A stimulating insulin vaccine

By Chris Cain, Staff Writer

A Dana-Farber Cancer Institute-led team has designed a modified insulin peptide-based vaccine that prevents type 1 diabetes in mice by inducing the production of immunosuppressive T_{reg} cells.¹ The group is now collaborating with the German Research Center for Environmental Health's Institute of Diabetes Research to identify peptides that have similar properties in humans.

Type 1 diabetes occurs when autoreactive T cells attack and destroy insulin-producing pancreatic β cells. Many of the genetic and environmental factors that trigger this immune assault are not well understood. However, multiple autoantigens have been identified that are strongly associated with the disease, including insulin itself.

Companies have taken two broad approaches to induce a state of immune tolerance that prevents or halts the destruction of β cells. The first approach is to develop vaccines using peptides derived from the autoantigens. The second is to use immunomodulating therapies such as antibodies that target CD3, a part of the T cell receptor (TCR) complex.

Both strategies have met with disappointment in the clinic in the last nine months.

This spring, **Diamyd Medical AB's** glutamic acid decarboxylase (GAD)-based vaccine failed to meet its primary endpoints in two Phase III trials. GAD is an autoantigen associated with type 1 diabetes.

Two immunotherapies fared no better. Last October, **MacroGenics Inc.** and **Eli Lilly and Co.'s** teplizumab anti-CD3 mAb failed in a Phase II/III trial. In March, **Tolerx Inc.** and **GlaxoSmithKline plc's** oteelixumab anti-CD3 mAb failed in a Phase III trial.

Harald von Boehmer, professor of microbiology and immunobiology at **Harvard University** and senior investigator at Dana-Farber, believes he has found a better way to induce immune tolerance. The key, he thinks, is found in the interplay between antigens and MHC II molecules, which together form a complex that agonizes TCRs. Previous work from von Boehmer's lab has suggested that strongly agonizing TCRs could effectively induce immune tolerance.²

Building on recent work that showed a 12-amino-acid insulin autoantigen does not interact strongly with MHC II molecules in diabetic mice,³ von Boehmer hypothesized that a modified insulin fragment that could more strongly interact with MHC II would be

able to more potently agonize TCRs and thus generate antigen-specific T_{reg} cells, which could suppress an autoimmune response.

Thus, the team designed a 12-amino-acid peptide that differs from the insulin fragment at one MHC II interaction position. This peptide induces T_{reg} cell production in mice with significantly greater efficacy than the unmodified peptide.

In the nonobese, diabetic (NOD) mouse model of type 1 diabetes, daily infusions of 5 μ g of the peptide completely prevented the development of the disease. However, the effect occurred only in mice with undetectable to moderate levels of anti-insulin antibodies. Autoantibody levels correlate with the development of type 1 diabetes, suggesting the approach might need to be used early in the disease to be effective.

"This is probably due to the fact that once a T cell is fully activated by antigen, it cannot be converted into a T_{reg} cell any longer. This approach has to be used early when there is still the potential to generate T_{regs} ," he said.

He also pointed out that a key to this strategy is that the dose of peptide used is subimmunogenic, meaning that it does not activate antigen-presenting cells or stimulate T cell activation, which he says is counterproductive to T_{reg} cell conversion.

Peptide-infused NOD mice had significantly greater numbers of pancreatic forkhead box P3 (Foxp3)-positive T_{reg} cells than noninfused NOD mice. This T_{reg} cell induction was further amplified when mice were treated with a mammalian target of rapamycin (mTOR; FRAP; RAFT1) inhibitor. Von Boehmer's group had previously reported that inhibitors of mTOR aid the conversion of naïve T cells into T_{reg} cells.⁴

The new results were published in *The Journal of Experimental Medicine*.

"The results are about as dramatic as you get. This is one of the most efficacious studies in this animal model—you don't have to use an adjuvant and you get results with 5 μ g of peptide," said George Eisenbarth, executive director of the Barbara Davis Center for Childhood Diabetes at the **University of**

Colorado Denver School of Medicine.

In collaboration with Aaron Michels, assistant professor of pediatrics and medicine at the University of Colorado Denver School of Medicine, Eisenbarth's lab is studying the molecular interactions between MHC, antigenic peptides and TCRs in the hopes of designing small molecules that could disrupt the interactions to treat diabetes.

Of mice and men

Although von Boehmer's modified peptide provides early protection in the diabetic mouse model, the key hurdle for translating this approach will be identifying modified insulin peptides that have the same T_{reg} cell-inducing potential in humans.

Humans express multiple MHC II alleles, many of which are structurally distinct from the single MHC II allele found in NOD mice. In addition, different individuals in a population can carry distinct

"This approach has to be used early when there is still the potential to generate T_{regs} ."

**—Harald von Boehmer,
Dana-Farber Cancer Institute**

variants of MHC II alleles, making it difficult if not impossible to design a 'one size fits all' peptide.

However, carriers of certain MHC II alleles are at high risk for type 1 diabetes, and von Boehmer noted that he intends to focus on those individuals.

He sees promise for translating this approach into humans because the natural insulin autoantigen that the modified peptide mimics has been found both in humans and in mice, and the high-risk human MHC II alleles are structurally related to the MHC II allele found in NOD mice.

To design and test new peptides, von Boehmer has teamed up with Anette Ziegler, director of the German Research Center for Environmental Health's Institute of Diabetes Research. Ziegler has access to clinical samples from patients at risk for type 1 diabetes and from patients at various stages of disease progression.

"We need to identify modified peptides that mimic the key autoantigens in man and then show *in vitro* and *in vivo* that we are able to find conditions that reproducibly result in efficient conversion of naïve, antigen-specific T cells to antigen-specific FOXP3 T_{regs}," said Ziegler.

She said because of diversity in autoimmune profiles, it is likely that one peptide won't work for all type 1 diabetes patients and the group may need to identify and combine several modified peptides.

Indeed, Matthias von Herrath, director of the Center for Type 1 Diabetes Research at the **La Jolla Institute for Allergy & Immunology**, wanted to see modified peptides tested in mouse models of diabetes expressing different MHC II molecules.

He also was concerned about safety, especially because the patients who would be given this vaccine might not yet have any disease

symptoms. Although autoantigen-based vaccines are intended to induce immune tolerance, patients must be closely monitored to ensure that the vaccine does not cause or accelerate an autoimmune reaction.

Von Boehmer did note that the modified peptide in fact decreased insulin autoantibody production.

Dana-Farber has patented the work, and the IP is available for licensing.

Cain, C. *SciBX* 4(29); doi:10.1038/scibx.2011.814
Published online July 28, 2011

REFERENCES

1. Daniel, C. *et al. J. Exp. Med.*; published online June 20, 2011; doi:10.1084/jem.20110574
Contact: Harald von Boehmer, Dana-Farber Cancer Institute, Boston, Mass.
e-mail: harald_von_boehmer@dfci.harvard.edu
2. Apostolou, I. & von Boehmer, H. *J. Exp. Med.* **199**, 1401–1408 (2004)
3. Stadinski, B.D. *et al. Proc. Natl. Acad. Sci. USA* **107**, 10978–10983 (2010)
4. Daniel, C. *et al. Proc. Natl. Acad. Sci. USA* **107**, 16246–16251 (2010)

COMPANIES AND INSTITUTIONS MENTIONED

Dana-Farber Cancer Institute, Boston, Mass.
Diamyd Medical AB (SSE:DIAM B), Stockholm, Sweden
Eli Lilly and Co. (NYSE:LLY), Indianapolis, Ind.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Harvard University, Cambridge, Mass.
German Research Center for Environmental Health, Munich, Germany
La Jolla Institute for Allergy & Immunology, La Jolla, Calif.
MacroGenics Inc., Rockville, Md.
Tolerx Inc., Cambridge, Mass.
University of Colorado Denver School of Medicine, Denver, Colo.