Neutralizing Anti-AAV Antibody Impact on Vector Transduction Following Intravitreal Administration of AAV in Non-Human Primates

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INTRODUCTION

Adeno-associated virus (AAV) is a non-pathogenic virus and a widely used gene therapy vector. Most people have been exposed to AAV early in life[1]. The host immune system reacts to AAV as a foreign entity and launches a humoral immune response towards the virus. As a result, the majority of people have pre-existing immunity (PEI) against AAV in the form of circulating capsid-specific neutralizing antibodies (NAbs) and/or memory cells, which generate a secondary antibody response in the event of a successive encounter with the virus. PEI against viral vectors is thought to be an impediment to gene therapy approaches, and even low NAb titers have been reported to prevent transduction. In ocular gene therapies, PEI does not impact subretinal vector delivery, whereas reports of transduction efficiency after intravitreal (IVT) injections seem to vary[2-5]. Correlation between pre-existing NAb titers and the extent of vector neutralization, however, has not been extensively characterized, especially in the ocular environment.

We determined AAV transduction efficiencies after IVT dosing in non-human primates (NHP) with varying levels of pre-dose systemic anti-AAV NAb titers. Further, intraocular titers were monitored throughout the study and compared with systemic titers.

METHODS

AAV vectors were manufactured by plasmid transfection in HEK 293 cells, purified by double iodixanol step gradient centrifugation, and formulated in balanced salt solution (BSS) containing 0.014% Tween 20. To determine anti-AAV NAb titers in samples, a standard *in vitro* assay was performed. Briefly, serially diluted serum or ocular fluid samples were mixed with AAV2-based viral vectors carrying a luciferase reporter transgene construct. The mixture was used to transduce cells, and neutralizing titers reported as the highest dilutions showing more than 50% inhibition of transduction after normalization to non-neutralized controls.

Adult male and female cynomolgus macaques (*Macaca fascicularis*) were screened for serum anti-AAV2-based virus NAbs prior to the study start and split into three groups of six animals. Groups 1 and 2 were dosed IVT with 1 x 10^{11} vg of AAV-hGFP vector into right eyes. Group 3 animals were first immunized by intramuscular (IM) injection of 2 x 10^{11} vg of AAV-luciferase vector, then dosed IVT 6 weeks later with 1 x 10^{11} vg of AAV-hGFP vector into right eyes. Ocular exams and fundus fluorescence imaging were performed regularly. Serum and vitreous humor samples from uninjected left eyes were collected at day of injection, Week 6 and Week 12 post-dose. Terminal (Week 12 post-dose) aqueous humor and vitreous humor samples were collected from both eyes. The study design is summarized in **Figure 1** and **Table 1**.

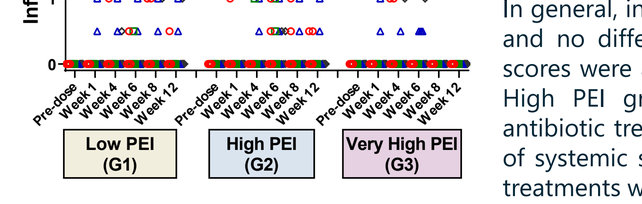

Table 1. NHP Study Design

Group	# of Animals	Pre-Dose Serum Titer	Dosing		Vg dose per Eye
			Right Eye	Left Eye	
Low PEI (G1)	6	<1:4	AAV-hGFP	N/A	1 x 10^{11}
High PEI (G2)	6	1:128-1:512	AAV-hGFP	N/A	1 x 10^{11}
Very High PEI (G3)	6	1:2,048-1:32,768	AAV-hGFP	N/A	1 x 10^{11}

Figure 1. NHP Study Design. Overview and timing of experimental procedures (top) and sample collection (bottom) during the study. IVT; intravitreal. IM; intramuscular. RE; right eye. LE; left eye. VH; vitreous humor. AH; aqueous humor.

RESULTS

All animals tolerated IVT AAV vector dosing well. No adverse events were reported during the study. Isolated incidents of mild ocular inflammation were detected and successfully treated with steroids (**Figure 2**). Anti-AAV NAb assays were performed for all collected samples as indicated in **Figure 3**.

Figure 2. Ocular Examinations. Ocular exams were performed using slit lamp and indirect ophthalmoscope prior to IVT dosing, at Weeks 1, 4, 6, 8 and 12, and as needed to monitor treatment response. Eyes were scored for aqueous flare (AF), aqueous cells (AC), vitreous cells (VC) and vitreous haze (VH). Inflammation score: 0.5=trace; 1=minimal; 2=mild; 3=moderate; 4=severe.

In general, inflammatory reactions in injected eyes were mild or less, and no differences between Groups were detected. Inflammatory scores were absent in uninjected eyes. Four animals in Low PEI and High PEI groups required early temporary topical steroid and antibiotic treatments; two of these NHP also received a single dose of systemic steroids. In all cases inflammation resolved quickly. No treatments were recorded for animals in Group 3.

Figure 3. NAb titers in serum and uninjected eyes. NAb titers were assayed in vitreous humor from uninjected eyes and from serum. Sample collection was done pre-study, pre-dose, at Week 6 and Week 12 (termination). Pre-dose vitreal NAb titers were below detection (<1:4) in all animals regardless of their serum titers, except for two animals in the Very High PEI (Group 3) with the highest serum titers (>1:16,384). While intraocular NAb titers did increase in the uninjected eye following IVT dosing in the contralateral eye, the titers were ~500 – 4000-fold lower compared to serum.

RESULTS continued

Pre-dose serum anti-AAV NAb titers, GFP expression and cumulative ocular inflammation scores in eyes dosed IVT with AAV are shown in **Table 2,** and **Figures 4 & 5**.
Parameters other than PEI alone are likely to determine transduction efficiency in IVT dosed primate eyes. Higher PEI does not increase inflammation.

Table 2. Pre-dose serum NAb titers, GFP expression and Inflammation in injected eyes

Low PEI			High PEI			Very High PEI		
Predose Titer	GFP	Inflam. Score	Predose Titer	GFP	Inflam. Score	Predose Titer	GFP	Inflam. Score
4	+++	1	256	+++	0	8192	+++	0
4	+++	0.5	256	+++	0	8192	---	0
4	+++	1	256	+++	0.5	2046	+++	0
4	+++	1	128	+++	0.5	2046	+++	0
4	+++	0.5	256	+++	0	16384	---	0
4	+++	0	512	---	1	32768	---	0
GFP signal: 1.6E6 ± 1.6E5			GFP signal: 1.1E6 ± 2.1E5			GFP signal: 1.4E6 ± 2.0E5		

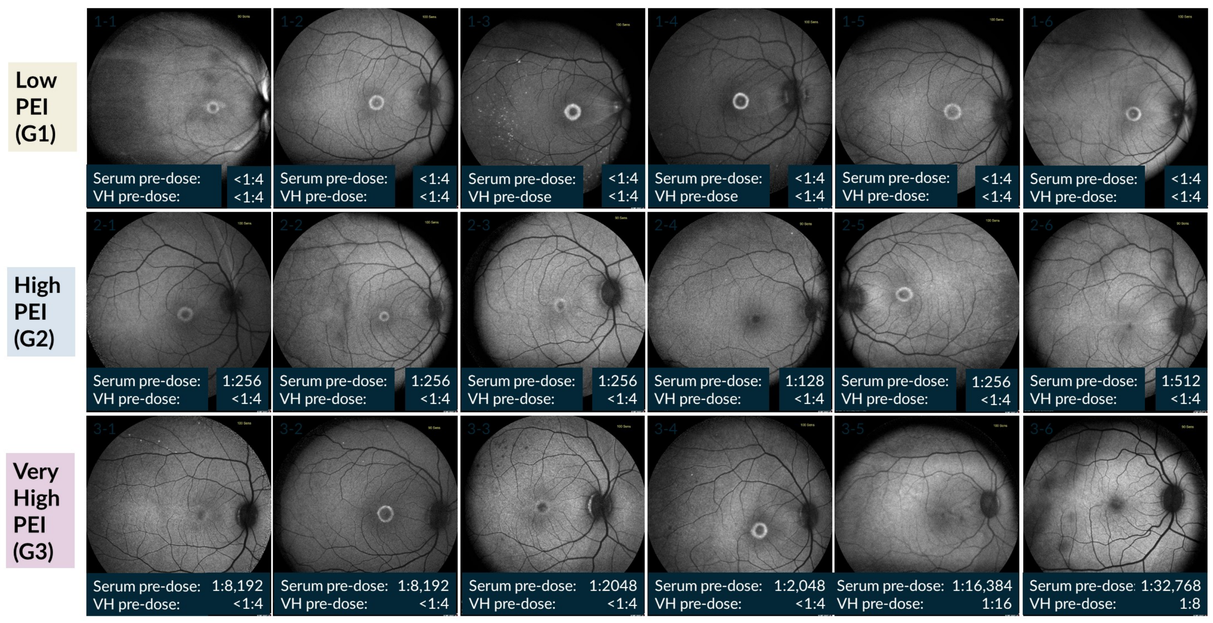

Figure 4. Fundus fluorescence images at Week 12 (termination). Images were collected during the study to assess transduction efficiency based on quantitative GFP expression (Figure 4B). hGFP expression was quantified using MetaMorph® image analysis software. Anti-AAV Nab titers of pre-dose serum and vitreous humor from uninjected eyes are depicted with each image. Eyes with robust fundus fluorescence were detected in all Groups in patterns typical of IVT delivery of rAAV viral vectors.

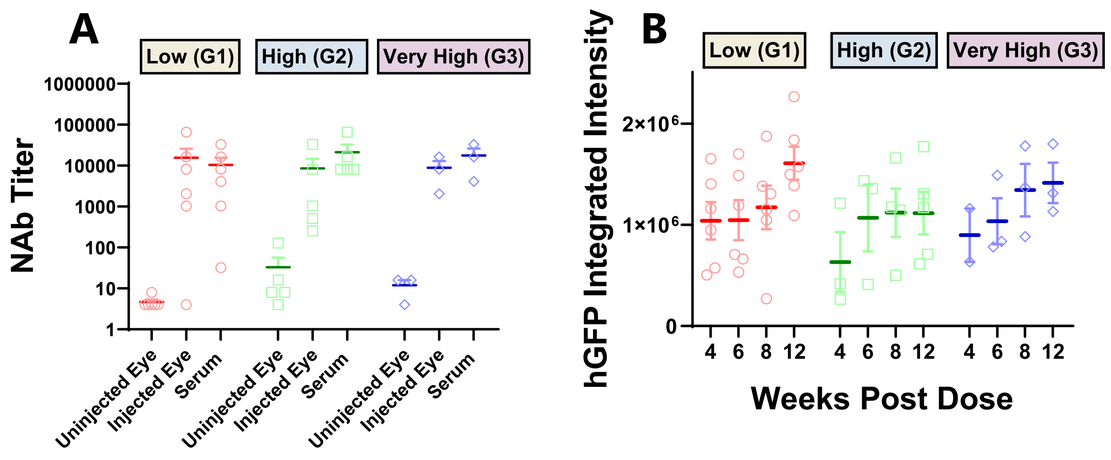

Figure 5. **A** Anti-AAV NAb titers in vitreous humor and serum at Week 12 (termination) of animals showing positive retinal hGFP expression. Anti-AAV Nab titers in injected and uninjected eyes do not show a correlation (Pearson correlation coefficient ρ ≈ 0.30).
B Quantitative hGFP expression post-IVT dose using MetaMorph® imaging software. No statistically significant differences were observed among hGFP expressing retinas in the three Groups.

CONCLUSIONS

- Anti-AAV NAb titers in pre-dose serum (PEI) did not result in increased inflammation and did not correlate to inflammation in the dosed eye (Figure 2)
- Very high serum anti-AAV NAb titers were not sufficient to block or impact efficiency of AAV transduction in the retina. This indicates that systemic anti-AAV NAb titers are not the sole predictor of transduction efficiency after IVT AAV dosing (Table 2 and Figure 4)
- IVT dosing of AAV increased anti-AAV NAb titer in serum and the injected eye (vitreous and aqueous) with good correlation between serum and ocular titers (Figure 5A). Ocular anti-AAV NAb titers obtained from aqueous humor were comparable to vitreous humor (data not shown), therefore aqueous humor sampling could be used to represent ocular anti-AAV NAb titers
- Independent of PEI, the anti-AAV NAb titer in eye and serum reaches similar levels, as does the retinal expression level of hGFP (Figure 5B)

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