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High-Level Transgene Expression in Nonhuman Primate Liver with Novel Adeno-Associated Virus Serotypes Containing Self-Complementary Genomes

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Adeno-associated virus (AAV) vectors are being considered for in vivo applications of gene therapy in the treatment of a variety of disorders. This study evaluates the biology of second-generation vectors based on the novel serotypes AAV7 and AAV8 and containing self-complementary genomes in the nonhuman primate liver. Stable levels of transgene expression were achieved in cynomolgus macaques and suggest efficiencies at least 2 log higher than what could be achieved with AAV2 vectors using traditional single-stranded genomes. Analysis of DNAs from tissues revealed high levels of vector in the liver that appeared proportional to the relative amounts of transgene expression.

The liver remains an important target for somatic gene transfer in the treatment of a variety of inherited and acquired disorders. The most promising vector system for achieving safe and stable transgene expression in the liver is based on adeno-associated virus (AAV), although the use of vectors created from the previously available isolates, AAV1 through AAV6, was disappointing because of poor transduction efficiencies in vivo (7, 8).

In order to improve the gene transfer efficiency with AAV vectors and to broaden their tropisms, we undertook a project to identify new AAV isolates from primates and used these to create novel vectors. Over 110 novel AAV sequences were recovered from human and nonhuman primate tissues as latent genomes forming a complex family of viruses that encompass five different clades (2, 4). Capsids from the novel AAVs were used to package vector genomes containing AAV2 inverted terminal repeats (ITRs). Vectors based on AAV serotypes 7 and 8 demonstrated substantial improvements in gene transfer efficiency in the murine liver (4). An alternative approach to improving AAV transduction was to create self-complementary(sc) AAV genomes that form double-stranded intermediates independent of second-strand synthesis. Hirata and Russell noted that AAV vector genomes of less than one-half the wild-type size were capable of being packaged as dimers (5). This biology was used by McCarty et al. to develop vectors capable of highly efficient transduction (6). Wang et al. mutated the D sequence of the ITR to favor the formation of self-complementary genomes (10). In these vectors, the terminal resolution site in one of the ITRs is deleted, resulting in a dimer capable of self-annealing. AAV2-based scAAV vectors demonstrated substantially improved gene transfer in various murine tissues, such as the liver, compared to traditional scAAV vectors (6, 10).

The purpose of this study was to evaluate the potential of combining the advantage of a novel AAV serotype with a scAAV vector and to test these second-generation concepts in a nonhuman primate model. We and Davidoff et al. (1, 3) recently showed that traditional scAAV vectors based on serotypes 7 and 8 do indeed lead to improved gene transfer in the nonhuman primate liver, although the improvements were less than those seen in mice. The beta subunit of macaque-derived choriogonadotropic (bCG) hormone was used as a reporter gene to avoid transgene responses in primates (3). Vectors were produced based on AAV2 single-stranded genomes transcapsidated with the AAV7 capsid and on self-complementary genomes cross-packaged with both the AAV7 and AAV8 capsids, in which bCG was driven from a cytomegalovirus-enhanced chicken β-actin promoter and a bovine growth hormone gene poly(A) sequence. Tail vein injections of these vectors into immunocompetent C57BL/6 mice (1.5×10^{12} genome copies/kg of body weight) led to 2- to 3-log increases in transgene expression with the self-complementary vectors compared to the single-stranded (ss) vectors (Fig. 1A). Threefold lower doses of the same lots of vectors (on a per-kg basis) were injected into the portal veins of cynomolgus macaques (2- to 3-kg captive-bred females of Indonesian origin, with two animals per group; all animals had no detectable neutralizing antibodies against AAV serotypes 7 and 8 at a 1:20 serum dilution prior to the study) (3). Levels of transgene product achieved with the ssAAV2/7 vector were below the limit of detection by 6 weeks (<50 relative units [rU]/ml), while the self-complementary vectors stabilized at levels 20- to 35-fold higher with the AAV2/8 vectors (1,000 and 1,740 rU/ml for animals AT3F and AT3E, respectively) and 80- to 120-fold higher with the AAV2/7 vectors (4,000 and 6,000 rU/ml for animals AJ28 and AJ7R, respectively), as shown in Fig. 1B.

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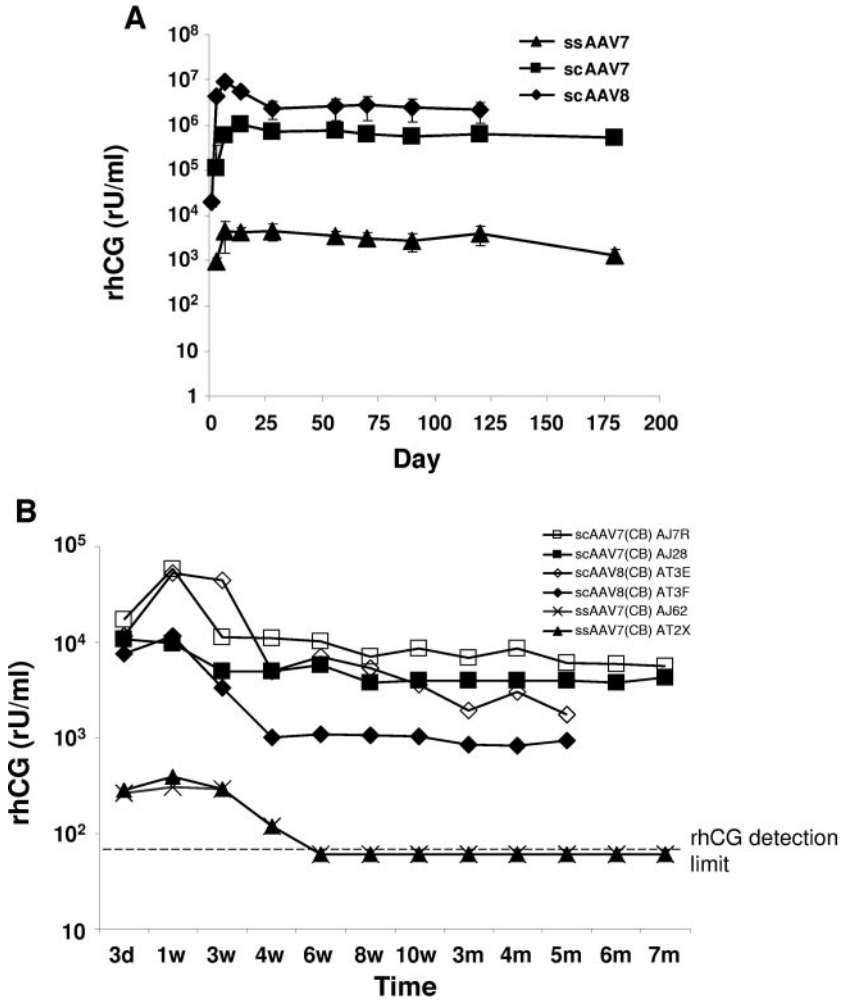

FIG. 1. Expression of bCG from AAV vectors in mouse and nonhuman primate livers. AAV7 vectors based on ss and sc genomes and an AAV8 vector based on an sc genome expressing bCG were injected intravenously into C57BL/6 mice (panel A, average ± 1 standard deviation; $n = 5$ per group) and cynomolgus macaques (panel B, $n = 2$ per group [each animal is presented separately]). The data presented are the serum levels of bCG measured by an enzyme-linked immunosorbent assay.

The combined advantages of AAV2/7 with the self-complementary genome resulted in fivefold higher levels of transgene expression in nonhuman primates over those we previously saw in another nonhuman primate liver study, where 20-fold more AAV2 vector with an ss genome was dosed (3). Even without considering the difference in promoter strength (cytomegalovirus-enhanced chicken β-actin promoter versus human thyroid hormone-binding globulin promoter), this translates to an estimated 2-log improvement in efficiency. This is consistent with the recent study of Nathwani et al. (9), who showed a high-level expression of human factor IX following AAV2/8-mediated gene transfer to the liver with an sc genome.

The present study also confirmed our previous finding, where, regardless of the vector genome structure (e.g., ss or sc), AAV2/8 outperformed an AAV2/7-based vector in the mouse liver (fourfold higher expression for sc vector comparison), but in the nonhuman primate liver, expression from the AAV2/7 vector stabilized at higher levels (an average of 5,000 versus 1,370 rU/ml in this study) (Fig. 1) (3). Our data again suggest that AAV2/7 should be considered a preferred vector for gene transfer in the primate liver.

The macaques were necropsied approximately 1 year after gene transfer (on day 355 for the AAV2/7 groups and day 315 for the AAV2/8 group), and tissues were harvested and analyzed for vector genomes. DNAs were harvested from the colon, heart, kidneys, four different lobes of the liver (left, right, middle, and caudate), lungs, muscle, and spleen and initially assayed for vector genomes by Taqman PCR using transgene-specific primers and probe, as described previously (3). Vector genome levels were found to be 2 to 3 log higher in the liver than in other tissues in all animals studied, based on numbers of vector genomes per 100 ng of tissue DNA (Fig. 2A). The abundance of vector was proportional to the level of steady-state transgene expression, with the highest level noted for scAAV2/7 vectors, at 13 to 16 vector genomes per diploid genome (Fig. 2B). DNA hybridization analyses were performed using restriction endonucleases that excise an internal fragment and a vector-specific probe (Fig. 2B). In each case, a single band of the predicted size was detected, whose intensity was compared against plasmid standards to quantify vector genomes (Fig. 2B). This estimate of vector abundance was consistent with the TaqMan measurements.

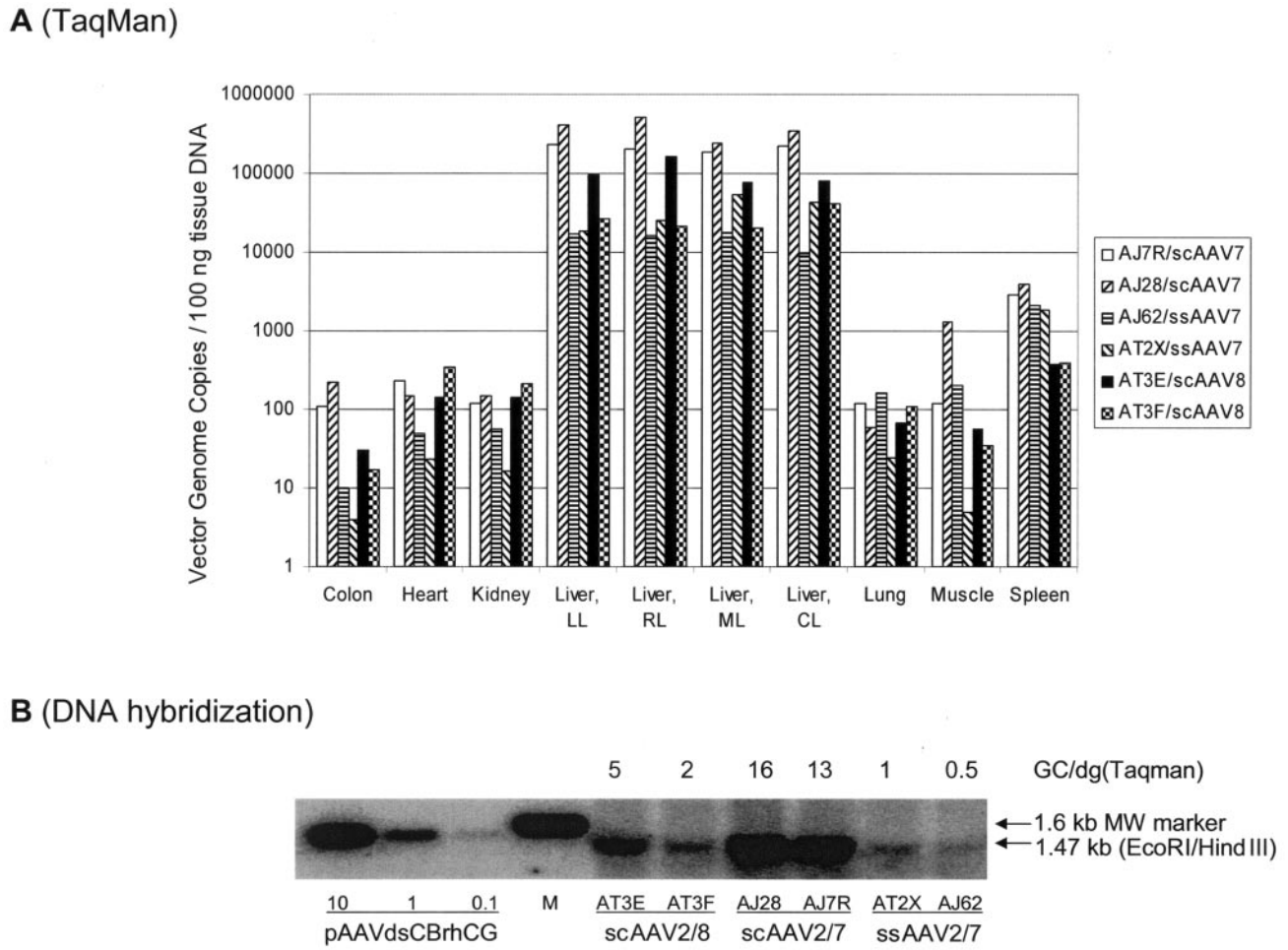

FIG. 2. Quantification of vector genomes in major organs from study animals. Total cellular DNAs were extracted from four lobes of the liver and from six other major organs that were harvested from the study animals at necropsy. The samples were subjected to vector genome quantification by real-time PCR (panel A) and DNA hybridization analysis (panel B). For the DNA hybridization experiment, the vector plasmid was used as a reference standard for vector genome copy numbers. A transgene probe was used for detection of a 1.47-kb internal fragment of the vector genome resulting from BamHI/HindIII digestion. GC, genome copies; dg, diploid genome.

Previous experiences with clinical translation of novel vector concepts have illustrated the importance of preclinical studies in large animal models, such as nonhuman primates. The improved transduction achieved in the nonhuman primate liver with the vectors described in this study could enhance the potential of their successful clinical application.

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