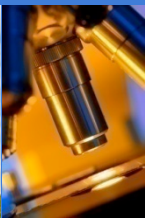


Disease-dependent and tissue-specific changes in gene expression in T1D

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Questions about T1D to be addressed by nPOD studies

We know lots of things about the pathophysiology of spontaneous autoimmune diseases including T1D or NOD disease; however several difficult questions remain including these three....

(1) What are the relevant genetic components of disease?

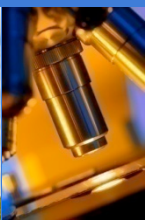
Are there “bad” genes or simply good genes behaving badly?

(2) Where does T1D start?

In the tissue targeted by the disease or elsewhere?

(3) When does T1D start and how can we identify the initiating event(s)?

Lets start with the first question:

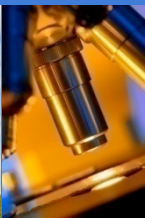


The initial nPOD question we addressed was...

Is it possible to identify human genes of relevance to T1D (as orthologs of NOD genes) that define actionable targets?
(*actionable: target for treatment to prevent hyperglycemia in T1D patients*).

GWAS has been a popular but, to me, an unproductive strategy, not one actionable T1D associated gene has been identified.

GWAS studies in autoimmune diseases support Einstein's definition of insanity; doing the same thing over and over again while expecting different results.



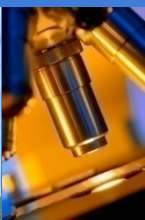
Disease-dependent and tissue-specific changes in gene expression in T1D

We used nPOD samples to identify genes in the PLN of T1D patients that were orthologs of genes whose expression changed in a disease-dependent and tissue-specific manner in NOD mice during destructive insulinitis before the onset of hyperglycemia.

These genes are probably not involved with disease initiation, but are involved in the progression of disease.

Tissue- and disease-specific gene expression patterns could define an actionable gene or set of genes to allow specific therapy to block the progression of insulinitis before beta cell destruction/hyperglycemia.

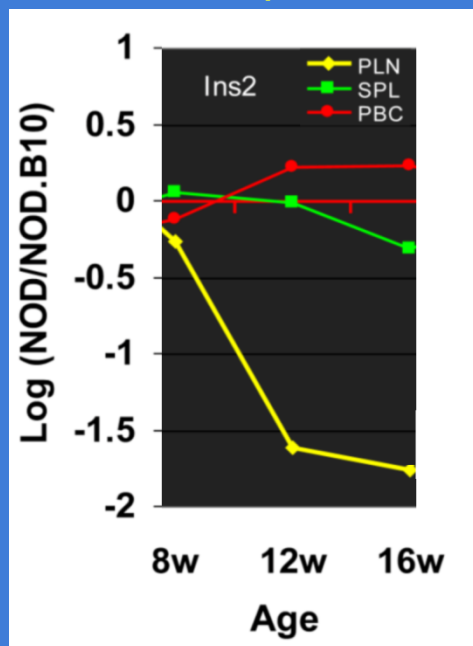
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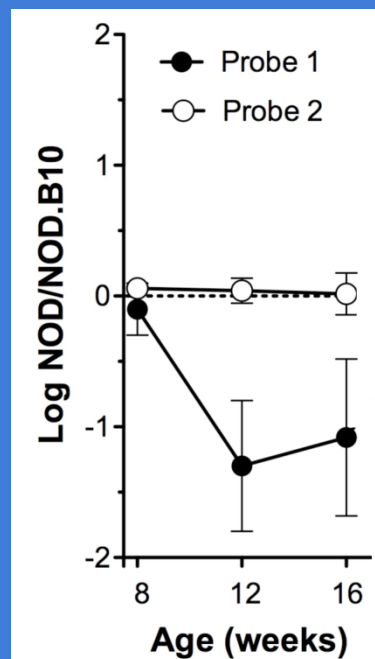
Tissue specific and age dependent changes in gene expression in NOD mice

Clin. Immunol; 2008 129(2): 195-201

Ins2 expression



Deaf1 expression (PLN)



Gene expression was measured using microarray technology from >5 mice at multiple time points comparing gene expression between NOD and NOD.B10 (arbitrarily set at 0)



What does Deaf1 do?

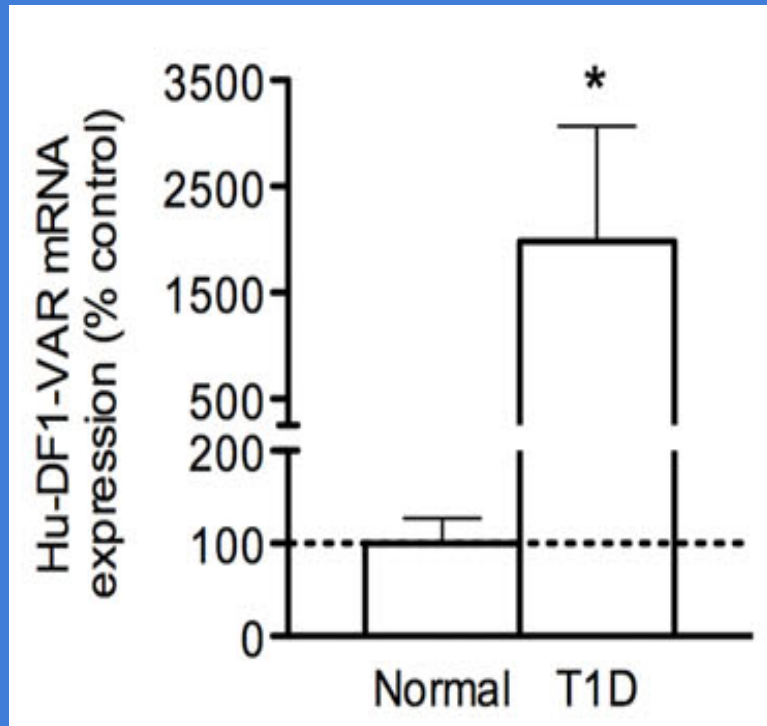
As demonstrated by our data published in 2009, the transcription factor Deaf1 serves a role in the periphery much like that of AIRE in the thymus, controlling transcriptional regulation of the ectopic expression of genes for self peripheral tissue antigens (PTAs).

Using nPOD samples, we translated these findings to identify disease-related, tissue-specific changes in orthologs of *Deaf1* and *PTAs (ins)* in man.

Yip et al., (2009) *Nature Immunology*, 10:1026-33



Disease- and tissue-specific changes were seen in nPOD samples, including *DF1-VAR* and *INS* expression in the PLN of T1D vs. control subjects (*Nature Immunol* 2009 9:1026-33)



Hu DF1-VAR is higher in the PLN of all five T1D vs. controls. (~20-fold)

Insulin mRNA			
Group	Age/Sex	PLN	SPL
ctrl	24/F	+	n/a
ctrl	30/M	+	n/a
ctrl	32/F	+/-	n/a
ctrl	30/M	+	+
ctrl	41/M	+	+
T1D	32/M	-	+
T1D	76/M	-	+
T1D	37/F	-	+
T1D	28/F	-	+
pre-T1D	7/M	-	+

INS was detected in the PLN of controls, but not T1D patients, and in the spleen of both groups. Thus there is loss of PTA expression with DF1-VAR.

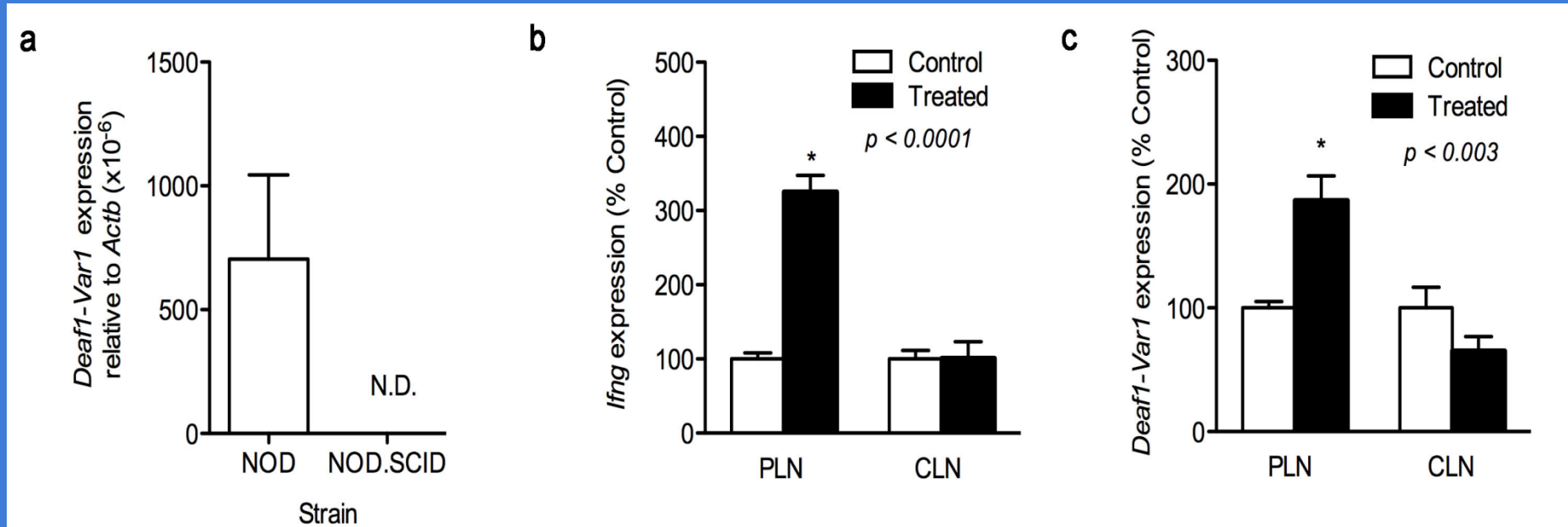


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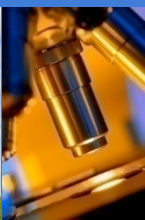
How/why are these genes actionable?

1) Splicing of *Deaf1* is driven by inflammation in NOD mice

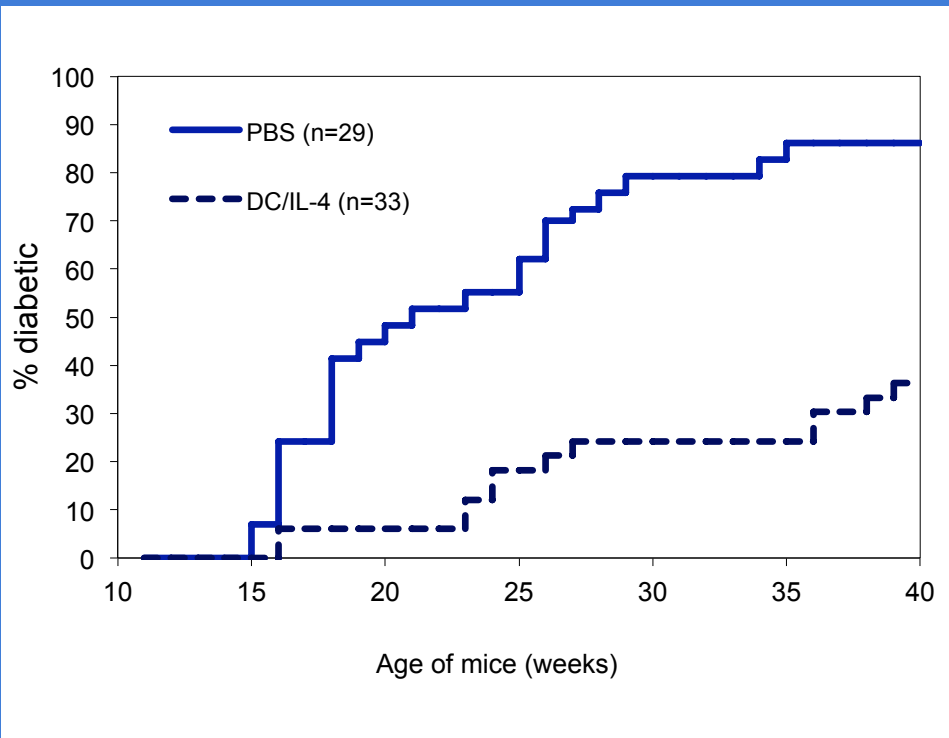


a) NOD.SCID mice don't splice *Deaf1*

b, c) splenocytes activated *in vitro* drive splicing in the PLN of NOD.B10



2) DCs transduced to express IL-4 prevent disease progression

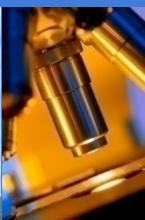


Dendritic cells transduced or transfected to express IL-4 delay and prevent the onset of hyperglycemia in most treated NOD mice.

Mol Ther. 2010 18:2112-20.

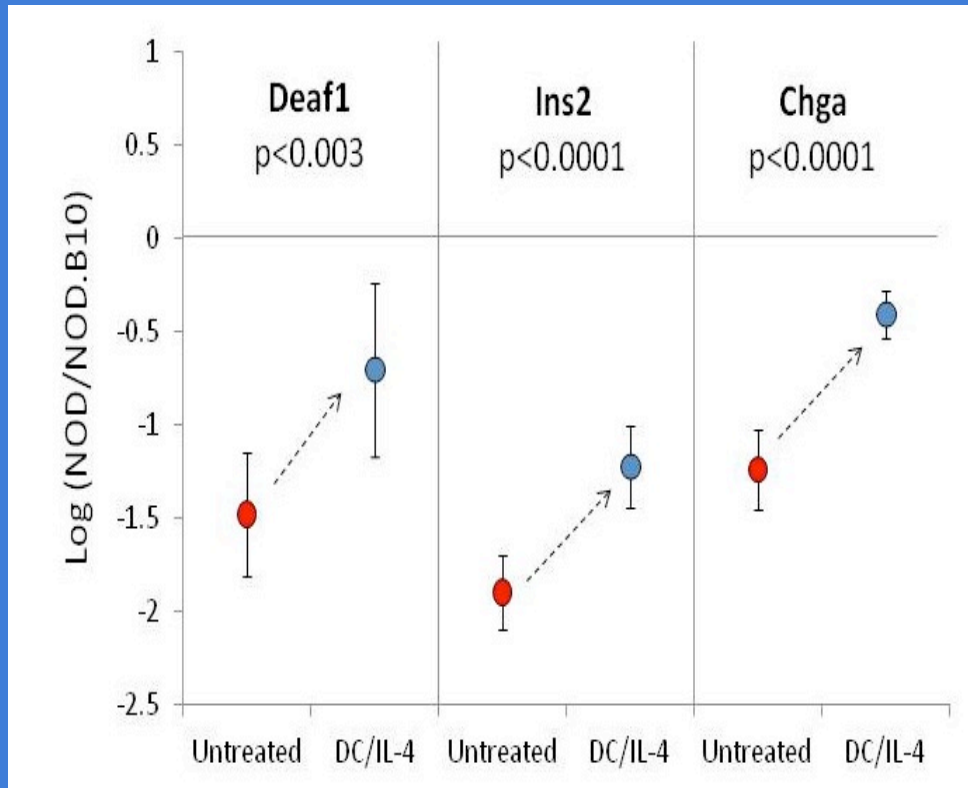
This treatment was initiated due to several published articles on hIL-4 and our own findings that *il-4* expression was diminished in the PLN of NOD mice compared to NOD.B10

Clin. Immunol. 2008 127:176-187.



(3) IL-4 treatment corrects the splicing of *Deaf1* and restores PTA gene expression in the treated NOD mice toward normal NOD.B10 levels

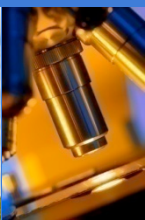
PLNs from 12-wk old NOD mice
 Untreated (n=6) versus DC/IL-4 day 3 (n=9)



DCs transduced to produce IL-4 were injected i.v. into 12 week old NOD mice that were sacrificed 3 days later and gene expression in the PLNs was assessed by microarray against the same NOD.B10 tissue. (*Deaf1*, *Ins2* and *Chga* are shown)

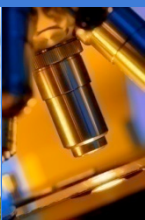


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In Summary

- (1) By comparing NOD to NOD.B10 mice, we were able to identify tissue-specific and disease-dependent changes in gene expression and, using nPOD samples, identified relevant orthologs in human T1D.
- (2) Actionable genes should be expressed in a disease-dependent and tissue-specific manner in **ALL** T1D patients to be considered a target for therapy.
- (3) We have developed therapies to block disease progression before beta cell destruction in NOD mice, can similar strategies work in man?



Acknowledgements

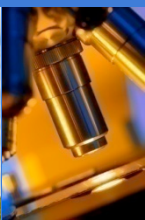
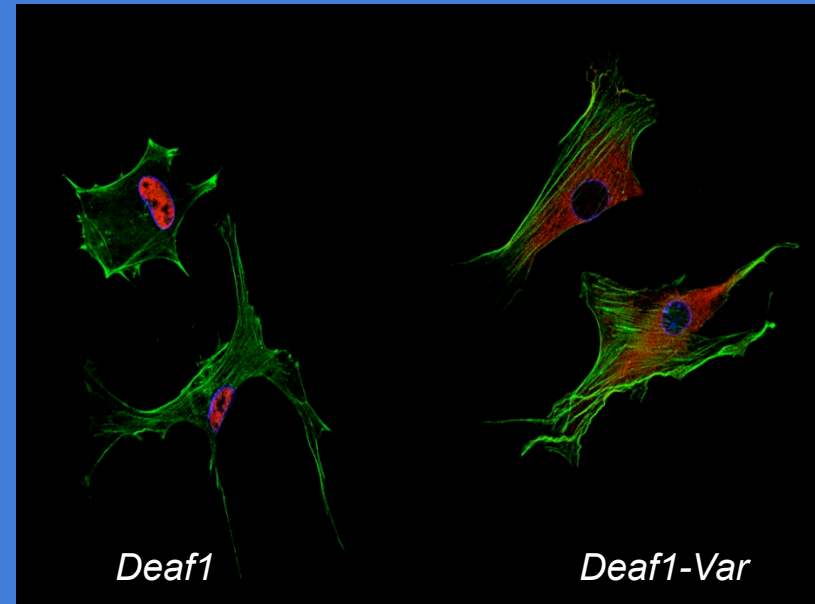
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Questions that should be addressed

- (1) What causes *DEAF1* splicing and could this “factor” be a target for therapeutic intervention in pre-diabetic patients?
- (2) Are there genes in the NOD, that as orthologs in human PBCs can be used as surrogates of *DEAF1* splicing?
- (3) Does ectopic expression of PTAs in the PLN lead to clonal deletion and/or Treg selection?
- (4) Could miRNAs lead to mRNA splicing events?

