed similar kinetics for OAV-mediated gene transfer, even though in vitro studies have shown that endogenous OAV promoters are not detectably active in some cell types (49). The hAd vector exhibited stable gene expression in scid mice, as shown previously (1), whereas OAV-mediated gene expression declined by ~50% over time, which may reflect the turnover of different cell types that were infected. Since the OAV vector contains all genes present in wild-type OAV, it is important to note that the mice showed no obvious ill effects from the dose of virus given, although more detailed studies are required to ascertain whether OAV has oncogenic potential.

The tissue distribution of the OAV vector in mice is different from that observed with hAd-derived vectors and therefore appears to be independent of the vascular architecture of the mouse and the liver in particular. We found that OAV-specific DNA was nearly equally distributed in all organs examined after systemic application, whereas hAd is mainly targeted to the liver. This most likely reflects differences in the structure of potential receptor ligands on the surface of OAV. Indeed, the OAV fiber protein is unique, and its penton protein lacks an RGD motif (47, 49). Such differences in the mechanism of OAV uptake by cells may permit OAV to target cell types and tissues that exhibit low susceptibility to infection by current hAd vectors because of the lack of receptors for hAd (2). However, further investigation is required to assess this potential of OAV vectors. It may also be possible to construct hybrid OAV vectors to target such tissues (53).

Although immunosuppressive approaches to circumventing the humoral immune responses have been reported in animal experiments (11, 18, 19, 29, 40, 56), such strategies obviously carry risks for diseased patients with respect to immunosurveillance of opportunistic infections. The results from our cross-administration experiments point to two immunological problems associated with adenovirus gene transfer. First, we demonstrated that the problem of preexisting antibodies against hAd can be overcome by use of the OAV vector, since the latter vector was not neutralized by antibodies to the former. Because OAV itself elicits neutralizing antibodies, however, repeated administration of OAV would be as problematic as a first administration of hAd in humans positive for hAd-neutralizing antibodies. Second, the problem of immunity against the transgene product became apparent in cross-administration experiments utilizing hAAT-expressing OAV and hAd vectors. Although gene transfer was successful, based on hAAT RNA analysis after readministration of the second vector, antibodies against the hAAT-protein precluded its detection in mouse sera, supporting increasing evidence that immunity against a heterologous transgene product, including hAAT (30), limits the duration of its expression (26, 31, 41, 43, 44). However, this problem can be avoided by delivery of a homologous gene product to mice (42). In our experiments, we excluded interfering immunity against the neoantigen by initial delivery of the *E. coli* β-galactosidase gene and subsequent transduction with the hAAT gene, and vice versa.

The study clearly demonstrates that antibody titers induced against either type of vector—even lower than those preexisting against hAd in some human individuals—are sufficient to completely block systemic readministration of the same vector. This might partially explain inconsistent results regarding the efficacy of hAd-mediated gene transfer in clinical trials, where the vectors have to travel at least some distance to reach the target cell (10, 51, 57). With respect to gene therapy strategies that aim at the local treatment of tumors (5, 25, 37), preexisting hAd antibodies have less impact on gene transfer efficiency. However, such strategies may not be appropriate for disseminated disease. In summary, our successful cross-administration of OAV vectors to hAd-immunized mice clearly reflects the human situation and points to the utility of OAV vectors as an alternative to hAd vectors in clinical applications.

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