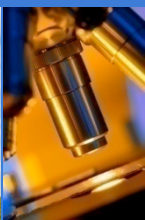




# Immune Responses to the Extracellular Domain of IA-2 in Type 1 Diabetes

Michael P. Morran, Leslie S. Satin, and Massimo Pietropaolo

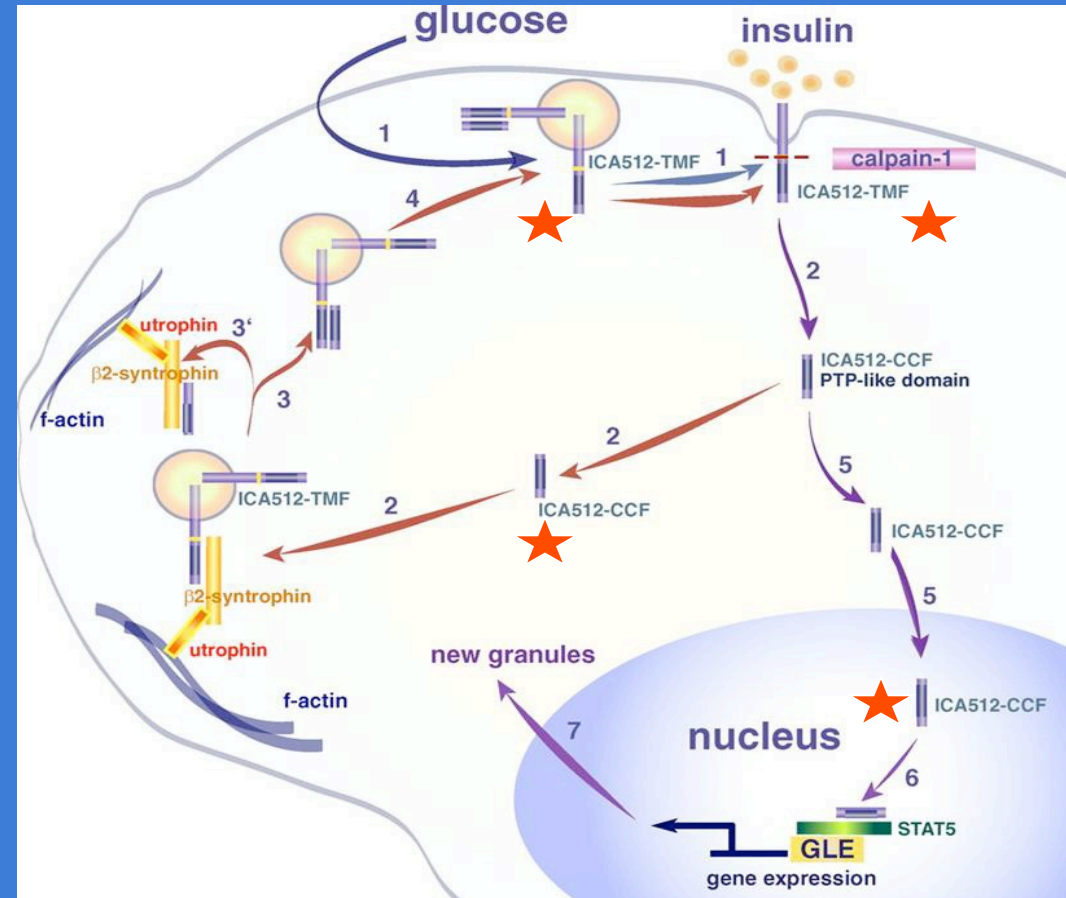
The Brehm Center for Diabetes Research, University of Michigan, Ann Arbor, MI



# $\beta$ -cell Insulin Secretory Machinery: A major target of T1D related autoimmune responses

## Islet Cell Antigen (IA-2 / ICA 512)

- Localized in Secretory Granules of  $\beta$ -cells.
- 50-60% of T1D patients are positive for IA-2 Auto-Abs.
- Conventional IA-2 biomarkers include: IA-2ic and ICA 512bdc.



Trajkovski, M. et al. *J. Biol. Chem.* 2008;283:33719-29

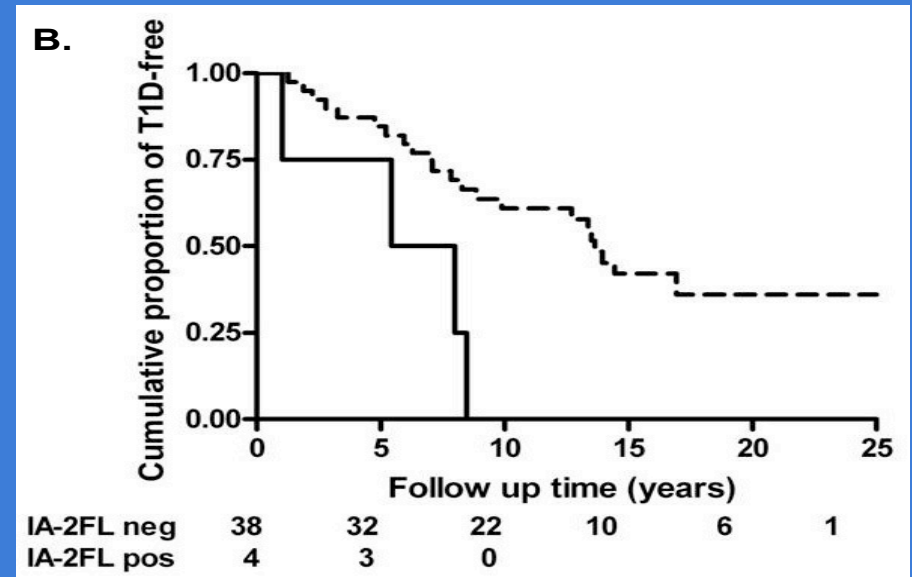
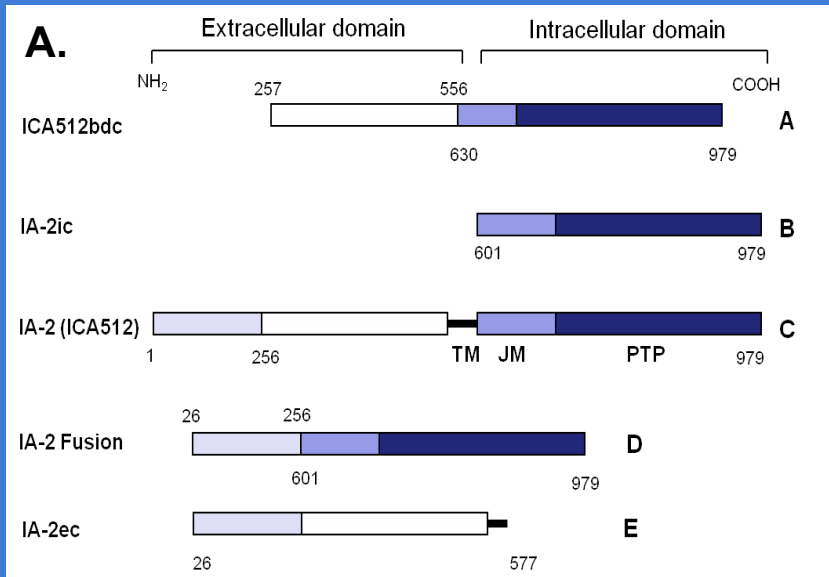


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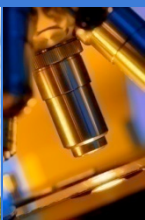


# Background

1. We previously identified a new candidate biomarker within the extracellular domain of IA-2 (IA-2ec; aa 26-577) in humans.
2. IA-2ec positive individuals illustrated a rapid acceleration of T1D onset compared to individuals reactive for conventional IA-2 biomarkers of T1D.



Morran MP et al. *Endocrinology* 2009,151(6): 2528-2537.

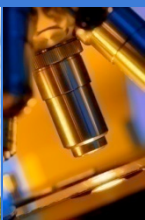


# Rationale

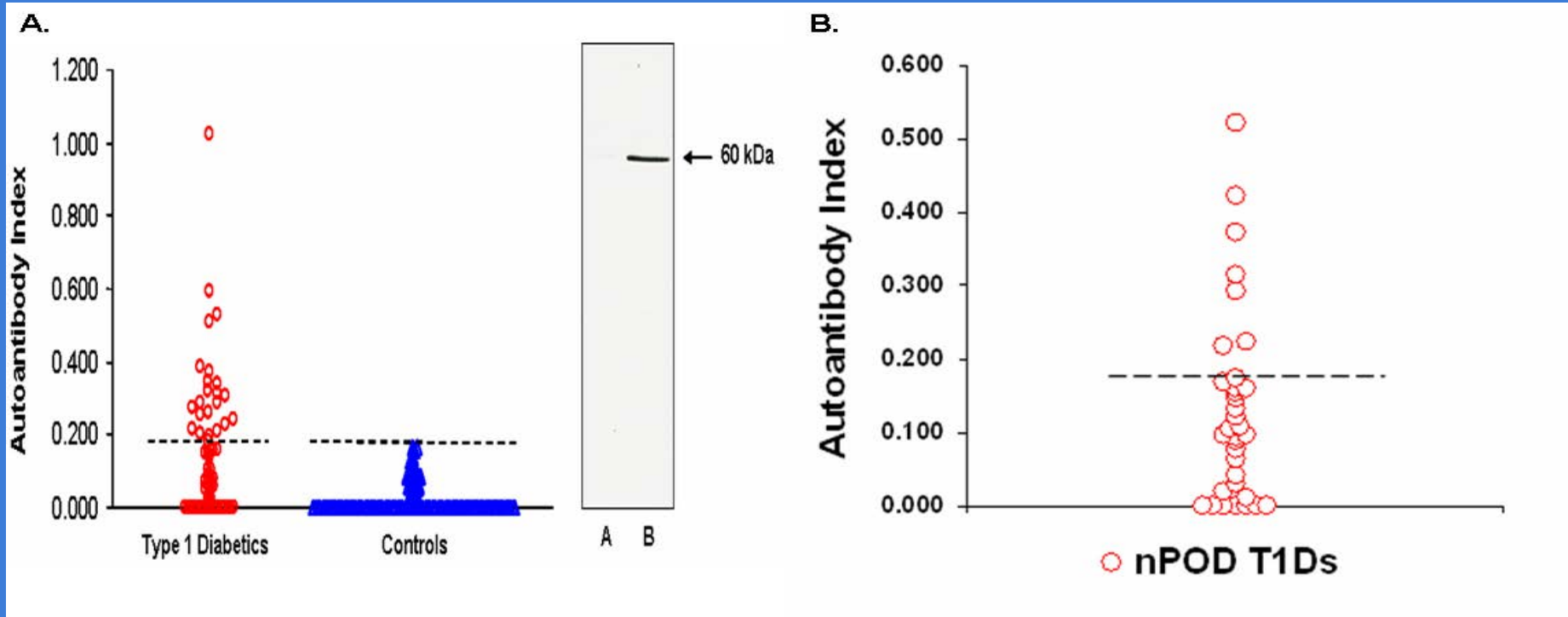
1. **Examples of antibody-mediated diseases include Graves', Myasthenia Gravis, Pemphigus Vulgaris, Goodpasture Syndrome and Lupus.**
2. **The ability to test and screen for the presence of T1D associated auto-Abs hold great predictive value, though it offers little insight into the pathogenic potential of auto-Abs in T1D.**

# Hypothesis

IA-2ec Auto-Abs have the potential for pathophysiological impact through alterations in cellular processes, which maybe a contributing factor to the rapid progression toward T1D observed in IA-2ec positive individuals.



# Results: RIA-Specificity

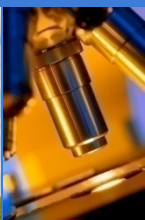


A. Autoantibodies against IA-2ec (residues 26-577), in newly diagnosed diabetic patients n=79 (○) and healthy control volunteers n=167 (△). Dashed lines represent the cut-off point of index=0.190. A total of 22 out of 79 (27.8%) were IA-2ec autoantibody positive.

B. nPOD diabetic subjects n=34 (○). A total of 7 out of 34 (20.6%) serum samples exhibited antibody responses.



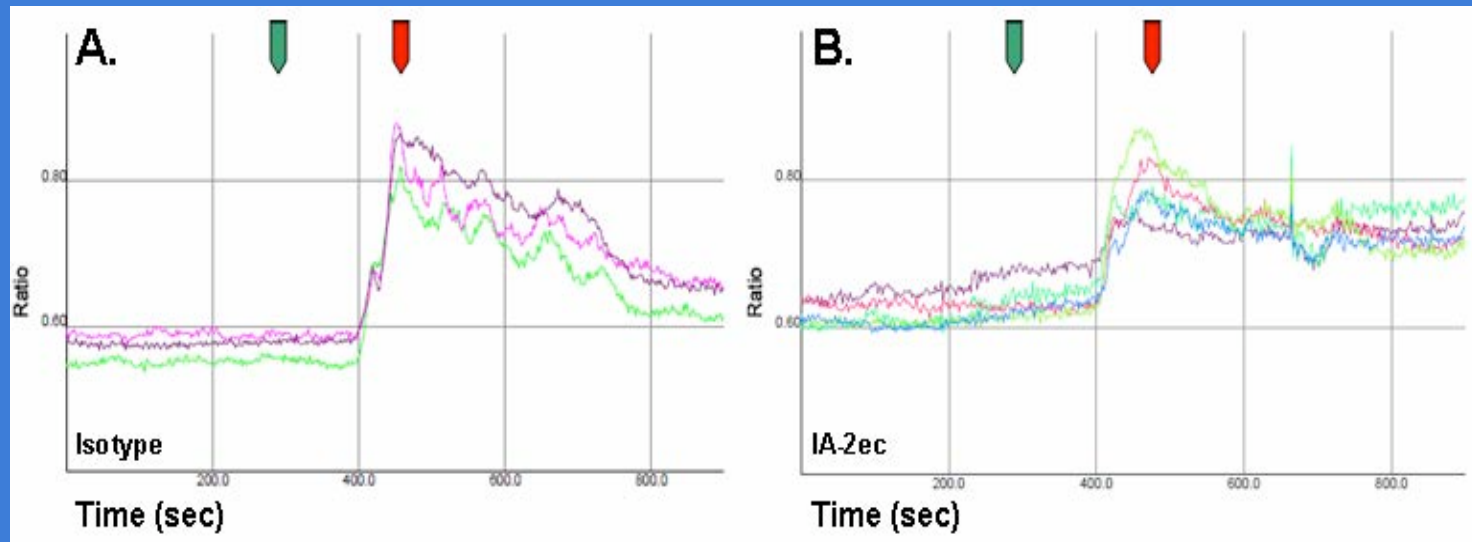
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# Results: Calcium Flux

- Analyze *in vitro* effects of IA-2ec Abs on cell processes including: Calcium Mobilization, Apoptosis, Autophagy, and Insulin Secretion.

## Initial Data Utilizing IA-2ec Specific Abs



### Methods - Calcium responses to 100mM K<sup>+</sup> in human SH-SY5Y Neuroblastoma Cells

Cells were pre-incubated 24 hours prior to analysis.

A) Cells exposed to 400ng/mL of normal goat IgG (isotype) overnight (sc-2028).

B) Cells exposed to 400 ng/mL of IA-2 (N-17) goat IgG overnight (sc-54678).

**Green** tab indicates when solution switch to high K<sup>+</sup> buffer.

**Red** tab indicates when high K<sup>+</sup> buffer was switched back to initial buffer.

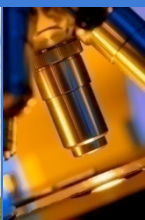
Y-axis Ratio is indicative of change in Fura-2 F340/F380.

# Conclusions

- We have identified a novel biomarker within the human IA-2ec domain of IA-2, which leads to an accelerated progression of T1D.
- Successfully identified IA-2ec positive subjects in the pool of T1D nPOD samples.
- Pathophysiological implications of IA-2ec Abs on  $\text{Ca}^{+2}$  flux seems to be insignificant despite some rather promising initial findings.

## Ongoing Research

1. We are continuing to investigate the potential pathophysiological role of IA-2ec antibodies in cellular processes including: Apoptosis, Autophagy, and Insulin Secretion.

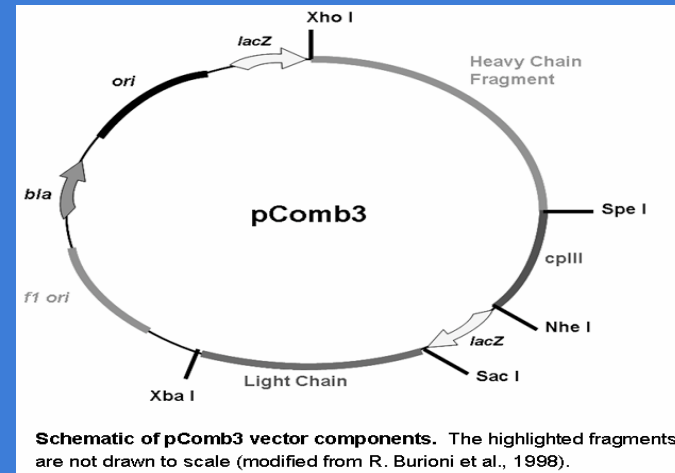
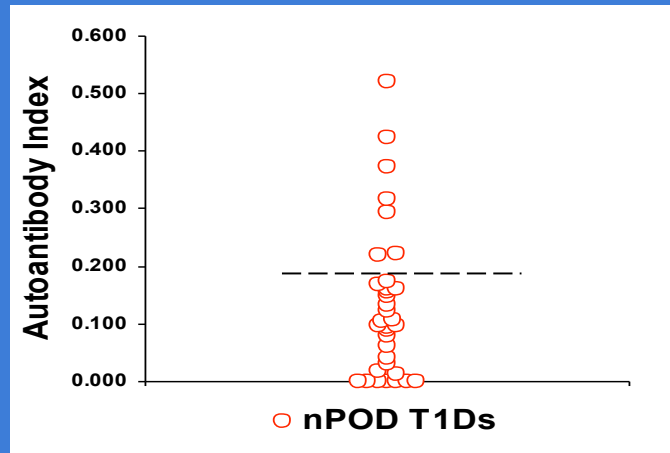


# Future Plan

The extraction of mRNA from nPOD tissue for combinatorial immunoglobulin library construction via a phage expression system.

## IA-2ec specific IgGs and Fab fragments can be utilized in the following:

1. Identification of antibody binding sequences.
2. Determination of possible Ab restriction of IgG H-and L-chains.
3. Analysis of pathophysiologic effects on target cells, i.e. beta cells.



Kang et al. *METHODS: A Companion to Methods in Enzymology*. 2 (2): 111-118, 1991.

Barbas et al. *METHODS: A Companion to Methods in Enzymology*. 2 (2): 119-124, 1991.



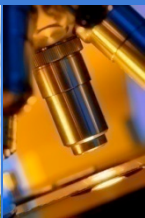
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# Questions



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