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Guinea Pig Adenovirus Infection Does Not Inhibit Cochlear Transfection with Human Adenoviral Vectors in a Model of Hearing Loss

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Routine surveillance of guinea pigs maintained within a barrier facility detected guinea pig adenovirus (GPAdV) in sentinel animals. These guinea pigs served as models of induced hearing loss followed by regeneration of cochlear sensory (hair) cells through transdifferentiation of nonsensory cells by using human adenoviral (hAV) gene therapy. To determine whether natural GPAdV infection affected the ability of hAV vectors to transfect inner ear cells, adult male pigmented guinea pigs (n = 7) were enrolled in this study because of their prolonged exposure to GPAdV-seropositive conspecifics. Animals were deafened chemically (n = 2), received an hAV vector carrying the gene for green fluorescent protein (hAV-GFP) surgically without prior deafening (n = 2), or were deafened chemically with subsequent surgical inoculation of hAV-GFP (n = 3). Cochleae were evaluated by using fluorescence microscopy, and GFP expression in supporting cells indicated that the hAV-GFP vector was able to transfect inner ears in GPAdV-seropositive guinea pigs that had been chemically deafened. Animals had histologic evidence of interstitial pneumonia, attributable to prior infection with GPAdV. These findings confirmed that the described guinea pigs were less robust animal models with diminished utility for the overall studies. Serology tests confirmed that 5 of 7 animals (71%) were positive for antibodies against GPAdV at necropsy, approximately 7 mo after initial detection of sentinel infection. Control animals (n = 5) were confirmed to be seronegative for GPAdV with clinically normal pulmonary tissue. This study is the first to demonstrate that natural GPAdV infection does not negatively affect transfection with hAV vectors into guinea pig inner ear cells, despite the presence of other health complications attributed to the viral infection.

Abbreviations: GFP, green fluorescent protein; GPAdV, guinea pig adenovirus; IFA, Indirect Fluorescent Antibody Test.

The mammalian auditory system is sensitive to dysfunction induced by aging, ototoxic drugs, infections, autoimmune disease, and traumatic overstimulation. Impairment of sensorineural hearing affects millions of people globally and is irreversible once auditory hair cells are destroyed. The guinea pig (*Cavia porcellus*) is the typical animal model for studying inner ear biology because the cochlea in this species is easily accessible. The cochlear hair cells are responsible for sensory transduction, with the outer hair cells more sensitive than the inner hair cells to acoustic trauma.

Guinea pigs serve as recipients of aural gene delivery, through use of a variety of viral vectors, which leads to in vivo transduction of multiple cell types in the inner ear.[3,12,16] Auditory hair cell replacement and improved hearing in deafened guinea pigs has been demonstrated by using human adenoviral (hAV serotype 5) delivery of *Atoh1*, a key regulator of hair cell development.[10]

Within our research colony of pigmented guinea pigs, routine surveillance detected guinea pig adenovirus (GPAdV) in sentinel animals. The unexpected finding of infection with a rarely reported guinea pig pathogen potentially compromised the surgical model and therefore warranted further investigation. Although clinical signs of GPAdV, particularly signs of respiratory distress, were not seen in the colony animals, laboratory personnel perceived a higher-than-anticipated loss of experimental animals during or shortly after the performance of surgical deafening procedures, particularly around the time of initial sentinel detection of GPAdV in the colony. Because of prolonged exposure to GPAdV-seropositive conspecifics in the animal housing room, and the knowledge that the laboratory also had worked extensively with recombinant hAV vectors, we wished to determine whether the presence of GPAdV negatively influenced the outcome of hAV vector gene transfer in the inner-ear epithelium.

Materials and Methods

Adult male pigmented guinea pigs (n = 7; age, approximately 5 mo; weight, 607 to 981 g; Elm Hill Breeding Labs, Chelmsford, MA) were maintained under a protocol approved by the University Committee on the Use and Care of Animals. Pigmented guinea pigs were used because of the greater ease of visualizing the stria vascularis, which serves as an essential landmark necessary for the surgical procedure. Health assessment profiles from the vendor reported that the guinea pigs were negative for Sendai virus, pneumonia virus of mice, reovirus, lymphocytic choriomeningitis virus, parainfluenza 3, guinea pig adenovirus, simian virus 5, and *Encephalitizoon cuniculi*. Animals were reported negative upon shipment for *Salmonella* spp., *Bordatella bronchiseptica*,

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β-hemolytic *Streptococcus*, *Streptococcus pneumoniae*, and *Pasteurella* spp. (testing performed by Taconic Anmed, Rockville, MD). Cytomegalovirus and parainfluenza virus were not routine pathogens for which test results were provided in this species.

Because they were shipped from an approved vendor, pigmented guinea pigs destined for experimental use bypassed quarantine housing on arrival and were placed directly into the barrier housing room. Prior to the onset of our study, sentinel albino guinea pigs (Hartley, Charles River Laboratories, Wilmington, MA) tested seropositive for GPAdV antibodies for 2 consecutive surveillance quarters, and the room was quarantined and classified as positive for GPAdV infection until all colony animals in the room were culled for experimental purposes. Subsequent shipments of sentinel animals were tested on arrival to the facility and found to be seronegative for antibodies to GPAdV, whereas incoming shipments of experimental animals continued to yield GPAdV-seropositive results on arrival.

To ensure that control animals were GPAdV-negative for comparison, we obtained guinea pigs (n = 5) of the same strain and from the same source as the sentinel animals (male Hartley; n = 5; weight, 390 to 430 g; Charles River Laboratories). Pigmented guinea pigs were unavailable through a vendor source other than the supplier of the experimental animals. Control animals were bled specifically for confirmation of GPAdV-negative status on arrival at the facility. These animals were housed for 1 wk prior to surgery in a room distinct from the experimental cohort. Control animals were reported to be negative for Sendai virus, pneumonia virus of mice, reovirus, lymphocytic choriomeningitis virus, guinea pig adenovirus, *Encephalitizoon cuniculi*, *Salmonella* spp., *Bordatella bronchiseptica*, *Mycoplasma pulmonis*, *Streptococcus* spp., *Helicobacter* spp., *Klebsiella* spp., *Pasteurella* spp., and *Clostridium piliforme*, according to provided health reports (testing performed by Charles River Laboratories, Kingston, NY).

Experimental animals were housed at a density of 1 to 4 guinea pigs per open-topped cage bin in a room where GPAdV-seropositive sentinels had been detected. Animals were fed a standard rodent diet (Guinea Pig Diet 5025, PMI Nutrition International, Brentwood, MO), had access to automatic waterers, and were maintained in a controlled environment compliant with recommendations promulgated by the *Guide for the Care and Use of Laboratory Animals*.[8]

Husbandry of control animals was crucial to ensure their GPAdV-negative status was uncompromised. These animals were housed on a different facility floor from that of the experimental colony. In addition, only one member of the research group interacted with these animals; control and experimental groups were not handled concurrently (either in the facility or in the laboratory spaces) during the course of the same working day.

Guinea pigs that had been housed in the GPAdV-positive room for longer than 7 mo were assigned randomly to 1 of 3 experimental groups: those in group 1 (n = 2) underwent chemical deafening; animals in group 2 (n = 2) underwent surgical adenoviral inoculation without prior chemical deafening; and guinea pigs in group 3 (n = 3) underwent chemical deafening followed by surgical adenoviral inoculation.

Guinea pigs in groups 1 and 3 (n = 5) were deafened bilaterally with a single subcutaneous dose of kanamycin (400 mg/kg, American Pharmaceutical Partners, Schaumburg, IL) followed 2 to 4 h later by ethacrynic acid (30 mg/kg, Sodium Edecrin, Merck, West Point, PA) injected into the jugular vein while animals were under anesthesia (40 mg/kg ketamine IP; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, and 10 mg/kg xylazine IP; AnaSed, Lloyd Laboratories, Shenandoah, IA). Deafening was verified by a negative Preyer reflex.

Animals in groups 2 and 3 underwent surgical inoculation with vector into the left ear; they were anesthetized as described, and a replication-deficient recombinant hAV (GenVec, Gaithersburg, MD) with a reporter gene for green fluorescent protein (hAV-GFP) under the control of a cytomegalovirus promoter was infused (10 µL; 1×10^{12} particles per mL) into the left cochlea through a cochleostomy as modified from previously described methods.[9] Briefly, the cochleostomy was drilled at the level of the apical turn through the bony wall of the cochlea and through the stria vascularis, which ensured that the hole opened into the scala media of the apex (Figure 1). Control animals underwent the same deafening and surgical inoculation with vector as described for group 3 animals. All animals received preemptive topical lidocaine (0.1 mL (of a 1% solution) SC; Hospira, Lake Forest, IL) at the site of incision prior to the procedure and then received buprenorphine (0.05 mg/kg SC daily for 2 d; Bedford Laboratories, Bedford, OH) after the surgical procedure for pain management.

Collectively, the timing of necropsies was scheduled over 2 consecutive days for all experimental animals. Briefly, animals were deeply sedated (50 mg/kg ketamine and 12 mg/kg xylazine IP) and decapitated, and temporal bones were removed for processing. The exposed cochleae were flushed gently with 4% paraformaldehyde, perfused for 2 h with paraformaldehyde, and processed for immunohistochemistry by described methods.[10] Terminal whole-blood samples were collected for analysis for antibodies against GPAdV. Tissues from brain, lymph nodes, lungs, heart, liver, kidneys, and nasal cavities were processed for histopathology. Collection of lung tissues was performed aseptically, and samples were frozen at –80.0 °C for later PCR amplification of adenoviral DNA by using cycle conditions and primers previously published.[18] Control animals were similarly necropsied at a time point distinct from the experimental animals, and lung tissues were harvested for histopathologic assessment.

Results

Recovery of the described guinea pigs from the deafening and surgical procedures was uneventful.

Group 1: Chemical deafening. Analysis of whole mounts of the organ of Corti from chemically deafened guinea pigs detected a few remaining hair cells only in the apical turn of the cochlea. In the 3 lower turns, no inner or outer hair cells could be detected (Figure 2 A, B). This distribution of hair cell loss was expected, because hair cells are most vulnerable to insult near the base of the cochlea. Evidence of chemical deafening viewed by whole-mount preparation showed loss of the regular geometric arrangement of cells, absence of the actin-rich cuticular plate and stereocilia, and a typical 'scar' morphology created by expanded supporting cells.[19]

Group 2: Inoculation with hAV. Evidence of hair cell loss was observed in the apical turn, primarily at the site where hAV-GFP inoculation occurred. This partial hair cell loss was due to the localized trauma of the injection surgery and has been noted in guinea pigs undergoing surgical inoculation of vector without prior deafening.[9,12,13] No detectable GFP expression (indicative of incorporation of the adenoviral construct) was noted in these animals. In guinea pigs that are not deafened, hair cells typically

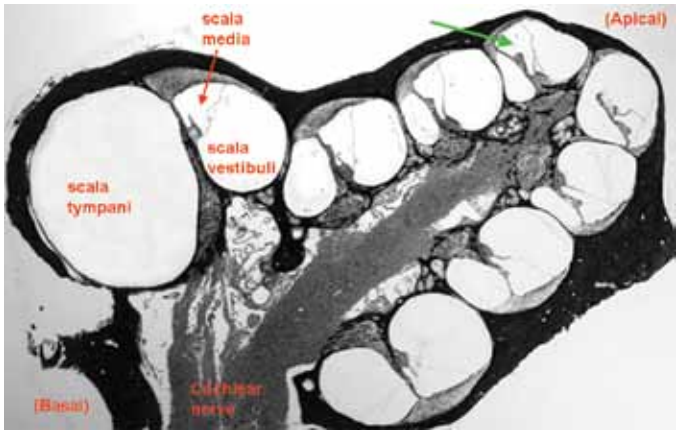

Figure 1. Light micrograph of a cross-section of the guinea pig cochlea at low magnification. The scala vestibuli and scala tympani are perilymph-filled fluid spaces that are connected at the apex. The scala media is filled with endolymph. During surgical inoculation, hAV-GFP is injected into the endolymph of the apex (green arrow). This image has been modified from reference 20 and is reprinted with permission.

show little or no expression of genes introduced by adenoviruses, but some supporting cells may express those genes.[12]

Group 3: Chemical deafening followed by hAV inoculation. Hair cell loss was evident in all animals (n = 3) and exhibited the same pattern as seen in group 1. GFP expression in all animals was revealed by epifluorescence in several types of supporting cells (Figure 2 C), as demonstrated previously.

In control animals, the transfection rate was similar to that typically seen for clinically normal experimental animals, with no surviving hair cells transfected and approximately 30% to 50% of supporting cells transfected successfully (Figure 2 D**)**. The anatomic markers typically used in the inoculation surgery were not visible in albino guinea pigs (the strain used as controls); therefore, cochlear inoculation was performed blinded at the time of surgery. However, during dissection to prepare the tissue for histologic examination, it was confirmed that all control guinea pigs were inoculated in the third turn, as were group 3 animals.

Transfection efficiency was assessed as an estimation of the relative presence (%) of fluorescence. Difficulties in quantifying transfection rates were compounded by the inherent disorganization of the supporting cells, as well as the undefined number of such cells in the ears.

Gross and histopathology. All experimental animals had gross evidence of mottled and diffusely reddened lung tissue, with ventrally distributed demarcations of abnormal parenchyma. On histopathology, lung tissue had areas of interstitial pneumonia, characterized by focal type II pneumocyte hyperplasia, heterophilic infiltration, and atelectasis. Presence of interstitial pneumonia was similar in all experimental animals and ranged in severity from mild and multifocal (Animal 1A) to severe and widespread (Animal 2B; Figure 3 A). Control animals overall had no evidence of pulmonary pathology (Figure 3 B**)**. Acute intraalveolar hemorrhage noted at necropsy in experimental and control animals was attributed to the method of euthanasia used. Lymph node sections from experimental animals revealed follicular and parafollicular lymphoid hyperplasia, which were highly suggestive of a response to an infectious agent; however no evidence of an etiologic agent (bacteria, fungi, or viral inclusion bodies) was noted in any of the evaluated sections. No animals had evidence of inflammatory disease in the nasal cavities. All experimental animals had evidence of segmental nephropathy, a common idiopathic lesion in this species. The application of the aminoglycoside kanamycin for chemical deafening may have contributed to the development of kidney disease, although mild nephropathy also was noted in group 2 animals, which did not undergo chemical deafening.

Serology and PCR analysis. Table 1 provides a summary of the experimental and control animal groups and GPAdV serology test results. Whole blood was collected at the time of necropsy, and serum samples were submitted to a contract diagnostic laboratory (Charles River Laboratories, Wilmington, MA) for detection of GPAdV antibodies by ELISA. Five animals tested GPAdV-seropositive, and another 2 animals were classified as 'interpreted nonspecific,' indicating that the samples were presumed to be GPAdV-positive yet required confirmation by another serologic assay. Indirect fluorescent antibody (IFA) assays, which confirmed the 5 positive ELISA results, demonstrated that the 2 animals initially deemed nonspecific were GPAdV-seronegative at the time of necropsy. Serology on whole blood from control animals was performed on arrival to the facility, and all animals were negative by IFA. PCR analysis of samples of lung tissue confirmed to be GPAdV-seropositive did not amplify GPAdV gene sequences.[11]

Discussion

In light of reports that subclinical infections with guinea pig adenovirus may interfere with laboratory studies,[2,4] we wished to determine whether exposure to GPAdV would interfere with experimental hAV gene therapy treatments. In particular, we wanted to determine whether important experimental data from the described colony animals could be retained, despite the concurrent identification of an infectious disease outbreak. Evaluation of whole mounts of the ears showed that hAV vectors readily transfected the supporting cells of ears in which hair cells had been destroyed by chemical exposure, similar to what has been described for animals known or presumed to be free of GPAdV infection. The cohort of control animals, verified to be GPAdV- seronegative prior to surgical experimentation, had a transfection level comparable to those animals that were GPAdV -seropositive. No apparent rejection of the recombinant hAV vector occurred, a finding that corroborates reports that adenoviruses are relatively host-specific and that sequences of GPAdV that are associated with type-specific antigenicity differ from those of human adenoviruses.[5] In addition, hyperimmune serum from experimentally infected guinea pigs has not been shown to cross-react with human adenovirus antigen in complement fixation assays.[14] The current study is the first to investigate GPAdV infection in a colony of guinea pigs that were routinely exposed to recombinant hAV for experimental purposes. We conclude that natural infection with GPAdV did not negatively affect experimental hAV transfection. The information presented indicates that guinea pigs from a colony with endemic GPAdV still have value as research subjects in studies involving hAV transfection.

Although adenoviral infections in guinea pigs in research colonies have been reported only rarely,[4,6,7,15] these infections may be more prevalent than typically recognized.[17] The disease tends to have an acute clinical course and has been associated with low morbidity and high mortality,[2,6,15,21] although we did not observe these features in our colony of adult animals exposed to GPAdV infection. Dunkin–Hartley weanling guinea pigs in a conventional

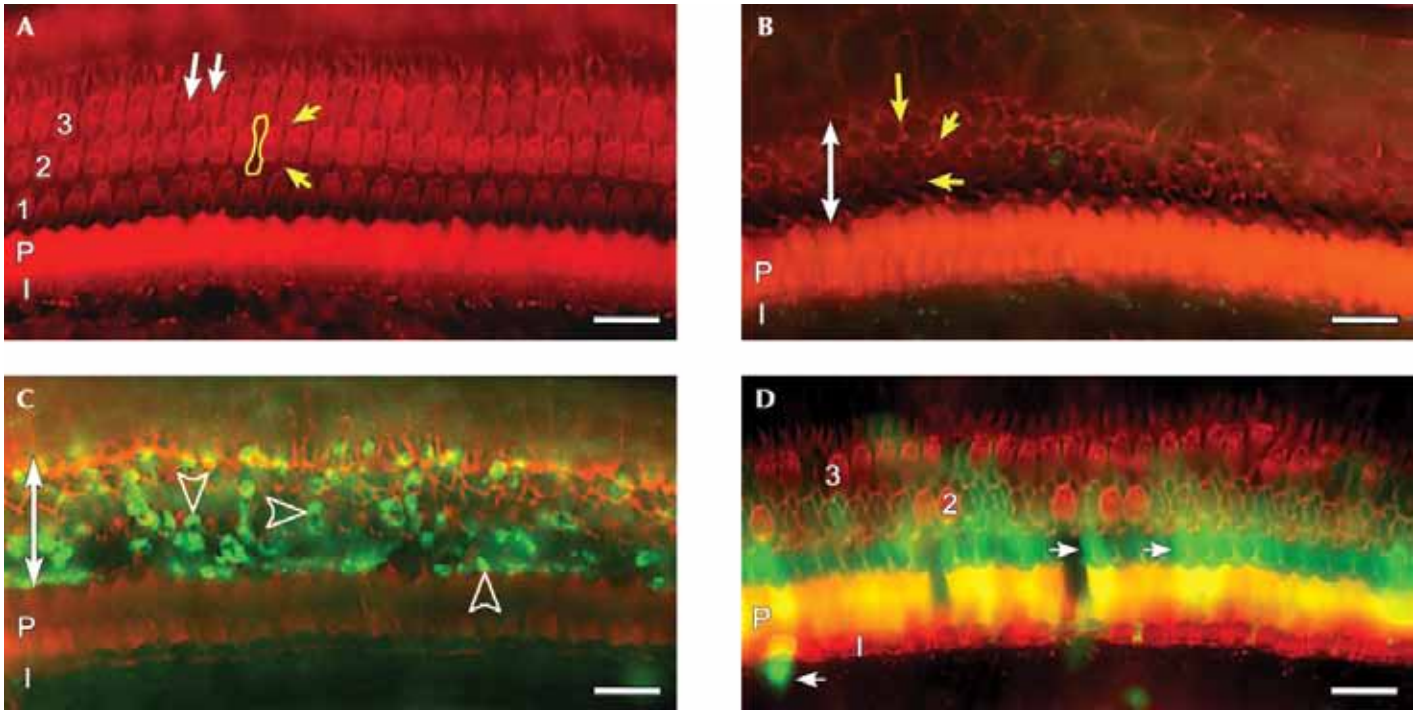

Figure 2. Representative whole mounts of the organ of Corti were stained with fluorescent-conjugated phalloidin and photographed in epifluorescence using electron microscopy. Phalloidin is a toxin that binds to the protein actin, which is present in high concentrations in cell junctions and the cuticular plate of the stereocilia. Scale bar, 20 µm. (A) Untreated control animal (GPAdV-seronegative) illustrating the normal arrangement of cells. Numbers 1 through 3 indicate the 3 rows of outer hair cells. Pillar cells (P) and the row of inner hair cells (I) also are indicated. White arrows point to the stereocilia bundles on the tops of 2 hair cells in the third row, which are the focal place of the image. The yellow dumbbell shape outlines a supporting cell intercalated between 2 second-row hair cells. Yellow arrows indicate junctional complexes between 2 supporting cells. (B) Group 1 animal (GPAdV-seropositive) that was chemically deafened. Within the organ of Corti, only the row of pillar cells (P) can be recognized. Hair cells are absent, and the approximate former locations of inner hair cells (I) and outer hair cells (double-headed white arrow) are indicated. Junctional complexes (yellow arrows) are still visible between surviving supporting cells. (C) Group 3 animal (GPAdV-seropositive) that was chemically deafened similar to Group 1 and subsequently inoculated with hAV-GFP. Supporting cells that were successfully transfected, resulting in GFP expression (revealed by epifluorescence), are indicated with arrowheads. (D) Treated control animal (GPAdV-seronegative) that was deafened and subsequently inoculated with hAV-GFP in the scala media, similar to Group 3. Arrows point to transfected pillar (P) and supporting cells expressing GFP. Surviving inner hair cells (I) and outer hair cells (2, 3) are never transfected.

colony were reported to present with sporadic death, occasionally subsequent to depression and dyspnea, but noticeably coincident with the initiation of experimental use and in the absence of respiratory bacterial infection.[7] Failure of experimental exposure to infect neonatal rats or hamsters demonstrated species-specificity of the virus.[14] Others have reported that adenoviral respiratory tract infections have been asymptomatic and only identified as an incidental finding at the time of necropsy.[2] GPAdV infection may become clinically symptomatic when animals encounter stressful circumstances,[7] which likely accounted for the investigative laboratory in our study perceiving a higher-than-expected loss of experimental colony animals after surgical deafening procedures.

Although GPAdV reportedly has a low level of contagion,[4] we found that all experimental guinea pigs, which all were housed in the same colony room in open-top cages, had evidence of lung pathology on necropsy. In previous studies, descriptions of gross lesions included sloughing of the epithelial lining of airways with multifocal reddening and consolidation of cranial (anterioventral) lung lobes.[7] Histopathologic lesions could include bronchiolitis, bronchitis, and catarrhal bronchopneumonia, along with demarcated areas of pulmonary condensation.[7] In our investigation, all animals demonstrated histopathologic evidence of pneumonia, indicative of sequellae to GPAdV infection. Other investigators have documented the appearance of basophilic intranuclear inclusion bodies within epithelial cells of occluded bronchioles for up to 30 d after detected infection,[2,7] but we were unable to identify similar viral particles in the examined tissues harvested from our infected animals. The agent may well have been cleared from the animals after an initial infectious phase, resulting in residual pulmonary lesions and the lack of detectable viral inclusion bodies.

Guinea pig adenovirus replicates in the upper respiratory tract of guinea pigs and PCR methods similar to those used in our study have been shown to amplify GPAdV within 15 d of experimental intranasal viral inoculation.[1] Our negative PCR results may be attributed to the length of time (4 to 7 mo) from initial detection of GPAdV in the colony to analysis of harvested lung tissues. The negative PCR results might also be attributed to clearance of the virus or absence of agent in the sections of submitted lung tissues. Work has been initiated to further investigate reliable methods for serologic monitoring through characterization of major GPAdV antigenic determinants.[5] Exposure to GPAdV in this colony was confirmed by ELISA and IFA in 5 of 7 animals; in the 2 that tested GPAdV -seronegative, we infer that the infection

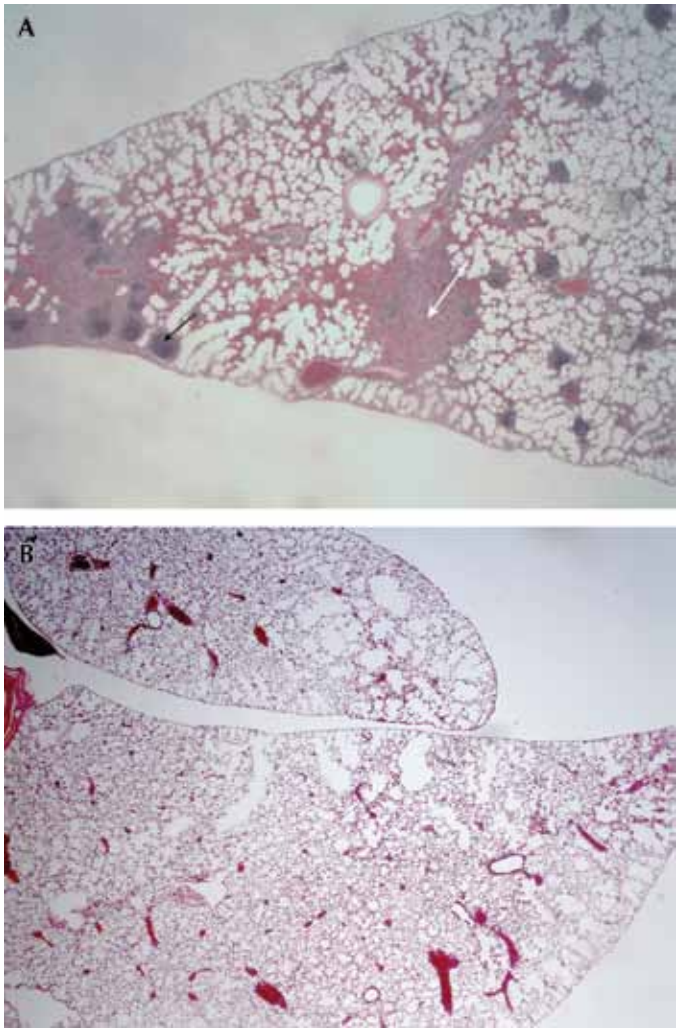

Figure 3. Representative histologic lung sections stained with hematoxylin and eosin. (A) GPAdV-seropositive animal (group 2, animal B); magnification, ×2. Lymphoid aggregates (black arrow) can be a normal finding in guinea pig lung tissue, but the severity here is indicative of a pathogenic response to infection. Areas of atelectasis and inflammation (white arrow) also are present. (B) Control GPAdV-seronegative guinea pig; magnification, ×2. No lung lesions were noted, except for focally extensive intraalveolar hemorrhage associated with the method of euthanasia.

had cleared over time, with lung lesions remaining as evidence of prior infection.

The guinea pigs described in the present study were maintained in a barrier facility in which GPAdV had never before been detected within the colony. We believe that infection with GPAdV was the result of the introduction of new animals carrying the infectious agent. Infection in these animals may have been missed because of the difficulty in detecting GPAdV serologically. Historically, diagnosis of GPAdV has been challenging and hindered by an inability to propagate the virus in tissue culture to establish homotypic antibodies.[6,14,22] In this study, we too were unable to isolate virus from lung tissue that was propagated in cell culture conditions.[11] The testing methods used by the vendor of these guinea pigs included standard reagents to mouse adenovirus (MAV 1/2) reagents to which GPAdV has been shown to weakly

Table 1. GPAdV serology test results

Group	Animal	Chemical deafening	hAV-GFP inoculation	GAPdV ELISA[a]	GAPdV IFA
1	A	+	not done	IN	–
	B	+	not done	+	+
2	A	not done	+	+	+
	B	not done	+	+	+
3	A	+	+	+	+
	B	+	+	+	+
	C	+	+	IN	–
Controls	1–5	+	+	not done	–[b]

IN, interpreted as nonspecific
[a]Performed on whole blood collected at the time of necropsy
[b]Performed on whole blood collected on arrival at facility.

cross-react. Although initial health reports from the vendor indicated that the experimental animals were GPAdV seronegative, further discussion and investigation of the testing methodology elucidated that the negative test results were inaccurate.

The outcome from this study provided retrospective assurance that despite the occurrence of the outbreak during an ongoing experimental protocol, the data obtained from GPAdV-infected animals was nonetheless valid. However, the underlying lung pathology observed in GPAdV-infected guinea pigs rendered them less appropriate than uninfected animals for general utility as research models. We recommend that guinea pig colonies with unexpected outbreaks of clinical or subclinical GPAdV be cleared of virus prior to experimental use in research animal protocols.

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References

1. **Butz N, Ossent P, Homberger FR.** 1999. Pathogenesis of guinea pig adenovirus infection. Lab Anim Sci **49:**600–604.
2. **Crippa L, Giusti AM, Sironi G, Cavalletti E, Scanziani E.** 1997. Asymptomatic adenoviral respiratory tract infection in guinea pigs. Lab Anim Sci **47:**197–199.
3. **Derby ML, Sena-Esteves M, Breakefield XO, Corey DP.** 1999. Gene transfer into the mammalian inner ear using HSV1 and vaccinia virus vectors. Hear Res **134:**1–8.
4. **Eckhoff G, Mann P, Gaillard E, Dykstra M, Swanson G.** 1998. Naturally developing virus-induced lethal pneumonia in 2 guinea pigs (*Cavia porcellus*). Contemp Top Lab Anim Sci **37:**54–57.
5. **Feldman SH, Sikes RA, Eckhoff GA.** 2001. Comparison of the deduced amino acid sequence of guinea pig adenovirus hexon protein with that of other mastadenoviruses. Comp Med **51:**120–126.

6. **Finnie JW, Noonan DE, Swift JG.** 1999. Adenovirus pneumonia in guinea pigs. Aust Vet J **77:**191–192.
7. **Harris IE, Portas BH, Goydich W.** 1985. Adenoviral bronchopneumonia of guinea pigs. Aust Vet J **62:**317.
8. **Institute of Laboratory Animal Resources.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
9. **Ishimoto S, Kawamoto K, Kanzaki S, Raphael Y.** 2002. Gene transfer into supporting cells of the organ of Corti. Hear Res **173:**187–197.
10. **Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, Brough DE, Raphael Y.** 2005. Auditory hair cell replacement and hearing improvement by *Atoh1* gene therapy in deaf mammals. Nat Med **11:**271–276.
11. **Kajon A.** 2008. Personal communication.
12. **Kawamoto K, Ishimoto S, Minoda R, Brough DE, Raphael Y.** 2003. *Math1* gene transfer generates new cochlear hair cells in mature guinea pigs in vivo. J Neurosci **23:**4395–4400.
13. **Kawamoto K, Yagi M, Stöver T, Kanzaki S, Raphael Y.** 2003. Hearing and hair cells are protected by adenoviral gene therapy with TGFβ1 and GDNF. Mol Ther **7:**484–492.
14. **Kunstyr I, Maess J, Naumann S, Kaup FJ, Kraft V, Knocke KW.** 1984. Adenovirus pneumonia in guinea pigs: an experimental reproduction of the disease. Lab Anim **18:**55–60.
15. **Naumann S, Kunstýr I, Langer I, Maess J, Hörning R.** 1981. Lethal pneumonia in guinea pigs associated with a virus. Lab Anim **15:**235–242.
16. **Patel NP, Mhatre AN, Lalwani AK.** 2004. Biological therapy for the inner ear. Expert Opin Biol Ther **4:**1811–1819.
17. **Percy DH**, **Barthold SW.** 2007. Pathology of laboratory rodents and rabbits, 3rd ed. Hoboken (NJ): Blackwell Publishing.
18. **Pring-Akerblom P, Blazek K, Schramlová J, Kunstýr I.** 1997. Polymerase chain reaction for detection of guinea pig adenovirus. J Vet Diagn Invest **9:**232–236.
19. **Raphael Y, Altschuler RA.** 1991. Scar formation after drug-induced cochlear insult. Hear Res **51:**173–183.
20. **Raphael Y, Altschuler RA.** 2003. Structure and innervation of the cochlea. Brain Res Bull **60:**397–422.
21. **Schoeb TR.** 2000. Respiratory diseases of rodents. Vet Clin North Am Exot Anim Pract **3:**481–496, vii.
22. **Vitalis TZ, Keicho N, Itabashi S, Hayashi S, Hogg JC.** 1996. A model of latent adenovirus 5 infection in the guinea pig (*Cavia porcellus*). Am J Respir Cell Mol Biol **14:**225–231.