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Raising a T_{reg} cell army

By Lev Osherovich, Senior Writer

Despite promising preclinical findings that raising levels of T_{reg} cells might have utility against autoimmune disease, obtaining enough of the rare cells from humans has been a major translational obstacle. Now, British and American teams have proposed *ex vivo* strategies to expand and activate T_{reg} cells harvested from patients and have shown that these cultured cells have efficacy in mouse models of graft-versus-host disease and graft rejection.[1-3]

Athelos, a new subsidiary of **NeoStem Inc.**, hopes to start clinical testing of an approach related to the new methods by year end.

The three studies "provide useful information on how to generate and potentially manipulate T_{reg} cells toward being whole-cell therapies," said Spiros Jamas, CEO of **Tempero Pharmaceuticals Inc.** Tempero is developing T_{reg} cell–targeting therapies for autoimmune disease.

Jamas added that the techniques "solve the issue of generating a sufficient number of T_{reg} cells that have the right functional properties and retain their suppressive activity" after cell culture.

"The significance is clear—you can now develop a clinical protocol that allows this type of cell therapy, which has been shown to be pretty effective in animal models, to be adapted to humans," said Jeffrey Bluestone, professor of medicine, pathology, microbiology and immunology at the **University of California, San Francisco**.

The trio of findings was reported in *Science Translational Medicine*.

Extract and expand

Two of the studies, from separate British teams, isolated T_{reg} cell cultures from the blood of human donors, activated the cells *in vitro* and introduced them into mice to prevent transplant rejection.

Giovanna Lombardi, professor of human transplant immunology at **King's College London** and the leader of one of the two teams, said her goal was to develop a cell-based alternative to immunosuppressants—the standard of care in solid organ transplantation.

"Although transplantation is a very successful procedure, the chronic use of immunosuppressants can lead to infection and cancer," said Lombardi. "We can make [immunosuppressive] cells that are specifically effective in transplant, thus avoiding the problems linked with immunosuppressive drugs."

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> *—Giovanna Lombardi, King's College London*

Lombardi's strategy was to isolate T_{reg} cells from a graft recipient and then expose the cells to donor-derived antigens presented by dendritic cells (DCs), thus activating an antigen-specific immunosuppressive response.

Her group used beads coated with antibodies to T_{reg} cell–specific cell surface markers to isolate dormant T_{reg} cells from human blood. The team then collected DCs from a sample of human skin and cocultured them with the T_{reg} cells, looking for signs of T_{reg} cell activation.

The team identified two surface markers, CD69 and transferrin receptor protein 1 (TFRC; TFR; CD71), that were induced in T_{reg} cells activated through contact with the foreign antigens presented by the DCs.

When enriched and transferred into humanized mice, these activated T_{reg} cells suppressed immunological assault by host immune cells on a human skin graft obtained from a different person. The animals had fewer signs of graft rejection than mice receiving saline control or a bulk population of unmanipulated T cells from the same donor.

A separate team led by the **University of Oxford**'s Andrew Bushell substituted the marker-based enrichment of active T_{reg} cells with a pharmacological approach. They used a phosphodiesterase-3 (PDE-3) inhibitor to increase T_{reg} cell activation in cell culture.

Bushell is a senior postdoctoral fellow and university research lecturer at the university's Nuffield Department of Surgical Sciences.

His team found that cultured T_{reg} cells treated with cilostamide, a PDE-3 inhibitor discovered in the 1970s at **Otsuka Pharmaceutical Co. Ltd.**, were more responsive to DC stimulation than vehicle-treated controls.

In a mouse model of human skin graft, cilostamide-treated human T_{reg} cells were more effective at suppressing effector T cell activity and graft rejection than untreated T_{reg} cells.

The two papers show "there are a lot of ways" to culture T_{reg} cells *ex vivo*, said Bluestone. He said an international consortium of academic researchers is already planning a clinical trial comparing these two methods against standard immunosuppressive therapy in renal transplant.

Lombardi did not patent her findings. Bushell did not disclose the patent status of his team's work.

T_{reg} cell horde

Meanwhile, a **University of Minnesota** team has worked out how to grow massive numbers of T_{reg} cells from peripheral blood and has used these cells to prevent graft-versus-host disease (GvHD) in mice.

The team was led by Bruce Blazar, professor of pediatrics and director of the Center for Translational Medicine at the **University of Minnesota Masonic Cancer Center**.

Blazar said previous efforts to isolate T_{reg} cells have not yielded enough cells for clinical use, primarily because of the difficulty of culturing T_{reg} cells in a way that encourages their proliferation.

"Researchers have struggled to make lots of these cells, which are resistant to proliferative cues," said Blazar. "We reasoned that other groups haven't succeeded" because they tried to stimulate T_{reg} cells under growth conditions that likely were nonpermissive.

Blazar's team crafted a stepwise strategy to expand the small quantity of dormant T_{reg} cells found in peripheral blood by activating them at exactly the right moment in their life cycle.

"The first advance was to carefully monitor the size of the cells and wait until they reached a nadir in size, when they would be most receptive to a proliferative signal," said Blazar.

The team then exposed the T_{reg} cells to an antigen-presenting cell line engineered to express stimulatory surface proteins that activate T_{reg} cells. Because other immune cells were present in the preparation, the team also administered rapamycin, an immunosuppressant that prevents the growth of most immune cells but does not affect T_{reg} cells.

Blazar's team repeated this cycle of size selection and growth stimulation four times, with each cycle yielding more T_{reg} cells. By the end of the fourth cycle, the team had hundreds of billions of T_{reg} cells, a quantity suitable for GMP-scale commercial production.

The resulting cells retained their immunosuppressive properties *in vitro* and extended the survival of mice in a model of GvHD compared with unmanipulated blood cell preparations.

Blazar thinks the cultured T_{reg} cells could be readily developed for GvHD, a life-threatening side effect of bone marrow transplantation in which transplanted immune cells turn against the recipient.

"The fact that Blazar could expand these cells to such a degree is certainly proof of concept that this could be feasible for ramping up to clinical studies," said James Ferrara, professor of pediatric oncology at the **University of Michigan Medical School**.

Ferrara said it remains to be seen whether Blazar's method creates T_{reg} cells with truly natural behavior when reintroduced into patients. Thus, clinical testing of cell therapies based on Blazar's approach should involve monitoring for signs of immune system derangement such as inflammatory- or autoimmunity-related cytokines, he said.

Blazar patented his findings, and the IP is available for licensing. He said "discussions are ongoing" to license his technology to an undisclosed company.

Auto vs. allo

Although all the proposed methods for amplifying T_{reg} cells ultimately have the same result—a large quantity of cultured T_{reg} cells—differences between the methods point to distinct clinical development strategies.

The approaches proposed by the two British teams involve isolating T_{reg} cells from individual patients, expanding the cells and reintroducing them to the same patient. These autologous approaches have the advantage of creating cells that are completely compatible with the donor, making them suitable for long-term prevention of graft rejection.

"The idea is to generate T_{reg} cells that are specific for the transplant patient and then to reinject them back into the patient after the transplant," said Lombardi.

In contrast, Blazar said that his method could be best suited for acute GvHD, delivering large quantities of allogeneic T_{reg} cells to transiently suppress the disease until the bone marrow graft takes root and the risk of GvHD diminishes. Indeed, most cases of GvHD occur in the first 100 days post-transplant.

Blazar envisions "an off-the-shelf product—a bank of cells that are partially HLA [human leukocyte antigen] matched with the recipient" transplanted along with the bone marrow to prevent GvHD or infused at the earliest signs of the disease.

"The least risky and most effective method would be autologous infusion," said UCSF's Bluestone. But he noted that producing T_{reg} cells from each individual patient "might cost $40,000–$50,000." With allogeneic cells, "there may be more risk but more of a commercial opportunity," he said.

Allogeneic T_{reg} cells that are not perfectly matched to donor bone marrow might themselves fall prey to immune cells, potentially limiting their efficacy.

Andrew Pecora, CMO of NeoStem's Progenitor Cell Therapy unit, thinks both methods have a place in the clinic.

"Allogeneic cells would be more desirable" from a cost perspective, said Pecora. But he added that despite the cost and complexity of growing up cell lines from each patient, "autologous therapy is already a business model." As an example he cited **Dendreon Corp.**'s Provenge sipuleucel-T, an autologous cell cancer therapy marketed for prostate cancer.

Pecora also heads NeoStem's Athelos unit, which has licensed IP on the *ex vivo* production of T_{reg} cells from an undisclosed source.

Athelos has T_{reg} cell–based therapies for GvHD, autoimmune disease and solid organ graft rejection in preclinical testing.

Pecora said the next challenge in this space "is to make certain that these cells can persist in an inflammatory environment *in vivo* and don't lose their regulatory ability or become effector cells."

Indeed, the long-term stability of *ex vivo*–produced T_{reg} cells reintroduced into the host remains unknown. Lombardi acknowledged that T_{reg} cells can gradually lose their suppressor activity and can even promote immune attack.

"There is a consensus that T_{reg} cells can revert into effector cells under stress conditions," said Lombardi. Thus, Lombardi is planning long-term preclinical studies to determine the stability of reintroduced T_{reg} cells.

Tempero's Jamas thinks the next big hurdle is to focus the T_{reg} cell response on a particular subset of antigens rather than to activate T_{reg} cells *en masse*. He cautioned that activating every type of T_{reg} cell at once might have undesirable immunosuppressive effects. "By focusing on a distinct subset of T_{reg} cells, this could be avoided," he said.

Tempero has small molecule compounds in lead optimization that activate particular subsets of T_{reg} cells to treat autoimmune disease.

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