

# A proinsulin encoding DNA plasmid vaccine decreases cellular and humoral autoimmune response to insulin and reverses established hyperglycemia in NOD mice

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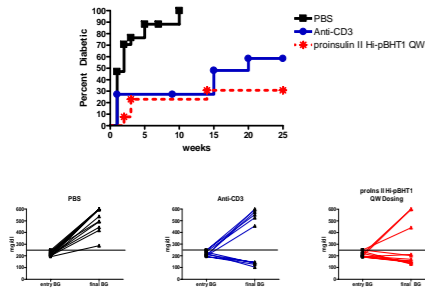
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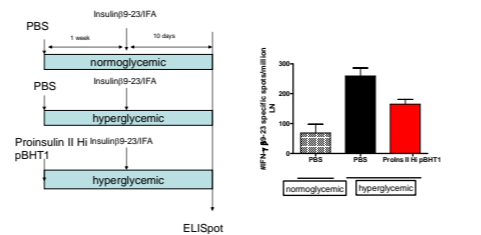
## Abstract

Proinsulin DNA was tested in hyperglycemic mice for ability to decrease diabetes onset. Animals were treated when blood glucose (BG) levels ranged between 190-250mg/dl. All 10 control mice progressed to persistent BG >250mg/dl by week 10, while 6/13 proinsulin DNA treated mice exhibited BG levels <170mg/dl at week 25 (p<0.0001). Hyperglycemic NOD mice treated with a proinsulin encoding DNA plasmid vaccine showed a significant decrease in B9-23 induced IFN-gamma ELISpot counts following a single administration of proinsulin DNA (p=0.02). When proinsulin DNA was administered to normoglycemic 8 week old mIAA positive NOD mice, a significant change in the rate of loss of insulin reactivity was observed in the DNA treated (p<0.0001) compared to the vehicle group (p=0.2) and diabetes onset was significantly delayed (p=0.05). In murine safety studies, repeated administration of proinsulin DNA both with and without parenteral insulin coadministration showed no evidence of sensitization to insulin therapy. In a non-human primate toxicology study, preliminary data show no signs of changes in mean fasting blood glucose levels after 4 weeks of dosing and no signs of allergic sensitization to insulin as measured by a skin test. This treatment was also shown to be safe in an animal model of latent gamma-herpes virus infection where repeated proinsulin DNA injections failed to induce viral reactivation (0/5 animals) while anti-CD3 treatment which induced viral reactivation in 4/5 animals. Therefore, proinsulin DNA efficiently ameliorates T1D onset in multiple model systems, effectively decreasing both the humoral and cellular response to insulin while inducing no signs of sensitization to parenteral insulin administration. A Phase I dose escalation placebo-controlled study in T1D patients is planned to begin in the US in late 2006.

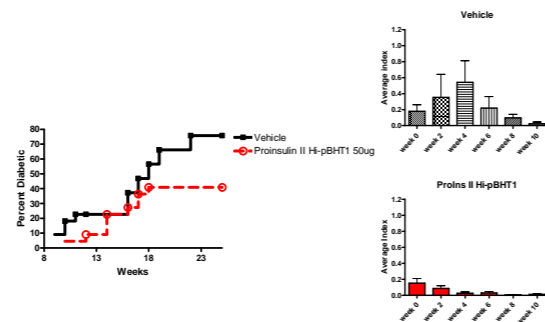
## Results



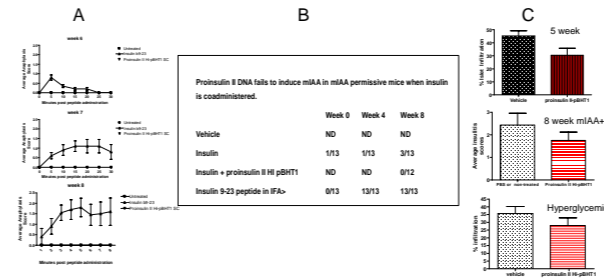
**Figure 1. Proinsulin II plasmid protects hyperglycemic NOD animals from diabetes onset and returns mice to normoglycemia.** Hyperglycemic NOD mice (blood glucose between 190-250mg/dl) were treated with either vehicle or 50ug proinsulin II plasmid by weekly IM injection. Anti-CD3 was administered by IV injection at 5ug for 5 consecutive days. Animals were monitored weekly for diabetes onset (blood glucose >250mg/dl for 2 consecutive weeks). DNA dosing was stopped after 8 weeks on study. Animals that were protected from diabetes onset for the length of the study returned to normoglycemic ranges.



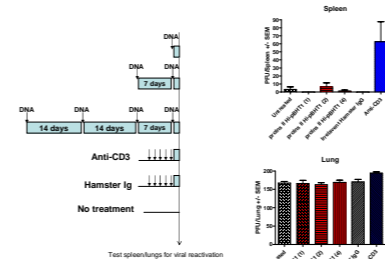
**Figure 2. Proinsulin II plasmid significantly decreases T cell immune response to the immunodominant region of insulin (β9-23).** Age matched NOD mice were identified as normoglycemic (BG <160mg/dl) or hyperglycemic (BG between 190-250mg/dl). Animals were treated with either a single administration of PBS or proinsulin II plasmid (50ug) by IM injection. One week later, animals were immunized with the β9-23 peptide in IFA. Draining lymph nodes were harvested 10 days later and LN cells were restimulated with β9-23 peptide in vitro and tested for IFN-gamma spot formation.



**Figure 3. mIAA levels are significantly decreased with proinsulin II plasmid treatment.** mIAA positive NOD mice were randomized between treatment groups. Groups were either treated with PBS or weekly IM injections of proinsulin II plasmid (50ug). Proinsulin II-DNA treated animals demonstrated a significant drop in diabetes onset and significantly depressed the average mIAA index of animals in the treated group.

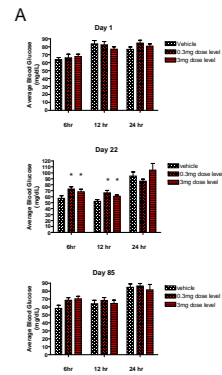


**Figure 4. Proinsulin II plasmid is safe in NOD mouse model.** (A) Proinsulin II DNA failed to induce any signs of anaphylaxis when administered with the identical dosing regimen of the insulin β9-23 peptide in which a fatal anaphylaxis is induced within 6 weeks of treatment. (B) Proinsulin II DNA failed to induce antibodies to insulin when coadministered to B6.NOD congenic mice which are permissive for mIAA development. (C) Proinsulin II DNA decreases insulinitis when administered to young normoglycemic NOD mice, eight week old mIAA positive NOD mice or hyperglycemic NOD mice.



**Figure 5. Anti-CD3, but not proinsulin II plasmid, induces viral reactivation.** NOD mice were infected with 400 PFU by IN with **γHV68** (γ herpes virus). After establishment of latency, animals were treated with either (1) irrelevant hamster IgG (2) hamster anti-mouse CD3 (3) one, two or four injections of proinsulin II plasmid (4) or left untreated. One day after the final treatment, animals were harvested and spleens and lungs plated for determination of plaque forming units indicative of viral reactivation.

**Figure 6. BHT3021 (human proinsulin plasmid) dose not induce hypoglycemia or DTH response to insulin.** (A) A 13-week repeat dose toxicity study was performed in a non-mouse model comparing vehicle, 0.3mg and 3mg BHT3021. In order to evaluate the ability of BHT3021 to induce hypoglycemia, blood glucose levels were monitored on day 1, 22 and 86 corresponding to 1, 4 and 13 total DNA injections. Animals were fasted overnight before administration of DNA followed by measurements of blood glucose levels at 6 hour, 12 hour and 24 hr post DNA administration. There were no significant changes in blood glucose levels in female animals (not shown). On day 22, males at the 0.3mg dose level showed a significant rise in BG which was not seen on day 86 and was not detected at the higher dose level. Therefore this change was not considered to be test article related and overall, there was no evidence for induction of hypoglycemia by BHT3021. (B) Furthermore, on day 89, animals were tested for delayed type hypersensitivity (DTH) reaction to intradermal delivery of lantus insulin (2 units). At 5, 15 and 30 minutes post insulin administration, clinical signs of DTH were measured including edema and erythema. Results in the table above were taken at the 30 minute time point and are representative of results at the 5 and 15 minute timepoints as well.



**B** T cell mediated DTH test after 13 weekly injections of BHT3021 (human proinsulin plasmid DNA)

Clinical Sign		Vehicle		0.3mg dose level		3mg dose level	
		Males	Females	Males	Females	Males	Females
Edema	None-normal	3/7	3/3	3/3	3/3	3/3	3/3
	Mild	0	0	0	0	0	0
	Severe	0	0	0	0	0	0
Erythema	None-normal	3/3	3/3	3/3	3/3	3/3	3/3
	Mild	0	0	0	0	0	0
	Severe	0	0	0	0	0	0

Note: None indicates no clinical signs. 3/3 time points post CD delivery of Lantus insulin: 5 minutes, 15 minutes and 30 minutes.

## Discussion

- Proinsulin II plasmid DNA reverses established hyperglycemia and decrease cellular and humoral response to insulin
- Proinsulin II plasmid is safe
  - No induction of anaphylaxis or DTH or hypoglycemia
  - No induction of insulin autoantibodies
  - No exacerbation of insulinitis
  - No reactivation of latent viral infection
- Phase I safety study in T1D individuals set to begin in early Q3 2006.