Dendritic cell dragnet

By Lev Osherovich, Senior Writer

The inability of peripheral immune cells to penetrate the CNS protects the brain against inflammation but has thwarted efforts to stimulate immunity against brain cancer antigens. Now a team at **Cedars-Sinai Medical Center** has devised a way around this barrier using an immunomodulatory peptide called FMS-like tyrosine kinase 3 ligand.¹

The finding is serendipitous for **Celldex Therapeutics Inc.**, which in-licensed FMS-like tyrosine kinase 3 ligand (FLT3LG; FLT3L) from **Amgen Inc.** last year in hopes of using the molecule to mobilize myeloid progenitor cells.

The brain, the testes and parts of the eye are thought to be impervious to normal immune cells and molecules, but the mechanisms behind this exclusion remain unclear. Pedro Lowenstein, professor of medicine and biomedical sciences and director of the Board of Governors Gene Therapeutics Research Institute at Cedars-Sinai, hypothesized that the relative inaccessibility of the brain to immune surveillance derives from a lack of dendritic cells (DCs), which are innate immune cells that scavenge and display antigens along with activators of the acquired immune system such as T and B cells.

Lowenstein's team previously had shown that expressing transgenic Flt3l stimulated immune activity and slowed tumor growth in a mouse model of brain cancer.² But the mechanism of this approach, as well as the safety of bringing immune cells into the brain, was unclear.

In a new paper published in the *Proceedings of the National Academy of Sciences*, the group thinks it found the answer: FLT3L breaks the immune privilege of the brain and seems to do so without triggering autoimmunity.

"We've been using FLT3L to treat brain tumors for a while, but we've never shown formally how it's supposed to act," Lowenstein told *SciBX*. "We wanted to see whether breaking immune privilege causes an autoimmune response in the brain."

Lowenstein's team used an adenovirus vector to transfect mouse brains with a model viral antigen. Because the antigen was hidden behind the blood brain barrier, the transfected mice did not show an immune response against it. However, when the mouse brains were co-transfected with Flt3l, the mice developed an immune response against the viral protein.

In addition, the team found evidence of DC infiltration into the brains of mice coexpressing Flt3l and the antigen but not into the brains of controls without Flt3l. Over time, T cells also migrated into the Flt3l-expressing brains but not into control brains.

The Flt3l-treated mouse brains also showed signs of a nonspecific immune reaction against brain proteins including myelin, which is the target of autoimmune attack in multiple sclerosis (MS). However, the

Box 1. Macrophage express to the brain.

Whereas Pedro Lowenstein of **Cedars-Sinai Medical Center** hopes to use gene therapy to deliver FMS-like tyrosine kinase 3 ligand (FLT3LG; FLT3L) to treat brain tumors, a **University of South Florida** team has hit upon an alternative approach for getting protein therapeutics into the brain—macrophage precursors.³

In a paper published last month in *The Journal of Neuroscience*, the Florida group showed that macrophage precursors transfected with a therapeutic transgene *ex vivo* and then transplanted back into mice could penetrate the brain to sites of injury and inflammation and deliver a β -amyloid (A β)-busting protein to inflamed Alzheimer's disease (AD) lesions.

Team leader David Morgan, professor of molecular pharmacology and physiology at the University of South Florida, said his method is better than gene therapy at "delivering potentially therapeutic genes and proteins to any sites in the brain that are experiencing inflammation."

Broader distribution of FLT3L or other therapeutic proteins throughout the brain could be useful if the cancer has spread beyond the tumor resection site.

Lowenstein countered that FLT3L-bearing macrophages could have the disadvantage of going to other tissues besides the brain, raising the risk that minor injuries and inflammation in the body could lead to autoimmunity.

Morgan's patent on the macrophage delivery method is available for licensing. —LZO

treated mice did not develop demyelinating disease, which, according to Lowenstein, suggests the immune attack was primarily directed toward the viral antigen and not toward self-proteins.

"Normally, you don't get an immune response in the brain because DCs aren't there," he said. "We found that there is an increased number of T cells that are specific for myelin proteins, but we don't see lesions, so there really isn't an autoimmune response."

Lowenstein thinks the findings validate using FLT3L in humans. He is working on a protocol for a Phase I/II trial of an adenovirus-encoded FLT3L transgene to treat advanced glioblastoma multiforme (GBM) and plans to submit an IND this year.

Mobilize and conquer

In the meantime, Celldex has IP that covers composition of matter on FLT3L.

Venky Ramakrishna, associate director of immunology, said the company picked up FLT3L not necessarily as a brain cancer vaccine adjuvant but instead as a way to mobilize myeloid progenitor cells. DCs are one of several types of immune cells that arise from these progenitors.

"FLT3L has great potential to interact with stem cells or anything from the bone marrow where we would want to repopulate progenitor cells," he said. "This molecule is very versatile."

Celldex's rindopepimut (CDX-110), a vaccine targeting epidermal growth factor receptor variant III (EGFRvIII), is in Phase IIb testing for GBM and is partnered with **Pfizer Inc.**

Using FLT3L together with CDX-110 "would be a synergistic way to enhance dendritic cells in the blood," said Ramakrishna. "It's a great adjuvant for glioblastoma."

He noted, however, that the signs of an immune reaction seen by Lowenstein's team raise potential concerns. FLT3L's ability to stimulate immunity in the brain is "a double-edged sword," said Ramakrishna. "You would want to pay attention to the inflammatory situation and to suppress autoimmunity."

Indeed, it's not yet known if long-term expression of FLT3L from Lowenstein's gene therapy vector would cause chronic immune activity in the brain. To answer this, the Flt3l-treated mice would need to be monitored for signs of late-onset brain inflammation or autoimmunity.

Finally, the real question is how to deliver FLT3L (*see Box 1, "Macrophage express to the brain"*). Ramakrishna said the molecule "is usually given subcutaneously, but if you administer it that way it's unlikely to find its way into the brain."

Lowenstein thinks adenovirus-mediated transfection of cells at

tumor resection sites would lead to production of enough FLT3L to attract DCs to the tumor site.

Cedars-Sinai holds a patent covering the use of FLT3L as an adjuvant to brain cancer therapy. The IP is available for licensing.

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Contact: Pedro R. Lowenstein, Cedars-Sinai Medical Center, Los Angeles, Calif.
e-mail: lowensteinp@cshs.org
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Contact: Dave Morgan, University of South Florida, Tampa, Fla.
e-mail: scientist.dave@gmail.com

COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Cedars-Sinai Medical Center, Los Angeles, Calif.
Celldex Therapeutics Inc. (NASDAQ:CLDX), Needham, Mass.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
University of South Florida, Tampa, Fla.