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# Ovine atadenovirus as a vector for gene transfer and vaccination

**Review Article**

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**Abbreviations:** gene-directed enzyme pro-drug, (GDEPT); human adenovirus, (hAdV); left hand, (LH); ovine atadenovirus type 7, (OAdV); purine nucleoside phosphorylase, (PNP)

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## Summary

**The use of adenoviruses as gene transfer vectors has become widespread within the last two decades. The commonly used vectors are based on human adenovirus type 2 and type 5 (hAdV-2 and hAdV-5) of subgroup C. Although these vectors are very efficient in transferring foreign genes into a variety of cell types, their use as gene transfer vehicles for clinical application in humans is compromised under some conditions due to wide-spread pre-existing immunity to these viruses in the population (Flomenberg et al, 1995; Horwitz et al, 1996; Chirmule et al, 1999). Therefore, and to allow for repeated vector administration into the same individual, vectors have been derived from less common human adenoviruses (Kass-Eisler et al, 1996; Mastrangeli et al, 1996; Vogels et al, 2003). Alternatively, gene transfer vectors based on adenoviruses of non-human origin have been developed and successfully tested in several animal models (Both et al, 2002a; Löser et al, 2002a). They combine the many favorable features of adenoviruses as gene transfer vectors with the lack of natural immunity to these viruses in humans. Here we review the features and utility of a novel vector based on ovine atadenovirus type 7 (OAdV) which has been developed for transient gene delivery purposes over the last several years.**

## I. Introduction

Adenovirus vectors are among the most efficient tools for gene delivery *in vivo*. However, the fact that adenoviruses are endemic in the human population means that natural immunity to human adenovirus (hAdV) vectors is common. Up to 95% of individuals experience adenovirus infections during early childhood and develop life-long B cell immunity and a long-lived CD4+ T cell response (Flomenberg et al, 1995). In addition to the presence of neutralizing antibodies (Horwitz et al, 1996), in vitro studies have shown that human adenovirus type 5 hemagglutinates human erythrocytes (Cichon et al, 2002) and is partially inactivated by the classical complement system (Löser et al, 1999). To overcome these inactivation mechanisms it might be necessary to elevate the dose of hAdV vector to achieve sufficient gene transfer which harbors the risk of undesired side effects, as observed in the clinic (Raper et al, 2003).

To circumvent the problem of anti-hAd immunity and to exploit possible alternative viral tropisms, gene transfer vectors based on adenoviruses from non-human mammals and from poultry have been developed. So far, adenoviruses from cattle (Mittal et al, 1995), dog (Kremer et al, 2000), pig (Reddy et al, 1999) and chimpanzee (Farina et al, 2001) as well as avian adenoviruses (Michou et al, 1999; Johnson et al, 2000; François et al, 2001) have been used for vector construction and the resulting vectors have been successfully applied to gene transfer studies in experimental animals. Many of the positive characteristics of hAdV derived vectors such as ease of genome manipulation, growth to high titers or broad cell type

tropism are maintained in these vectors. Except for the aviaadenoviruses the above vectors are derived from members of the mastadenovirus genus. They therefore have high homology with the well-characterized human adenoviruses which first defined this genus. Probably because of this, antibodies directed against human, bovine and porcine adenoviruses showed reciprocal cross reactivity although they were not neutralizing (Moffatt et al, 2000). Nevertheless, such cross-reactive antibodies might still be capable of activating the complement system following cross-infection. Complement activation occurs in sera from individuals with anti-hAdV immunity after contact with human adenoviruses from a different serotype (Cichon et al, 2001). In addition, growth of some recombinant animal mastadenoviruses is trans-complemented by hAdV-5 E1 genes (Xiang et al, 2002) which might allow vector multiplication in man following opportunistic infection with a human adenovirus.

## II. OAdV genome analysis and gene function

Arguably, the adenovirus vectors that are the most phylogenetically distinct from the mastadenoviruses are derived from OAdV, a sheep atadenovirus (isolate 287) classified as serotype 7 (Boyle et al, 1994). On the basis of sequence homologies in the protease and hexon genes OAdV was grouped together with viruses from other species, including cattle (BAdV 4 to 8), deer (OdAdV-1), duck (DAdV-1) and snake (SAdV-1), into the new genus atadenovirus (Benkö and Harrach, 1998), the name of which was based on the observation that the genomes of these viruses have a high AT content. To distinguish OAdV from ovine adenoviruses in the mastadenovirus genus, OAdV is now referred to as ovine atadenovirus and has been designated as the prototype of the genus.

The OAdV genome is about 30 kb in size, the genome has been completely sequenced (gene bank accession number U40938) and shows the typical organization of an adenovirus genome (**Figure 1**). Whereas the structural proteins as well as gene products necessary for virus DNA replication (located in the center of the genome) show more or less homology to the respective proteins in mastadenoviruses, there are major differences in the regions flanking this core region (Vrati et al, 1995, 1996a and b). The most striking difference of OAdV to hAdV-5 is the lack of an E1 region: OAdV does not contain genes that show any homology to the E1A genes of human adenoviruses or to the CELO functional homologues, the Gam-1 and orf-22 genes. Although the left hand (LH) region contains three potential open reading frames (LH1 to 3), two of the postulated gene products (LH1 and LH2) show no significant homology to any known protein outside the atadenovirus genus. Only LH3 has some limited homology to the adenovirus E1B 55 kDa protein, but the significance of this finding remains to be elucidated. However, the E1B 55 kDa protein is known to be involved in p53 degradation, and infection of ovine cells with OAdV results in a temporal decrease in p53 protein but not RNA (Kümin and Löser, unpublished results) suggesting the presence of some p53 down-regulating activity coded by OAdV or induced by OAdV infection.

Beside the lack of a typical E1 region, an E3 region (in adenoviruses typically located between the pVIII and fiber genes) is missing from the OAdV genome, and the potential E4 region is less complex and at a different genomic position than that of hAdV-5.

In the RH region, which is unique to atadenoviruses, three of five potential gene products could encode proteins with functional F-box motifs which are predicted to facilitate degradation of cellular protein(s) (Both, 2002b). However, most of the RH region can be deleted from the OAdV genome (leaving only the RH1 gene intact) with no obvious negative consequences for virus growth in vitro (Xu et al, 1997). In addition to these differences, OAdV is missing a virus-associated RNA gene (Venktesh et al, 1998) and genes for the structural proteins V and IX. However, the left end contains a gene in the right to left orientation that encodes the structural protein p32K, which is characteristic of the genus.

## III. Genome engineering and vector development

In contrast to hAdV-5 derived vectors that can package DNA up to 105% of their genome size (Bett et al, 1993), OAdV is capable of packaging up to 114%. This was shown by the rescue of a virus in which 4.3 kb of plasmid DNA was inserted into the OAdV genome without deleting any viral sequences. Therefore, most OAdV vectors contain, in addition to the inserted transgene, all viral sequences necessary for productive replication in permissive cells. They are grown in established ovine cell lines that do not contain viral sequences. CSL503 lung and HVO156 skin cells (Pye, 1989, Löser et al, 2002b) are derived from fetal sheep tissue, have a fibroblast phenotype and support growth of wild-type and recombinant OAdV to a similar degree (**Figure 2**). A complete cytopathic effect develops in about 96hr after infection at ~1$TCID_{50}$ unit per cell.

Up to now, three insertion sites for transgenes have been identified within the OAdV genome (sites 1, 2 and 3; **Figure 1**) (Vrati et al, 1996c; Xu et al, 1997). Site 1 is located between the genes coding for pVIII and fiber. In mastadenoviruses, the (non-essential) E3 region of ~3 kb is located at this position, but OAdV has only a short intergenic region of ~200 bp. Site 2, which is located within the non-essential RH open reading frame 2, is a unique *Sal*I site near the right hand end of the OAdV genome at nt 28.675. Site 3 is situated at position 26.677 between the RH and E4 transcription units. Insertion of short polylinkers or an expression cassette for human $\alpha_1$-antitrypsin into either of these sites did not interfere with virus rescue and growth. In addition, deletion of ~2 kb of DNA between sites 2 and 3 which removed most of the RH region had no major impact on vector growth (Xu et al, 1997, Löser et al, 2003).

Initially, vectors with transgene expression cassettes inserted into site 1 were constructed. These vectors, most of which used the RSV 3'LTR promoter were shown to be superior with respect to transgene expression levels compared to vectors containing the same transgene

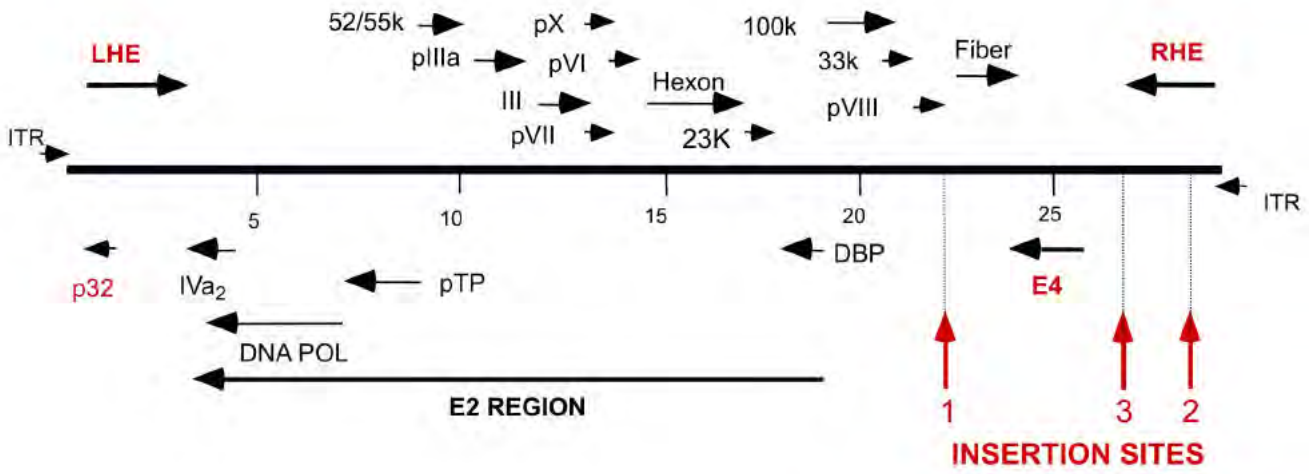



**Figure 1.** OAdV genome organization. The regions/genes which are characteristic for atadenoviruses are highlighted in red. Other open reading frames (ORFs) show significant homology with mastadenovirus genes. Sites 1 to 3 for the insertion of foreign DNA are indicated.

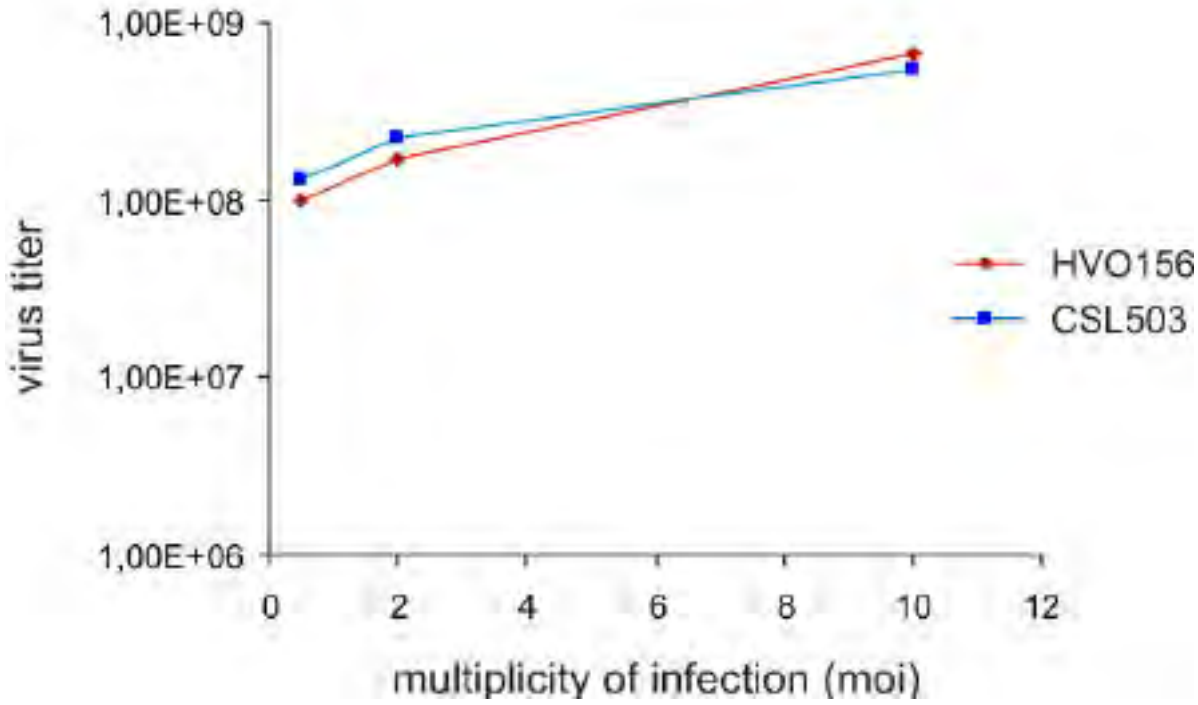



**Figure 2:** Growth of OAdV on sheep cell lines CSL503 and HVO156. 2 x $10^5$ cells were infected at multiplicities of infection (moi) of 0.5, 2 or 10, respectively, with wild-type OAdV and virus titer was determined in cell lysates 90 hours later by an end point dilution assay on HVO156 cells. At a moi of 2 each cell produced about 1,4 x $10^3$ (HVO156) or 1,8 x $10^3$ (CSL503) infectious particles.

inserted into site 2 or 3 (Löser et al, 2003). However, although some vectors with site 1 insertions could be rescued and propagated in a stable manner (Rothel et al, 1997; Xu et al, 1997; Khatri et al, 1997; Hofmann et al, 1999), several vectors proved to be unstable and showed major rearrangements within the transgene after a few passages (Löser et al, 1999 and 2003; Both, unpublished results). Although the exact reason for vector instability is unknown this finding underlined the importance of testing OAdV vector integrity by extensive passaging. Insertion of transgenes into site 2 has been performed, but rarely due to the limited availability of restriction sites (Lockett and Both, 2002, Löser et al, 2003). The preferred site for gene insertion is now site 3. Using either classical cloning methods or a highly effective cosmid-based system for vector construction, a variety of vectors with genes inserted into site 3 were constructed and rescued. These carried genes that encoded reporter or potentially therapeutic proteins, enzymes for cancer therapy or viral antigens for potential use in vaccination. Site 3 vectors propagated in a stable manner and grew to high titers on the producer cell lines. The orientation of the transgene

within site 3 had little or no impact on rescue efficiency, virus growth or transgene expression levels (Voeks et al, 2002; Löser et al, 2003; Wüest et al, 2004; Wang et al, 2004; Löser et al, unpublished observations).

*In vitro*, OAdV derived vectors have been used to infect a wide variety of human and rodent cell lines as well as primary cells from mouse and men. The spectrum of cells infected by OAdV and hAdV5 overlaps to some extent but there are significant differences in efficiency of infection. For example, infection of 293 or A549 cell lines by OAdV is very poor, whereas mouse C2C12, hamster CHO cells or the mouse prostate cancer cell line RM1 were infected to a higher degree by OAdV than by hAdV - 5 (Voeks et al, 2002, Löser et al, unpublished results). This is almost certainly due to the use of different receptors for cell entry. Although the OAdV receptor has not been identified it is clearly different from the Coxsackie and adenovirus receptor (CAR, Bergelson et al, 1997) which is used by some human and non-human adenoviruses including hAdV-5 (Roelvik et al. 1998, Soudais et al, 2000; Tan et al, 2001, Cohen et al, 2002). During simultaneous infection of CSL503 cells OAdV and hAdV-5 did not compete for entry, and exchange of the OAdV fiber protein cell-binding domain with that of hAdV-5 resulted in a hybrid virus with a clearly altered cell tropism (Xu and Both, 1998). In our hands, greatest gene transfer and expression in non-ovine cells was observed in cell lines of a fibroblast phenotype (Kümin et al, 2002) with transgene product secretion of up to 50 pg per cell attained within 24 hours. This very high level of transgene expression in vitro is probably due to a moderate amount of viral DNA replication in these cells, albeit without virus production (Kümin et al, 2002). Such replication might also be responsible for the cytopathic effect observed in some non-ovine cell lines after infection with high doses of OAdV (Khatri et al, 1997).

## IV. *In vivo* utility of OAdV vectors

The rationale for exploiting OAdV vectors was the finding that they are not cross-neutralized by pre-existing antibodies to hAdV-5 (Hofmann et al, 1999). In addition, no complement activation was observed in human sera containing antibodies to several hAdV serotypes when incubated with wild-type or recombinant OAdV (Löser and Cichon, unpublished results). The presumption, that pre-existing anti-hAdV immunity does not interfere with OAdV-mediated gene transfer was proved by cross-application experiments in mice. Mice pre-immunized with hAdV-5 developed neutralizing antibody titers comparable to those measured in randomly selected human sera and sera pools. These mice could not be infected systemically or locally by an hAd5 vector carrying a different transgene but were susceptible to gene transfer mediated by OAdV vectors (Hofmann et al, 1999; Wüest et al, 2004).

Systemic infection of mice, rats and rabbits with moderate doses of OAdV derived vectors resulted in high plasma levels of secreted transgenes such as human $\alpha_1$-antitrypsin (haat) or clotting factor IX which declined to background levels within three weeks after infection, presumably due to an immune response directed against the vector and/or the secreted transgene product (**Figure 3**).

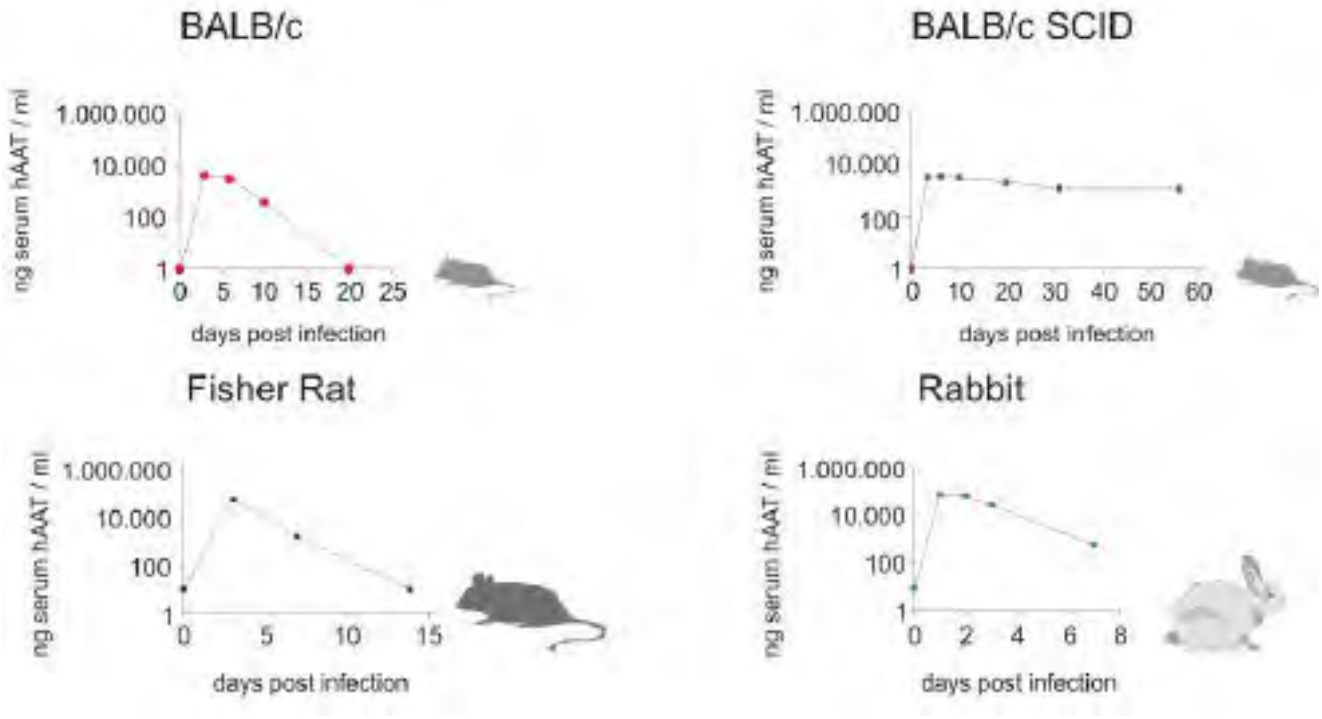
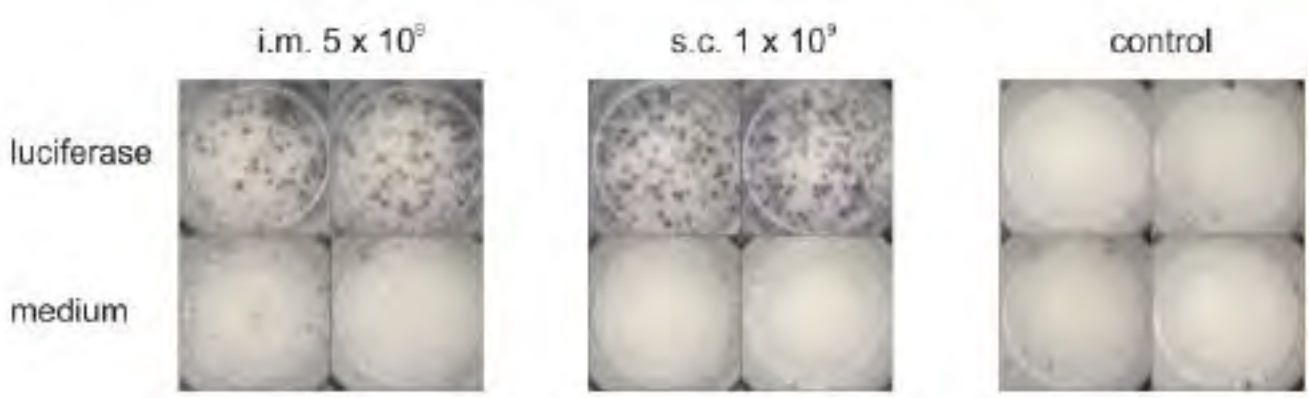



**Figure 3.** OAdV gene transfer *in vivo*. BALB/c mice (wild type or SCID, 5 animals per group), Fisher Rats (6 animals) or New Zealand White Rabbits (3 animals) were intravenously injected with OAdV-haat, a vector expressing the human haat cDNA under control of the RSV 3' LTR. hAAT serum levels were determined by ELISA at the time points indicated, values are means from the number of animals indicated. Virus doses were 5 x $10^9$ infectious particles (mice) and 2 x $10^{10}$ per kg infectious particles (rats and rabbits). Modified from Hofmann et al, 1999, and Löser et al, 2002a.

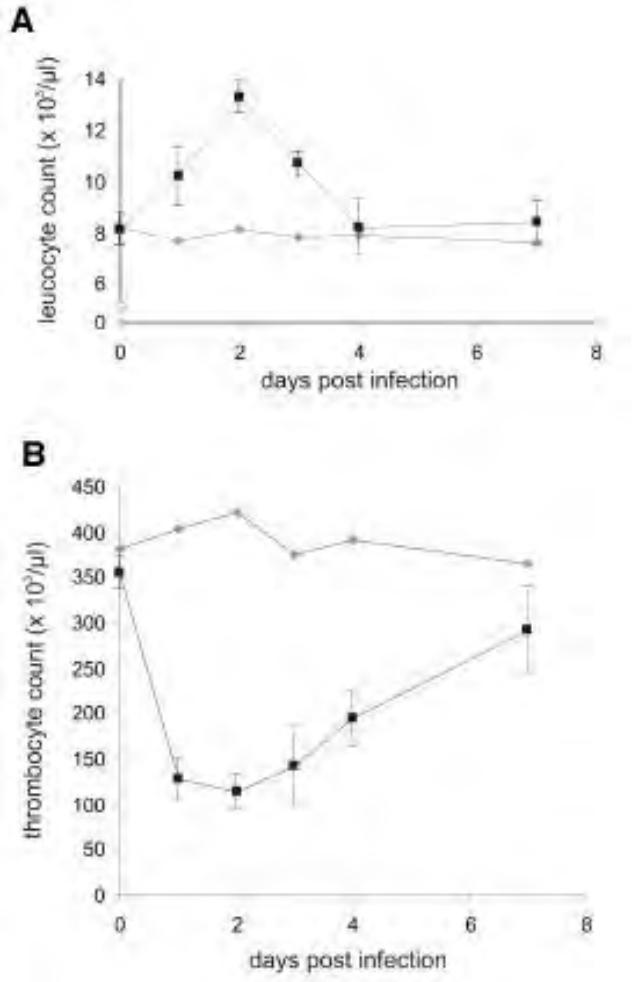
Independently of the infection route, the vector distributed evenly to many organs after intravenous, intraportal or intraperitoneal injection, indicating that OAdV receptor is widely distributed in many organs (Löser et al, 1999). The OAdV tissue distribution was similar in mice, rats and rabbits, which is in sharp contrast to the situation with hAdV-5 derived vectors that showed different tissue tropism in mice and rabbits (Hofmann et al, 1999; Cichon et al, 1999; Löser et al, 2002a), probably due to differential CAR expression. Thus, the tissue distribution of OAdV and hAdV-5 vectors in vivo confirms the presumption that these viruses utilize different receptors for cell entry.

Local injection of OAdV into liver, skeletal muscle or solid tumors of mice also resulted in high transgene expression levels (Löser et al, 2000, Martiniello-Wilks et al, 2004a, Löser et al, unpublished observation). Transgene expression following injection of an OAdV vector into mouse skeletal muscle was at least one $\log_{10}$ greater than expression from the same transgene delivered by a hAdV-5 vector. Transgene expression again declined rapidly, due to loss of vector DNA, even though OAdV gene expression was undetectable by RT-PCR. However, vector clearance remained incomplete after intraportal or intramuscular injection even when an empty OAdV vector was used suggesting that induction of T cells directed against the expressed transgene might substantially contribute to the loss of vector DNA and/or destruction of vector infected cells. The development of a strong T cell response to the transgene product was confirmed by injecting mice intramuscularly or subcutaneously with an OAdV vector expressing the luciferase gene and performing ELIspot analysis of luciferase-specific, IFN-γ secreting T cells (**Figure 4**). Similar results were observed after local injection of OAdV vectors coding for certain HCV antigens into mice suggesting the usefulness of OAdV vectors in vaccination (see below).




| Vector, administration route, particle number | specific spots per $10^6$ splenocytes | % of spots in positive control (ConA) |
|---|---|---|
| OAdV-luc, i.m. ($5 \times 10^8$) | 161 ± 38 | 7.2 |
| OAdV-luc, s.c. ($1 \times 10^9$) | 260 ± 29 | 11.6 |
| OAdV-control, i.m. ($5 \times 10^8$) | -13 ± 14 | 0 |

**Figure 4.** OAdV mediated induction of a T cell response specific for the transgene product. OAdV-luc which contains the firefly luciferase cDNA under the control of the RSV 3'LTR was injected into the quadriceps muscle (intramuscular, i.m., $5 \times 10^9$ infectious particles) or into the plantars (subcutaneous, s.c., $1 \times 10^9$ infectious particles). OAdV-control, which contains the RSV 3' LTR but no transgene was used as a control (i.m., $5 \times 10^9$ infectious particles). After 11 days, spleen cells were used for ELIspot analysis of IFN-γ secreting T cells specific for luciferase. Activation of T cells was performed by over-night incubation of splenocytes with (luciferase) or without (medium) luciferase protein at 10 μg/ml. The upper panel shows the outcome of a typical experiment, mean results are given in the table below (mean values from double measurements in three animals per group with standard deviations). As a positive control, splenocytes were non-specifically activated with ConA.



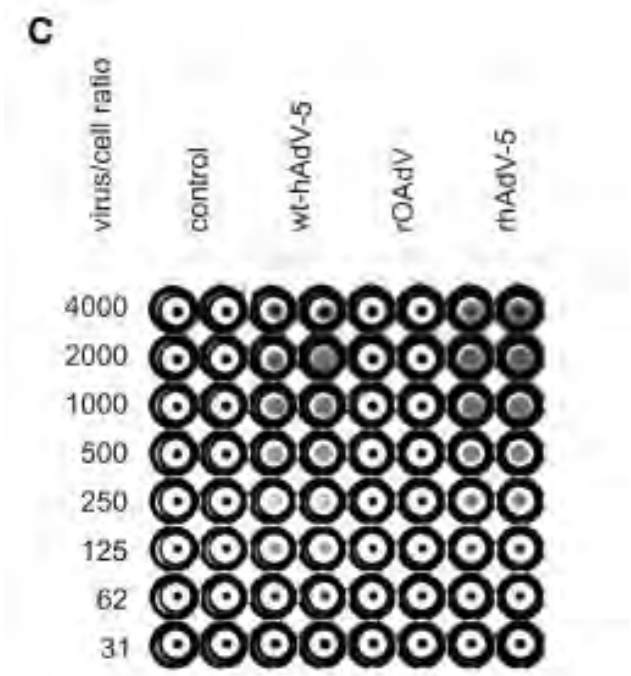




**Figure 5:** Hematological effects of OAdV infection. (**A and B**) Rabbits were infected with OAdV-haat as in figure 3 and thrombocyte and leucocyte counts were determined in blood samples at the time points indicated. Visual investigation of the blood smear at 24 and 48 hours p.i. revealed that only a low fraction of the "leucocytes" were erythroblasts. Black squares, rabbits infected with OAdV; gray diamonds, rabbit injected with buffer. (**C**) OAdV does not hemagglutinate human erythrocytes *in vitro*. 50 µlof a 1% suspension of washed human erythrocytes were mixed with 50 µl virus suspension (recombinant OAdV, rOAdV, or recombinant hAdV-5, rhAdV-5) in 96 well plates at the virus-cell ratios indicated. After two hours of sedimentation plates were inspected for hemagglutination patterns. Whereas hAdV-5 clearly showed hemagglutination, OAdV showed none even at the highest virus-cell ratio.

## V. Safety profile of OAdV vectors

Systemic administration of hAdV vectors at high doses can induce toxic side effects. Intravenous injection of rats with about 2 x $10^{10}$ infectious particles per kg of an OAdV vector that contained the haat gene resulted in a moderate increase in the GOT level which peaked at day 7 post injection at 92 U/ml and normalized to pre-injection levels of 38 U/ml by day 15 in all but one animal. GPT levels were also increased (up to 37 U/ml) but peaked at day 10 (Löser et al, unpublished observations). Since hAdV-5 vectors were also reported to induce severe hematological changes in rabbits, especially erythroblastosis (Cichon et al, 2001), and a gene transfer study in man with fatal outcome reported high vector concentrations in lymphohematopoietic organs (Raper et al, 2003) we checked rabbits for hematological side effects after systemic infection with 2 x $10^{10}$ infectious particles per kg of the OAdV-haat vector. This vector dose was sufficient to produce high serum levels of about 100 µg/ml hAAT protein by day 2 post infection. In the infected, but not in control animals, a low and transient increase in leucocyte number was observed apparently due to virally induced leucocytosis (**Figure 5a**). Notably, in contrast to the situation after infection with hAdV (Cichon et al, 2001) erythroblasts did not dominate the leucocyte fraction, indicating that no major damage to bone marrow had been caused by infection with OAdV. As was the case with hAdV, a transient moderate drop in thrombocyte count that normalized by 4 days after infection was observed in OAdV-infected rabbits (**Figure 5b**). However, in sharp contrast to hAdV-5 vectors that are known to hemagglutinate human erythrocytes in vitro, no binding of OAdV to human erythrocytes was observed (**Figure 5c**). Thus, although OAdV induced certain side effects in experimental animals, these were moderate and transient at the doses used. Moreover, the experiments in rats and rabbits were performed with vector doses that produced very high levels of the transgene product in serum (up to several hundred µg/ml of hAAT) and that probably represent the maximum dose that would be required for adequate expression.

As mentioned above, currently available recombinant OAdV vectors contain the full length OAdV genome extended by the transgene cassette that includes regulatory sequences such as a promoter and polyadenylation signal. Deletion of about 2 kb of genome

between insertion sites 2 and 3 removes a large part of the RH region, but the resulting vectors are not compromised in their ability to replicate on ovine cell lines. Thus, it was necessary to assess the potential risk of vector replication on non-ovine, especially human cells. In several studies it was shown that OAdV did not replicate productively in any non-ovine cell line investigated (Khatri et al, 1997, Kümin et al, 2002). However, viral DNA was replicated following infection of many cell types, although to very different degrees. DNA replication was most efficient in human and rat cells of fibroblast origin, where up to an 84-fold increase in vector DNA was achieved within 72 hours. Replication and early viral gene expression was particularly inefficient in cells of liver origin (Khatri et al, 1997; Kümin et al, 2002). However, even in cells where early gene expression and viral DNA replication occurred the translation of late genes, posttranslational processing and/or nuclear transport of late gene products was defective. Consequently, productive virus replication was blocked. OAdV remained replication abortive even after several passages in human cells (Khatri et al, 1997; Lockett and Both, 2002). The abortive replication properties of OAdV renders assays for replication-competent viruses unnecessary and unachievable in human cells. The high replication rate of OAdV vector DNA in some cell types (which has not been observed for E1-deleted human adenovirus vectors in non-transformed cells such as fibroblasts) might contribute to the high transgene expression observed after OAdV mediated gene transfer in vivo by elevating the number of copies of template DNA for transcription. On the other hand, the expression of viral genes observed *in vitro* could also be responsible for inducing an immune response against OAdV *in vivo*.

OAdV does not contain genes with homology to adenovirus E1 genes, the products of which can cooperate with E4 gene products to transform rodent cells in vitro. OAdV was therefore tested for transforming activity in standard transformation assays on rat embryo cells. In contrast to hAdV-5, which efficiently caused colony formation, neither infection with OAdV nor transfection of cells with plasmids carrying the complete or partial OAdV genome sequences produced transformed foci (Xu et al, 2000). The lack of OAdV genes with functions homologous to those of the hAdV-5 E1 genes was also confirmed by the finding, that a recombinant OAdV carrying the hAdV-5 E1A region, but not a control vector, was able to trans-complement replication of an E1A-mutated hAdV-5 in human cells (Lockett and Both, 2002). In the reverse experiment, replicating hAdV-5 was not capable of trans-complementing OAdV replication in three human cell types. This finding invalidates apprehensions that recombinant OAdV vectors could replicate *in vivo* during an opportunistic infection by a human adenovirus.

Nevertheless, the safety of OAdV vectors could be further enhanced. For example, since adenoviruses have evolved mechanisms to drive host cells into S phase the identification of gene products of OAdV which interfere with the E2F pathway is of particular interest. One such protein encoded by the OAdV genome is the E43 protein that directly binds to the E2F-1 protein of ovine and human cells (Kümin et al, 2004). E43 was shown to induce E2F-dependent transcription and was able to strongly activate the OAdV E2 promoter which contains a non-palindromic E2F site. Activation of the E2 promoter is a very early and important event in the viral replication cycle. No stimulation of the E2 promoter or of a synthetic E2F-dependent promoter was achieved by over-expression of the OAdV LH1-3 genes that were initially thought to be functionally equivalent to the mastadenovirus E1 genes. Mutation of the E43 gene (by the introduction of stop codons), but not of the other E4 genes, resulted in a dramatic reduction in virus replication. Thus, because of its essential nature it should be possible to provide the E43 gene in *trans* in a packaging cell line. This may also reduce viral gene expression and the immunogenicity of OAdV vectors.

## VI. Potential applications of OAdV vectors

OAdV is an efficient gene transfer vector that can produce high level, but transient expression of a gene in animals after systemic or local delivery. Favored applications for this vector system currently include tumor gene therapy and vaccination. For both applications, vectors have been successfully tested in mouse models.

As mentioned above, OAdV was able to induce a strong $TH_1$ cell response against the transgene product after local injection. Strong CD4+ and CD8+ responses correlate with the clearance of viral infections such as hepatitis C virus (HCV) (reviewed in Ward et al, 2002), and induction of a strong and sustained T cell response against multiple HCV epitopes is thought to be necessary for overcoming chronic HCV infection. In mice, OAdV vectors coding for non-structural genes of HCV induced a strong T cell response to the respective HCV proteins which persisted for at least 10 weeks (Wüest et al, 2004; Wüest and Löser, unpublished results). This immune response was obtained by both intramuscular and subcutaneous injection of the vector. Importantly, the development of OAdV-mediated T cell immunity to HCV was not affected by pre-existing, neutralizing anti hAdV-5 antibodies in mice at titers comparable to those found in the human population. In contrast, this immunity markedly diminished the ability of an equivalent dose of a hAdV-5 vector carrying the same HCV genes to induce HCV-specific T cell immunity. This finding is in agreement with studies of a human adenovirus-based anti SIV vaccine in macaques which was far less efficient when animals were pre-immunized with hAdV (Casimiro et al, 2003). Similarly, vaccination of hAdV-5 pre-immunized mice was effective with a chimpanzee adenovirus vector carrying the HIV-1 gag gene but not with an equivalent hAdV-5 vector (Fitzgerald et al, 2003). These findings and our results again underline the necessity to develop vaccination vectors which are not based on hAdV-5.

OAdV vectors have been also applied to a gene-directed enzyme pro-drug (GDEPT) strategy for the treatment of prostate cancer. Vectors carrying the *E. coli* gene for purine nucleoside phosphorylase (PNP) were injected directly into the tumor and the pro-drug (fludarabine) was applied for a period of five days. This

pro-drug is converted to a toxin by the expressed enzyme thus causing the death of infected and nearby cells. Significant retardation of tumor growth was obtained in mice where human prostate cancer cells were implanted subcutaneously in nude mice (up to 75% reduction in tumor size) (Voeks et al, 2002) as well as in a transgenic mouse model for a human adenocarcinoma of the prostate (Martiniello-Wilks et al, 2004a). Prostate-specific transgene expression in both androgen-dependent and -independent prostate cancer cell lines was also demonstrated, thus increasing the safety of the vector system if the vector should escape from the injection site in the prostate (Wang et al, 2004). Importantly, treatment of the primary tumor in the prostate had a significant impact on the development of lung pseudo-metastases in an immunocompetent mouse model (Martiniello-Wilks et al, 2004b). Substantial effort has also gone into vector production and scale-up (Both et al, unpublished results) in preparation for a phase I/IIA clinical trial for prostate cancer.

## VII. Conclusions

The understanding of the biology of OAdV and the development of the virus as a vector system has progressed steadily over the last decade. Although intriguing questions about the biology remain to be elucidated, as a vector OAdV has proved to be safe and efficient *in vitro* and *in vivo*. Depending on the mode of administration the lack of anti-OAdV immunity in the human population should provide an initial advantage compared with vectors derived from human adenoviruses. Moreover, the distinct cellular tropism of OAdV may allow for gene transfer into cell types that are not susceptible to infection by hAdV vectors. If OAdV proves to be safe in the clinic its value as a vector will be considerably enhanced.

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