Acute Complement Activation and Subacute CTL Formation: A Sequential Two-Hit Model for Hepatotoxicity Following High-Dose AAV Administration

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ABSTRACT

Marked hepatotoxicity has been reported in some human subjects after high dose systemic administration of rAAV products characterized by early (acute, 1wk) complement activation and delayed (subacute, 4 - 8wks) cell-mediated immunotoxicity. A 'two-hit' model is proposed involving sequential humoral and cellular immune responses focused on hepatocytes that combined can result in severe liver injury. Hit one is activation of the classical pathway of complement (C') by antibody binding to cell surface-bound AAV particles leading to damage of hepatocytes by the C' membrane attack complex. Relevant AAV doses ranging from E14 to E15 cp/kg correspond to MOIs from approx. 3E4 to 3E5 cp per hepatocyte may saturate internalization pathways so that cell-surface AAV is still present when nascent capsid-specific IgM appears (est. 5d). Key parameters influencing the magnitude of hit one include; a) total capsid dose, and b) serotype-specific kinetics of AAV uptake by hepatocytes, which both would affect such immune complex formation. Hit two is the generation of capsid-specific cytotoxic T lymphocytes (CTLs) that then further damage AAV-transduced hepatocytes presenting capsid peptides on MHC Class 1 molecules. Key parameters influencing the magnitude of hit two include; a) total capsid dose determining the number of hepatocytes that become CTL targets, and b) number of TLR9-binding CpG motifs in the vector DNA genome that trigger and amplify CTL formation. This two-hit model for liver damage is consistent with available data reporting moderate acute and severe subacute stage immunotoxicities reported using high doses of AAV.

FIG 1. ADAPTIVE IMMUNE RESPONSES TO rAAV

	HUMORAL	CELLULAR
*	hepatotoxicity	*
AAV CAPSID	complement activation	loss of transgene expression
	blocks re-administration	hepatotoxicity
	capsid IgM, IgG	capsid CTLs
	[ ] virus protein (capsid)	*[ ] TL9 PAMP DNA (CpG)*
TRANSGENE PRODUCT	*non-self aa sequence*	
	ectopic expression	
	ADA / inhibitors	autoimmune sequelae?
	break (establish?) tolerance	

Four arms of the adaptive immune response to recombinant AAV vectors and the product of the vector genome and proposed consequences are shown. Propose key triggers for each arm are are shown in red font. The model for hepatotoxicity presented herein is based on the humoral and cellular adaptive responses to AAV capsid (*). []: concentration.

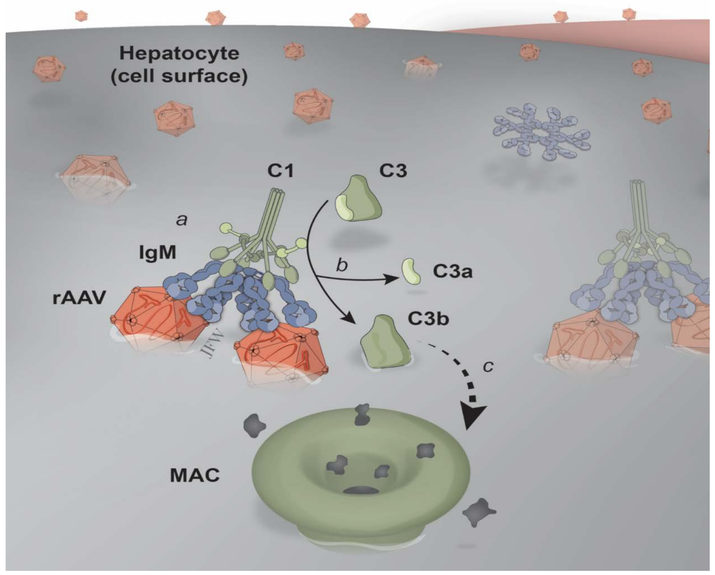
FIG 2. MODEL FOR HEPATOXOCITY AFTER HIGH DOSE rAAV ADMINISTRATION

	HIT 1	HIT 2
TIMEFRAME:	3-7 DAYS POST rAAV ADMIN	1-3 MONTHS POST rAAV ADMIN
HALLMARK:	COMPLEMENT ACTIVATION	CAPSID CTLs and ALT
PRIMARY TRIGGER:	CAPSID CONCENTRATION	TLR9 PAMP DNA CONCENTRATION
THRESHOLD:	*> 10^{14} CP/KG*	*rAAV / HUMAN Me$^-$CpG > 7**
SECONDARY FACTORS:	?	CAPSID DOSE CAPSID SEROTYPE

*Wright (2020) *Mol Ther* 28:1756, NRF_3

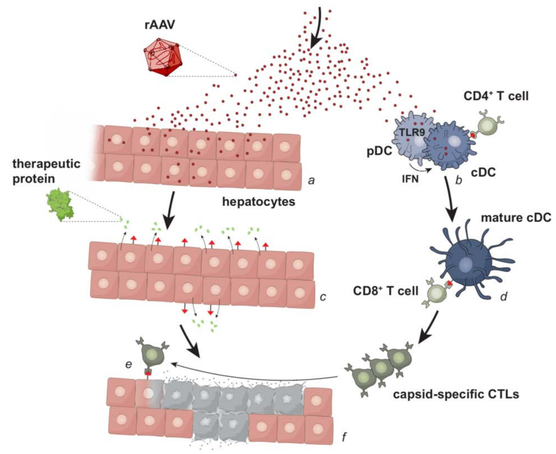
A schematic summary of the two-hit model is shown. The first hepatotoxic event (HIT 1) occurs in an acute timeframe (3-7 days) corresponding to formation of capsid IgM (naïve) or anamnestic IgG formation in response to rAAV administration. At very high rAAV doses (10^{14}-10^{15} CP/KG) a key assumption is that a fraction of rAAV particles are still resident at the hepatocyte surface in an overlapping timeframe and act as IgM/G binding ligands on the cell surface. The hepatocyte surface-bound immune complexes activate complement (see *Fig 3*) resulting in C' opsonization and/or a first round of cell damage. The second event (HIT 2) occurs in a delayed, sub-acute timeframe (1-3 months) corresponding to the previously reported and characterized formation of capsid CTLs (see *Fig 4*) and leading to a second round of cell damage.

FIG 3. HEPATOTOXICITY HIT 1: ACUTE COMPLEMENT ACTIVATION



High dose rAAV administered systemically (10^{14} -10^{15} CP/KG) is hypothesized to oversaturation hepatocyte receptor-mediated vector uptake, resulting in an extended residence duration for some particle that overlaps with formation of capsid antibodies. Cell surface immune complexes formed by capsid-specific IgM/G are proposed to bind complement component C1 (**a**), activate C3 by the classical pathway (***b***) leading to cell surface covalent deposition and opsonization by C3b, and potential formation of membrane attack complexes (MAC) (***c***).

FIG 4. HEPATOTOXICITY HIT 2: SUBACUTE CTL FORMATION



Systemically administered AAV vectors transduce hepatocytes (***a***), but are also taken up by pDCs, where vector genome Me$^-$CpG (TLR9 PAMP DNA) activates the MyD88 pathway, and licenses cDCs (***b***). Transduced hepatocytes secrete therapeutic protein, but also display capsid-derived peptides (red) via MHC Class 1 (***c***). Mature cDCs activate capsid-specific CD8+ T cells leading to CTLs (***d***) that damage the transduced hepatocytes (***e***).

CONCLUSIONS

- AAV gene therapy has demonstrated definitive therapeutic potential for many diseases and has been validated by the licensure of three products to date.
- However, immunotoxicities remain a challenge for some AAV gene therapies, especially those requiring high doses and likely contribute to severe adverse events
- The preliminary model presented requires additional data is required to support or refute the hypotheses proposed. Sharing of the following data by sponsors of ongoing clinical trials can help elucidate immune / hepatotoxicity pathways and advance the field and improve patient safety:
  - Total capsid dose (including empty capsids) in investigational products;
  - Quantification of TLR9-stimulatory CpG motif content in AAV expression cassettes using a sequence-blinded, standardized method (e.g. NRF_3);
  - Precise timing and magnitude of immunological adverse events (e.g. complement activation, capsid CTLs, ALT levels).

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