SciBX
Science-Business eXchange

A tolerant alternative to immunosuppression

By Kai-Jye Lou, Staff Writer

Two research teams have shown that temporary monotherapy with human α_1-antitrypsin can lead to long-term protection against inflammation-mediated and T cell–mediated destruction of islet β-cells in mice. The results, which were achieved without the use of immunosuppressants, could bolster the prospects for islet transplantation in patients with type 1 diabetes by overcoming two of the main drawbacks associated with such therapy: immunosuppressant use and eventual loss of graft function.

Both groups are already planning clinical trials to evaluate AAT in type 1 diabetics.

Human α_1-antitrypsin (SERPINA1; AAT) is an anti-inflammatory serine protease inhibitor that is marketed as an enzyme replacement therapy for patients with a defective version of the protein (AAT deficiency). The **FDA** has approved three AAT products: Aralast from **Baxter International Inc.**, Prolastin from **Talecris Biotherapeutics Inc.** and Zemaira from **sanofi-aventis Group**.

In a paper published in the *Proceedings of the National Academy of Sciences*, researchers at the **University of Colorado Health Sciences Center** and **Ben-Gurion University of the Negev** showed that AAT monotherapy resulted in dose-dependent increases in acceptance rates of allogeneic islet β-cell grafts and normal glucose levels in diabetic mice.[1] The grafts persisted for the duration of the 12-week experiment, whereas all grafts in albumin-treated controls were rejected within 12 days.[1]

The research group was led by Charles Dinarello, a professor of medicine at the University of Colorado.

The accepted grafts remained functional and maintained normal glucose levels following cessation of AAT, whereas removal of the grafts led to hyperglycemia. Importantly, mice receiving a second islet graft from the same source, following removal of the initial graft, did not require additional doses of AAT for immune tolerance. In contrast, mice receiving islet β-cells from a different source quickly rejected the second graft—a result that suggests the induced immune tolerance of grafted cells is specific to the original tissue source.

Separately, researchers at **Harvard Medical School** published in *PNAS* that AAT monotherapy halted the autoimmune reactions that lead to the destruction of native islet cells. The treatment also partially restored native islet β-cell mass and normal glucose levels in 14 of 16 nonobese diabetic (NOD) mice, a significant improvement compared with what was seen in the 150 untreated controls, which all remained hyperglycemic ($p<0.0001$).[2]

The research group was led by Terry Strom, scientific director of the Transplant Institute at **Beth Israel Deaconess Medical Center** and a professor of medicine at Harvard Medical School.

Similar to Dinarello's group, Strom's team showed that diabetic mice receiving AAT and islet β-cells from a syngeneic source accepted the graft and had normal glucose levels. Grafts in untreated controls were destroyed.

Both *PNAS* papers showed that AAT does not directly affect T cells. Instead, AAT shifts the body's cytokine balance from proinflammatory to anti-inflammatory. The anti-inflammatory environment prevents the maturation of dendritic cells (DCs) and encourages the conversion of naïve T cells into protective T regulatory cells that prevent effector T cells from attacking the islet β-cells.

Lowering the number of mature DCs also downregulates the conversion of naïve T cells into effector T cells (*see* **Figure 1, "Protecting islet cell grafts using AAT"**).

> **"Clearly AAT not only alters autoimmunity but also transplant immunity."**
> *—Terry Strom, Beth Israel Deaconess Medical Center*

"The most important finding is that we do not see dendritic cells mature—they process foreign antigens from the allograft but present them to naïve T cells in an immature state, thus inducing antigen-specific tolerance," Dinarello told *SciBX*.

Strom noted that his team became interested in this area after Dinarello shared some initial observations; he thinks the results reported in *PNAS* by Dinarello's group complement the work from his own group. "Clearly AAT not only alters autoimmunity but also transplant immunity. The underlying mechanism in both cases appears to involve a beneficial modification of the inflammatory milieu in which autoimmunity or transplant immunity is spawned," Strom told *SciBX*.

Away with immunosuppression

Strom and Dinarello also noted that the results reported in the *PNAS* articles were achieved without the use of potentially toxic immunosuppressant drugs. Normally, patients receiving transplanted islet cells would need to take an immunosuppressant cocktail for the life of the graft.

NIH's **National Institute of Diabetes and Digestive and Kidney Diseases** warns that the use of immunosuppressive drugs in patients receiving pancreatic islet transplantation is known to increase both susceptibility to infections and cancer risk.

Moreover, a paper in *Diabetes* by Canadian researchers showed that only about 10% of islet cell transplant recipients using the standard immunosuppressant drug cocktail remained free of the need for insulin injections at a five-year follow-up.[3]

Thus, said Robert Elliot, medical director and cofounder of **Living Cell Technologies Ltd.**, "immunosuppression has yielded few results" from the attempts to treat type 1 diabetes "along the immune track."

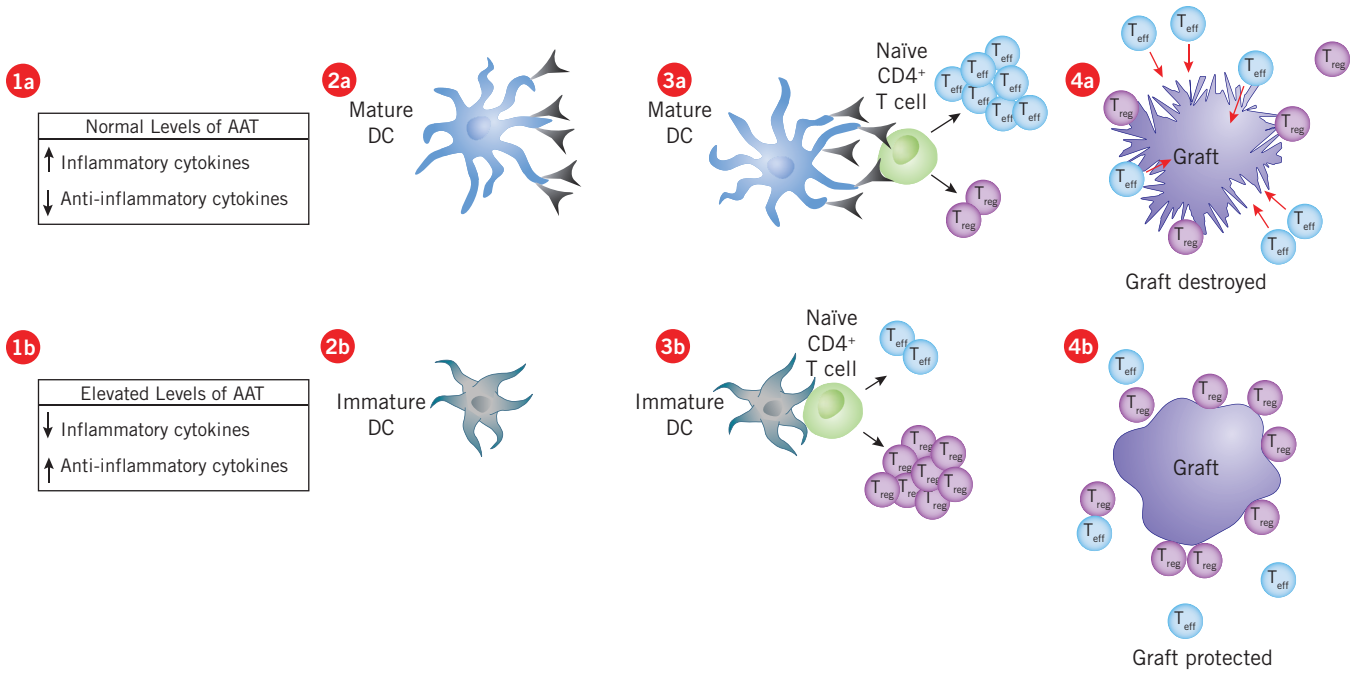

Figure 1. Protecting islet cell grafts using AAT. Human α_1-antitrypsin (SERPINA1; AAT), which does not directly act on T cells, has anti-inflammatory properties that are believed to promote host immune tolerance toward islet β-cell grafts by expanding the population of T regulatory (T_{reg}) cells and decreasing the number of effector T (T_{eff}) cells.

Two papers in *PNAS*[1,2] now show that treatment with AAT can shift the cytokine environment from proinflammatory [**1a**] to anti-inflammatory [**1b**].

Immature dendritic cells (DCs) collect antigens from the islet β-cells. In a proinflammatory environment, DCs will mature and express an array of surface molecules that encourage the conversion of naïve T cells into T_{eff} cells [**2a**]. When inflammation is blunted by the addition of AAT, the DCs remain in an immature state and fail to express the surface molecules needed for T_{eff} cell conversion [**2b**].

The antigen-bearing DCs engage naïve T cells and imprint them to recognize the foreign antigen. When engaged with a mature DC, a naïve T cell is encouraged to differentiate into a T_{eff} cell [**3a**]. When engaged with an immature DC, the naïve T cell will tend to differentiate into a T_{reg} cell [**3b**].

Without a sufficient population of antigen-specific T_{reg} cells, the T_{eff} cells invade and kill the islet β-cells [**4a**]. However, when sufficient T_{reg} cells physically reside in the vicinity of the islet β-cells, they disarm the incoming T_{eff} cells [**4b**]. In addition, subsequent islet β-cell grafts from the same donor may be accepted without the need for repeated treatment, as demonstrated in the two *PNAS* papers.

Living Cell's DiabeCell encapsulated porcine pancreatic islet cells are in Phase I/II testing. The capsules are intended to ensure the cells are not recognized as foreign by the recipient's immune system, thus circumventing the need for immunosuppressants.

"All drugs currently approved to prevent transplant rejection are immunosuppressive, but the real problem is that these drugs are also toxic," said Dinarello. "AAT does not suppress the immune system, so you don't have the standard problems associated with immunosuppressive drugs."

Eli Lewis, a lead author on the Dinarello paper and director of the Clinical Islet Lab at Ben-Gurion University of the Negev, noted that "it is quite unique that a genuinely anti-inflammatory molecule was able to re-edit the immune system to achieve antigen-specific tolerance mediated by T regulatory cells." Immunosuppressive therapy, he said, only delays the immune system from recognizing the foreign tissue.

"The most surprising aspect is that the AAT can redirect, through changes in inflammation, the activity of tissue-destructive T cells toward a tissue-protective mode," said Strom.

Easy dose it

Even if AAT can take immunosuppressants out of the picture, a question going forward is whether—and at what dose—AAT will be able to improve on the success rates for islet cell transplant therapies.

"The five-year islet graft survival rates have been reported as being very low" in humans, noted Lewis. "We are hoping that the behavior of the mouse immune system, especially chosen in our study to be that of healthy wild-type strains, reflects that of humans. We believe only a clinical trial can provide the answer, a particularly feasible option considering the safety record of AAT."

He added: "We chose AAT for a reason. Aside from being such a

powerful anti-inflammatory agent, it has been used in patients for over two decades as enzyme replacement therapy. Studies for up to 13 years clearly show that these patients exhibit no compromise in their inflammatory responses."

In the diabetes setting, Lewis said, a few weeks of AAT treatment "appears to be sufficient for graft acceptance and induction of immune tolerance."

Nevertheless, Ingrid Stuiver, senior director of research at **MicroIslet Inc.**, cautioned against directly translating results from murine models to humans.

"Studies in several different animal models such as the diabetes-prone BB [BioBreeding] rat or nonhuman primates might be enlightening," she told *SciBX*. "We would like to see the preclinical work include a larger subset of animals to assure reproducibility."

The company is planning its IND submission to enter the clinic with its MicroIslet-P microencapsulated porcine islet cell implant.

Maria Koulmanda, a lead author on the Harvard *PNAS* paper, also noted AAT potentially can induce T cell tolerance toward harmful pathogens. Given the potential for pathogen tolerance, she said the clinical trial protocol involving AAT therapy would specifically exclude patients with active infections.

Koulmanda, is associate professor of surgery at Harvard and director of nonhuman primate research at Beth Israel Deaconess Medical Center.

AAT designs

Dinarello said his group is planning a clinical trial to evaluate AAT in patients with type 1 diabetes undergoing islet cell transplantation. "The real question is how long you have to give AAT," Dinarello told *SciBX*.

Similarly, Strom's group is working on clinical trial designs for AAT-based therapies to treat new onset type 1 diabetes.

The University of Colorado has a patent covering inhibitors of serine protease activity and their use in treatment of graft rejection and promotion of graft survival. The work is available for licensing from the University of Colorado Office of Technology Transfer.

Strom said a patent application has been filed covering the use of AAT in type 1 diabetes and autoimmune diseases and is available for licensing from the Technology Ventures Office at Harvard.

Lou, K.-J. *SciBX* 1(40); doi:10.1038/scibx.2008.963
Published online Nov. 6, 2008

REFERENCES

1. Lewis, E.C. *et al. Proc. Natl. Acad. Sci. USA*; published online Oct. 13, 2008; doi:10.1073/pnas.0807627105
   Contact: Charles A. Dinarello, University of Colorado Health Sciences Center, Denver, Colo.
   e-mail: cdinarello@mac.com
2. Koulmanda, M. *et al. Proc. Natl. Acad. Sci. USA*; published online Oct. 13, 2008; doi:10.1073/pnas.0808031105
   Contact: Terry B. Strom, Beth Israel Deaconess Medical Center, Boston, Mass.
   e-mail: tstrom@bidmc.harvard.edu
3. Ryan, E.A. *et al. Diabetes* **54**, 2060–2069 (2005)

COMPANIES AND INSTITUTIONS MENTIONED

Baxter International Inc. (NYSE:BAX), Deerfield, Ill.
Ben-Gurion University of the Negev, Beer-Sheva, Israel
Beth Israel Deaconess Medical Center, Boston, Mass.
Food and Drug Administration, Rockville, Md.
Harvard Medical School, Boston, Mass.
Living Cell Technologies Ltd. (ASX:LCT; Pink:LVCLY), Hawthorn, Australia
MicroIslet Inc. (OTCBB:MIIS), San Diego, Calif.
National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Md.
National Institutes of Health, Bethesda, Md.
sanofi-aventis Group (Euronext:SAN; NYSE:SNY), Paris, France
Talecris Biotherapeutics Inc., Research Triangle Park, N.C.
University of Colorado Health Sciences Center, Denver, Colo.