Poster #226

Sandhoff (*hexb*$^{-/-}$) mice mount an immune response towards the novel human variant HexM protein when treated with scAAV9-*HEXM*

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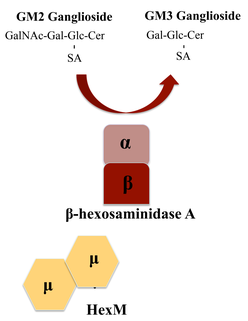
INTRODUCTION



Ganglioside Degradation & GM$_2$ Gangliosidosis Disorders

- The enzyme β-hexosaminidase A, HexA, degrades GM2 gangliosides
- HexA consists of α and β subunits
- GM2 gangliosidoses disorders result from an accumulation of GM2 gangliosides in the brain due to a deficient HexA
- Sandhoff disease is due to a defective β subunit of the HexA
- Symptoms include decreased motor function, attentiveness, and visual impairment
- Unfortunately, this disease often has fatal consequences

HexM Protein

- The hybrid protein HexM, a variant of HexA, consists of two μ subunits
- A single μ-subunit exhibits both α and β properties of HexA
- The gene encoding for the μ-subunit fits the cargo capacity of self-complementary adeno-associated virus 9 (scAAV9)

AAV9-HexM as a Therapeutic Approach

- scAAV9 has had major potential in crossing the blood brain barrier
- A therapeutic approach to treat Sandhoff disease is by testing the integration of the AAV9-HexM viral vector
- A previous study, using the AAV serotype 9 (AAV9) vector expressing HexM showed successful long-term correction of SD in the murine model

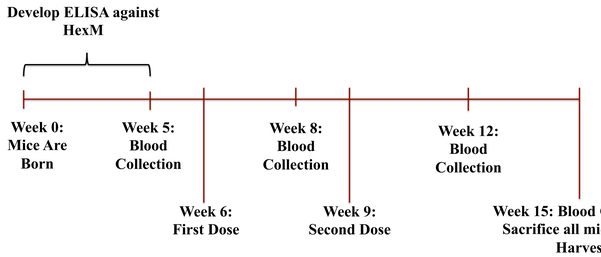
Immune Response to AAV9-HexM

- An important risk associated with viral vectors is the potential of the host to cause an immunological defense against the vector or transgene products if seen as foreign
- This could contribute to the pathogenesis of the disease
- It is imperative to perform immunological studies to test the immune response when being administered the treatment

HYPOTHESIS

The administration of AAV9-HexM viral vector for the treatment of Sandhoff Disease will trigger an immune response against the HexM transgene product in the adult *HEXB* deficient mouse model.

METHODS



B-cell Response: **Indirect Enzyme Linked Immunosorbent Assay (ELISA)**

- Technique used to detect the presence of antibodies in the blood to the HexM protein

T-cell Response: **Enzyme-Linked Immunospot Assay (ELISPOT)**

- Measure production of cytokine IFN-γ in splenocytes of study mice

Group	Cohort #	Genotype	Number of Mice	First Dose	Second Dose	Endpoint
Positive Control	1	Knockout	6	HexM + CFA	HexM + IFA	15 Weeks
	2	Knockout	6	HexM	HexM	15 Weeks
	3	Heterozygous	4	HexM + CFA	HexM + IFA	15 Weeks
Negative Control	4	Knockout	3	Vehicle + CFA	Vehicle + IFA	15 Weeks
	5	Knockout	3	Vehicle	Vehicle	15 Weeks
	6	Heterozygous	3	Vehicle + CFA	Vehicle + IFA	15 Weeks
Experimental	7	Knockout	3	AAV9-HexM	X	9 Week
	8	Knockout	3	AAV9-HexM	X	15 Weeks
	9	Knockout	3	AAV9-HexM	AAV9-HexM	15 Weeks
	10	Knockout	3	AAV9-HexM	HexM	15 Weeks

RESULTS

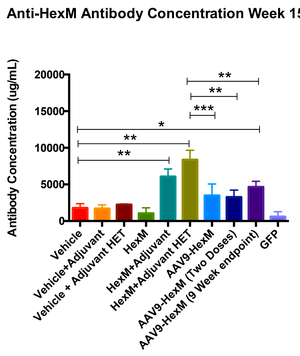


Figure 1. Anti-HexM antibody concentration (ug/mL) in serum of mice euthanized at 15 weeks. High antibody production against HexM is observed in AAV9-HexM treated mice.

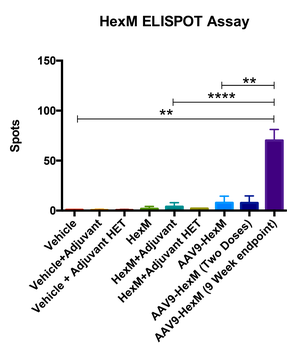


Figure 2. Spot formation from ELISPOT assay measuring IFN-γ production in splenocytes of experimental mice. A significant increase in IFN-γ cytokine production is observed in AAV9-HexM treated mice 3 weeks after vector treatment.

In order to improve the treatment benefits of gene therapy, it is imperative to investigate the suppression of this heightened immune response. Current immunological studies are investigating the effects of immunosuppressants, rapamycin and prednisone, on the heightened immune response following AAV9-HexM treatment in Sandhoff mice.

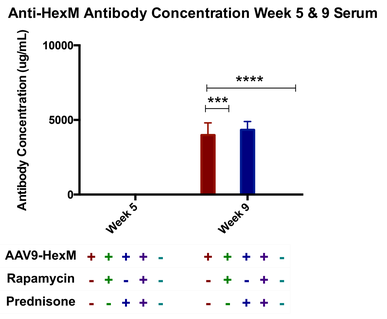


Figure 3. Anti-HexM antibody concentration (ug/mL) in 5 week serum and 9 week serum of experimental mice. A significant increase in antibody production is observed in AAV9-HexM mice treated with no immunosuppressants and AAV9-HexM mice treated with prednisone alone. AAV9-HexM mice treated with rapamycin alone and rapamycin and prednisone in combination showed no increase in anti-HexM antibody response.

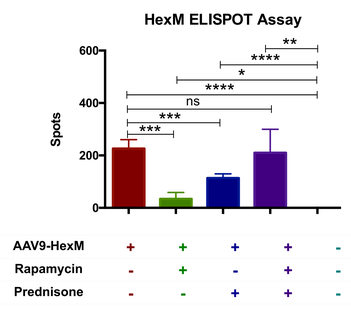


Figure 4. IFN-γ production measured in splenocytes isolated at 9 weeks of age (3 weeks after vector treatment. A significant increase in cytokine production is observed in AAV9-HexM mice treated with no immunosuppressants, and AAV9-HexM mice treated with both rapamycin and prednisone in combination. Low levels of IFN-γ production is detected in mice treated with prednisone alone and the lowest levels of IFN-γ is observed in mice treated with rapamycin alone.

DISCUSSION

- Evident increase in anti-HexM after administration of AAV9-HexM gene therapy treatment in both knockout and heterozygous mice
- High IFN-γ production observed in splenocytes of mice 3 weeks after the vector injections, however not 9 weeks after treatment
- Observations support hypothesis that AAV9-HexM treatment of Sandhoff disease will trigger an immune response against the transgene product HexM

Future Directions:

- Current immunological studies are investigating the effects of immunosuppressants, rapamycin and prednisone, on the heightened immune response following AAV9-HexM treatment in Sandhoff mice.

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