AAV-Dependent Complement Lysis of Cultured Hepatocytes

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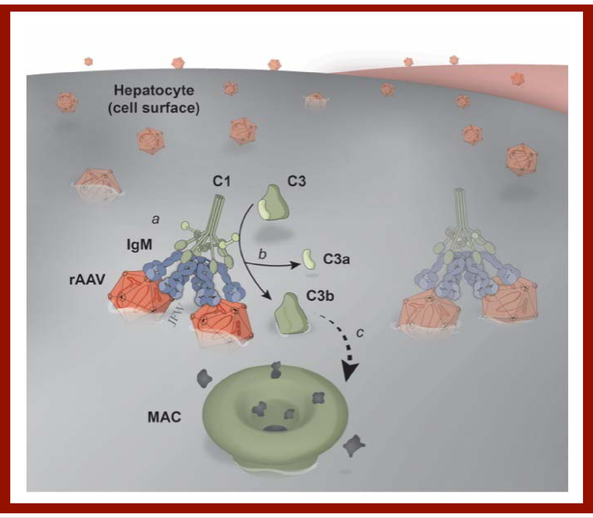
As vector dose has increased, complement activation has been reported more frequently.

Sponsor	Vector	High Dose (x10[12]):		C' Act'n	Hepato (ALT)	AEs
		(vg/kg)	(~cp/kg)	(acute)	/ Renal Tox	(*est.* grade)
Pfizer	AAVSPK-FIX Padua	0.5	1.5-2.5	NR	**+/-**	**1/2**
UCL, St Jude	AAV8-FIX	2	1 - 10	NR	**+/-**	**1/2**
Shire (335)	AAV8-FIX Padua	3	NR	NR	**+**	**2**
Dimension	AAVrh10-FIX	5	NR	NR	**+**	**2**
Uniqure	AAV5-FIX Padua	20	40	NR	**+/-**	**1/2**
Biomarin	AAV5-FVIII	60	60	NR	**+**	**2**
Avexis	AAV9-SMN1	110+	NR	NR	**++**	**2/3**
Solid	AAV9-mDys	200	200+	**++**	**++**	**3/4**
Pfizer	AAV9-mDys	300	NR	**++**	**++**	**3**
Audentes	AAV8-Mtm1	300	NR	**++**	**+++**	**4/5**

Modified from Wright (2020) *Mol Ther* 28:701
Paulk (2020) *Gen Eng Biotech News* July
Wilson and Flotte *Hum Gene Ther* 31:695
Ronzitti *et al (2020) Frontiers Immunol* 11:260

Increased hepatotoxicity, complement activation, and severe adverse events have been reported with high dose systemic AAV administration in clinical trials. The events that initiate complement activation are uncharacterized in this context.

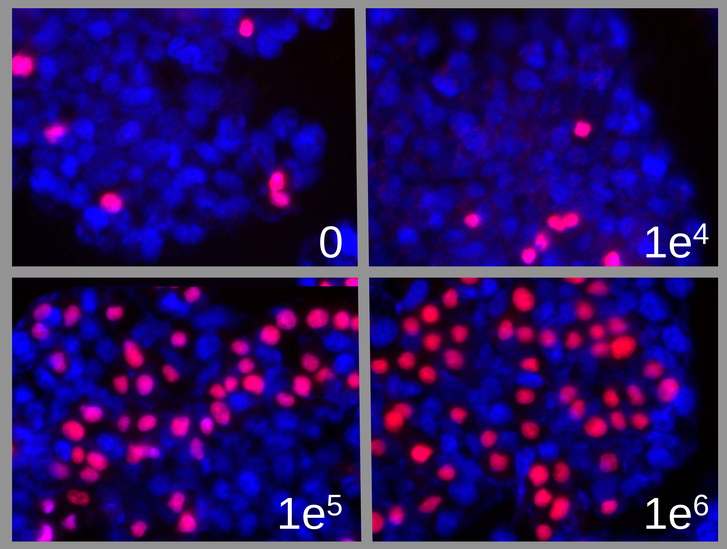
We hypothesize antibodies bound to densely concentrated AAV on hepatocyte surfaces can facilitate lytic immune complex formation.



Methods

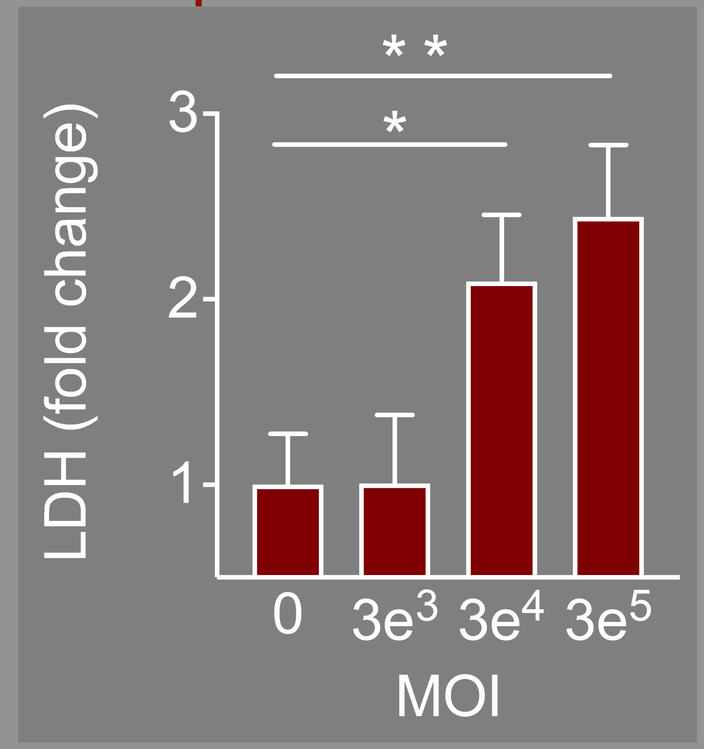
To test our hypothesis, we cooled cultured HepG2 cells to 4ºC to halt endocytosis and applied AAV-GFP at MOIs estimated to correspond to systemic administration dosing. After an hour, we exchanged AAV-media with 4ºC media containing 50mg/mL human polyclonal IgG (IVIG) predicted to contain AAV2 antibodies. One hour later we exchanged IVIG-media with human complement resuspended in gelatin veronal buffer, and incubated under standard culture conditions for 8 hours.

More dead HepG2 cells are observed at higher AAV MOI



Representative images of HepG2 cells under 40x magnification show propidium Iodide (magenta) intercalated in the DNA of dead cells, while live cells exclude the stain. Hoechst (cobalt) is intercalated in all nuclei. As MOI (lower right in each image) increases the percentage of dead cells also rises.

Hepatocyte lysis increases in a dose dependent manner



We assayed the percentage of HepG2 cells lysed by measuring lactate dehydrogenase (LDH) released in media/total LDH released after subsequent Triton X-100 lysis. Over two fold more lysis occurred when MOI was increased from $3e^3$ to $3e^4$ ($p=0.04$). A 2.5 fold increase was observed at MOI of $3e^5$ ($p=0.007$).

Conclusion

Our preliminary data suggest circulating antibodies could recognize AAV capsids densely concentrated on the surface of hepatocytes and initiate membrane attack complex formation via the classical complement activation pathway. Further investigation is needed to characterize the relationship between the kinetics of AAV endocytosis and complement activation