Induction of Immune Tolerance towards the Human HexM Enzyme following Intravenous Gene Transfer in β-hexosaminidase Knock-out Mice

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PROBLEM STATEMENT

The adaptive immune system identifies foreign proteins and mounts a response to rid the body of these "non-self" proteins and the cells that harbor them. While essential for combating pathogens, an immune response can also counteract therapies that introduce proteins intended to treat disease. Immune responses have been broadly reported following delivery of Enzyme Replacement Therapies in individuals lacking the enzyme (i.e., cross-reactive immunologic material (CRIM) negative). Similarly, immune responses could impact effectiveness of gene therapies expressing "non-self" proteins.

Disease and Study Background:

A deficiency of β-hexosaminidase A (HexA), Figure 1, causes a toxic accumulation of GM2 ganglioside, severe neurodegeneration, and death by 5-years in infants completely lacking this enzyme.

The Sandhoff (SD) knock-out mouse model of this disease shows a severe phenotype with death occurring at ~16 weeks of age. Intravenous (IV) gene transfer of a variant human HexA enzyme, called HexM, using adeno-associated viral (AAV) vectors has been shown to be beneficial in treating these mice. Yet, these mouse studies have also shown an immune response to the expressed human variant enzyme and the AAV capsid, which we hypothesized could be impacting therapy effectiveness. Here we investigate methods for inducing immune tolerance towards the human enzyme expressed from an IV injected scAAV9/*HEXM* vector in mice.

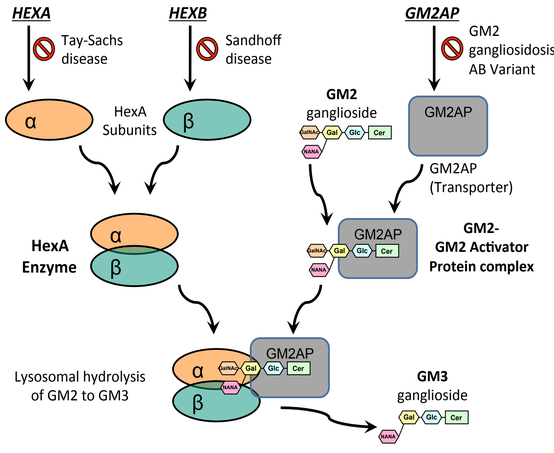


Figure 1: GM2 gangliosidosis. GM2 ganglioside hydrolysis requires active HexA enzyme in conjunction with the GM2 Activator Protein. The most prevalent forms of the GM2 gangliosidosis diseases are caused by mutations of *HEXA* or *HEXB genes*, which encode the α-and β-subunits of the HexA heterodimer, and are commonly called Tay-Sachs and Sandhoff disease, respectively.

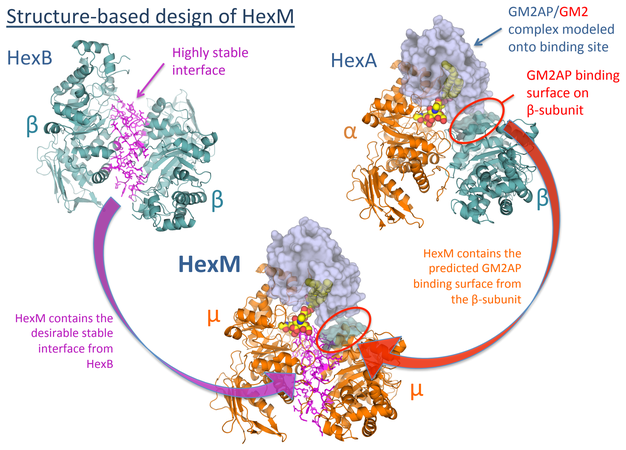


Figure 2: Model of engineered HexM homodimer (patent pending). Shown is the active HexM quaternary complex. The μ-subunit of HexM is derived from the α-subunit (orange) of human HexA, which was modified to include the stable homodimer interface (magenta) formed between the β-subunits (teal) of human HexB and a region from the β-subunit predicted to interact with GM2AP (grey) and GM2 (spheres). The 22 amino acid changes are primarily internal to the HexM conformational structure.

Gene Vector Design

The gene for the HexM μ-subunit (*HEXM*) is packaged in an AAV serotype 9 with a self-complimentary construct, which has been shown to cross the blood brain barrier and enter the central nervous system, which is the primary gene transfer target.

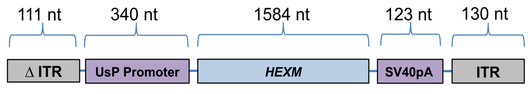


Figure 3: Design of *HEXM* self-complementary AAV9 vectors.

IMMUNE SUPPRESSION DRUG BACKGROUND:

Recent research has demonstrated that the combined effect of rapamycin plus prednisone deplete pre-existing levels of antibodies in mice [1]. Here we investigate whether the combination of these two immune suppression drugs can block an immune response, increase levels of regulatory T-cells, and induce immune tolerance towards a vector expressed human variant protein, HexM.

Rapamycin:

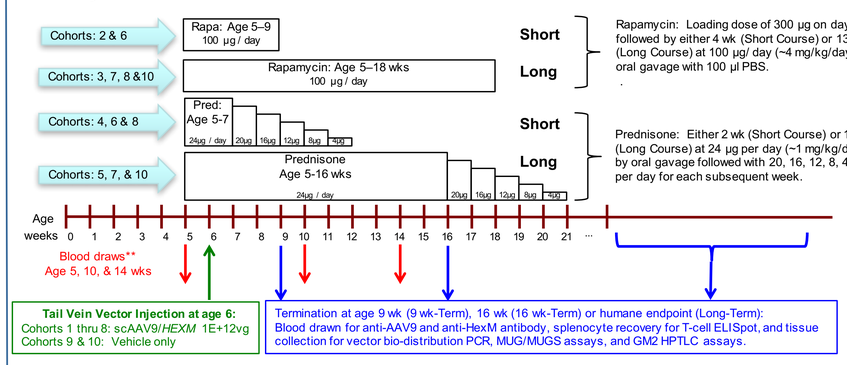
- Clinically used for immune suppression in organ transplant procedures.
- Inhibits lymphocyte activation and proliferation that occurs in response to antigenic and cytokine stimulation.
- Conjugates to form an immunosuppressive complex that inhibits the activation of the mammalian "Target Of Rapamycin", a key regulatory kinase which suppresses cytokine-driven T-cell proliferation and progression of the cell cycle.
- Shown to have a positive effect on the level of regulatory T-cells, which play a key role in inducing and maintaining immunologic tolerance [2, 3].

Prednisone:

- Has beneficial anti-inflammatory effects in targeting the functions of monocytes and macrophages and reducing the number of CD4 T-cells.
- Inhibits the synthesis of certain cytokines involved in the control of lymphocyte differentiation and participate in the regulation of T cell development by controlling apoptotic death of immature thymocytes.
- High doses have been shown to result in a rapid, dramatic, and transient increase in circulating regulatory T cells [4], but others have disputed this finding [5].

STUDY DESIGN

Study Timeline:



* Prednisone given once per day at beginning of the light cycle (i.e., approximately at nadir of cortisol cycle and peak of blood lymphocyte cycle).

** Blood drawn in sufficient amount from each mouse to conduct HexM specific T-cell IFNγ ELISpot, anti-HexM antibody ELISA, and HexM MUG / MUGS, liver function assays, and Anti-AAV9 antibody.

Cohorts: All cohorts use Sandhoff Mice:

Cohort #	Vector Injection	Immunosuppressant Regimen		Term at Age 9 wk (n)	Term at Age 16 wk (n)	Long-Term Follow-up (n)	Cohort Rationale
		Rapamycin	Prednisone**				
1	AAV9/*HEXM*	--	--	4	6	6	Immune Response Positive Control
2	AAV9/*HEXM*	Short	--	0	6	6	
3	AAV9/*HEXM*	Long	--	4	6	6	
4	AAV9/*HEXM*	--	Short	0	6	6	
5	AAV9/*HEXM*	--	Long	4	6	6	
6	AAV9/*HEXM*	Short	Short	0	6	6	
7	AAV9/*HEXM*	Long	Long	4	6	6	
8	AAV9/*HEXM*	Long	Short	0	6	6	
9	Vehicle Only	--	--	4	6	6	Immune Response Negative Control
10	Vehicle Only	Long	Long	0	6	6	Only Immune Suppression
				20	60	60	Total

Study Limitations:

- Only a small number of assays have been completed in some cohorts.
- Assays for anti-AAV9 antibodies and liver function are still in progress.
- Assays were conducted at timepoints that may not have detected peak/valley levels.
- Only one dose level and starting time of rapamycin and prednisone was studied.
- Additional T-cell categorization is still in progress.

RESULTS – From Terminations at 16 Weeks of Age

Figure 4-A

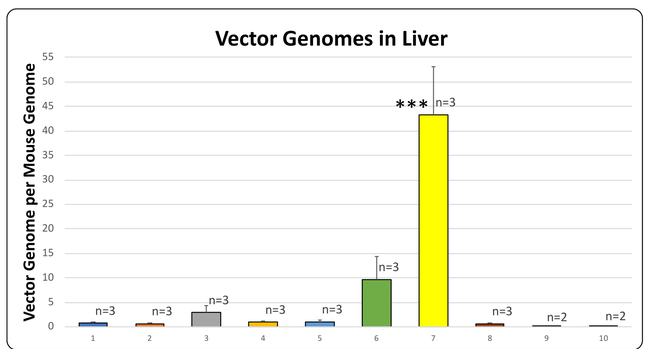


Figure 4-B

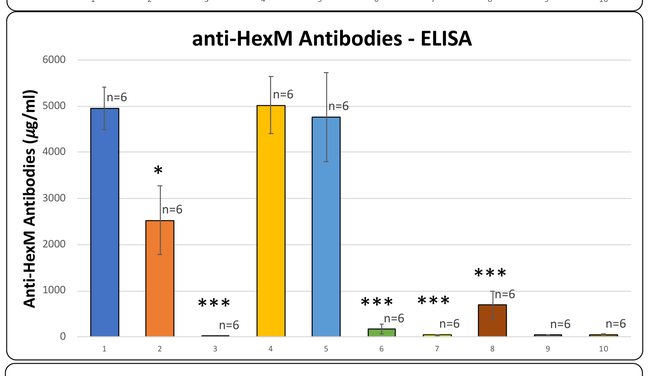
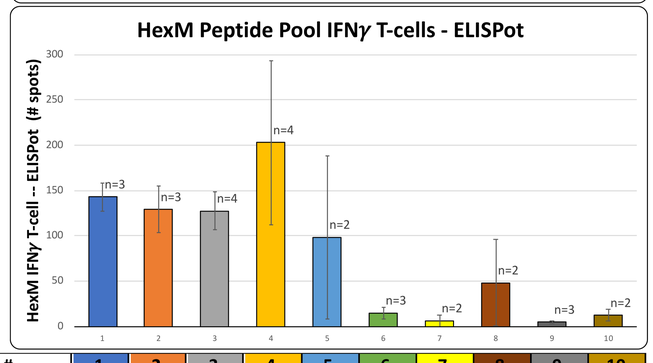


Figure 4-C



Cohort #	1	2	3	4	5	6	7	8	9	10
scAAV9/*HEXM*	+	+	+	+	+	+	+	+	--	--
Rapamycin	--	S	L	--	--	S	L	L	--	L
Prednisone	--	--	--	S	L	S	L	S	--	L

Figure 4. Assessment of mice terminated 10-weeks after vector injection. (A) Mice administered with the long regimen of both rapamycin and prednisone (Cohort #7) showed greater vector genomes per mouse genome than treated mice without immune suppression (Cohort #1). (B) Sera anti-HexM antibodies levels were lower in all cohorts with a regimen of rapamycin compared to Cohort #1. (C) One-way ANOVA for T-cell levels across cohorts #1 - #8: p<0.097. (Error bars = SEM. Significance in treated cohorts with respect to Cohort #1: * p ≤ 0.05 ; ** p ≤ 0.01; *** p ≤ 0.001 using Tukey-Kramer Post hoc.)

RESULTS – At the Humane Endpoint

Figure 5-A

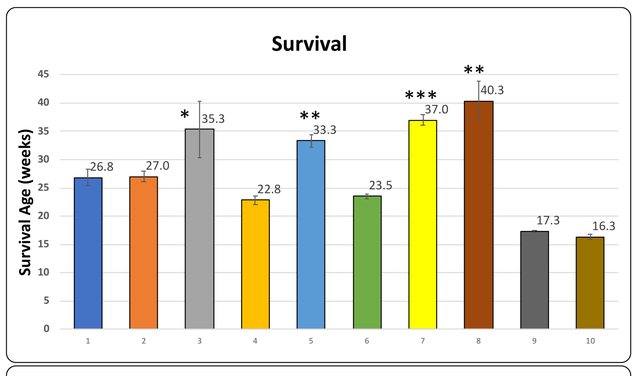


Figure 5-B

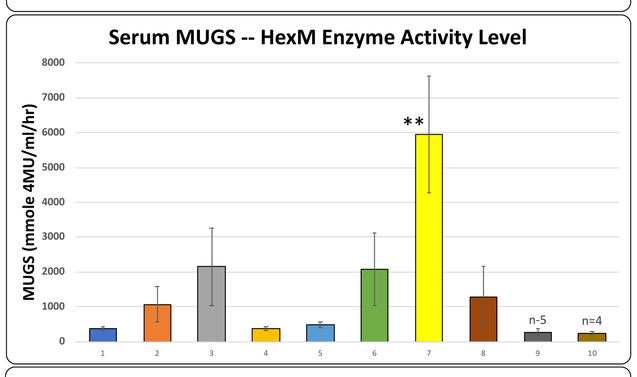


Figure 5-C

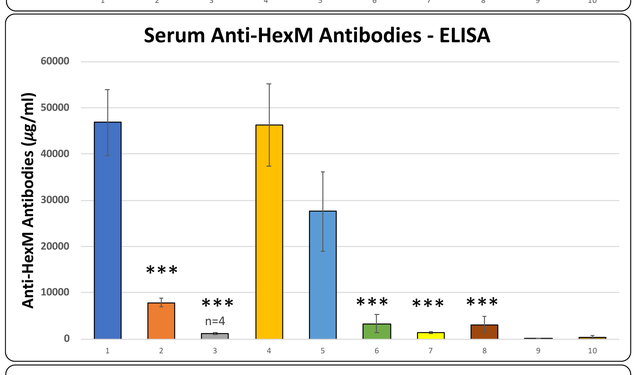
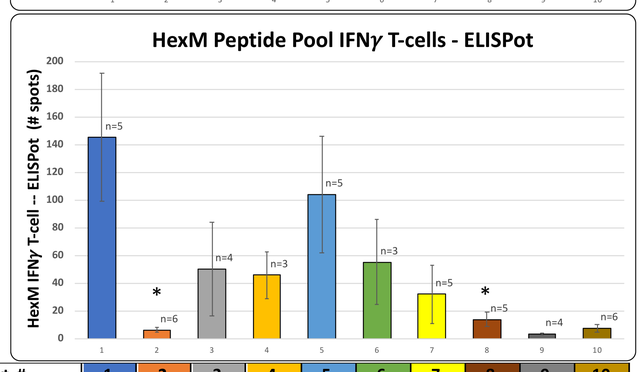


Figure 5-D



Cohort #	1	2	3	4	5	6	7	8	9	10
scAAV9/*HEXM*	+	+	+	+	+	+	+	+	--	--
Rapamycin	--	S	L	--	--	S	L	L	--	L
Prednisone	--	--	--	S	L	S	L	S	--	L

Figure 5: Assessments at humane endpoint. Compared to treated mice without immune suppression (Cohort #1), the additional administration of a long-regimen of both rapamycin and prednisone (Cohort #7) resulted in a significant survival benefit (p<0.001), and at the humane endpoint, showed a reduction in HexM-specific antibodies (p<0.001) and IFNγ T-cells with elevated circulating HexM enzyme activity levels (p<0.01). (The n for each cohort is 6 if not marked otherwise. Error bars = SEM. Survival significance in Figure 5-A compares treated cohorts with Cohort #1 and uses Log-Rank test of Kaplan Meier survival. In Figures 5-B, 5-C, and 5-D, the significance of treated cohorts compared to Cohort #1: * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001 using Tukey-Kramer Post hoc.)

CONCLUSIONS

- Vector genomes per mouse genome in liver tissue taken from mice terminated at 16 weeks of age (10-wks after gene transfer) were greater in mice administered with an ongoing regimen of rapamycin and prednisone (Cohort #7). This suggests the other treated cohorts may have experienced a cytotoxic response to cells expressing HexM protein.
- Diminished levels of anti-HexM antibodies concurrent with elevated HexM activity in serum at the humane endpoint, weeks after immune suppression regimen cessation, implies that **a long-regimen of prednisone and rapamycin induces immune tolerance towards the vector expressed human HexM "non-self" protein in Sandhoff mice.**
- In general, *in vivo* gene transfer studies and biodistribution assessments expressing "non-self" proteins should consider an immune suppression regimen to avoid cell loss and diminished therapy effectiveness.

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