OF FLIES, MICE & MEN

Could a signaling pathway discovered decades ago in fruit flies hold the key to preventing deadly bone marrow transplant complications?
More than a century ago now, a professor at a small college in Olivet, Michigan, discovered a hereditary mutation in his fruit flies that created small notches on the edges of their wings.

The observation was a clue that ultimately led scientists to an important signaling pathway that has been preserved across millions of years of evolution, carrying vital messages between cells in organisms ranging from millimeter-long nematodes to humans.

Today the Notch pathway is known to play many critical roles in health and disease — including in the differentiation of embryonic cells, immune cell development and response, and the promotion of certain cancers.

An international team of scientists led by the University of Michigan Life Sciences Institute has been compiling evidence that targeting Notch might prevent a deadly complication of bone marrow transplants known as graft-versus-host disease. Targeting Notch also shows promise for reducing organ transplant rejection and for helping multiple sclerosis patients by reducing the damage their immune systems cause to their central nervous systems.

“When a pathway has been preserved by biology for a long time like this and is used over and over by nature for different purposes, it’s usually a sign that its function is vital and profound,” says Ivan Maillard, M.D., Ph.D., a hematologist and researcher whose lab at the LSI is leading the work. “And so we take inspiration from this pioneering work in flies, and we explore biological questions in mouse models, with an ultimate goal of finding ways to target these pathways in human diseases.

“The work around Notch is an example of how basic science can point the way toward improving human health by trying to answer fundamental questions about important biological mechanisms, like how the immune system is regulated,” adds Maillard, an associate professor in the U-M Medical School Department of Internal Medicine’s Division of Hematology/Oncology and in the Department of Cell and Developmental Biology.

A FOUR-LETTER DISEASE

Patients with blood cancers like leukemia, lymphoma and myeloma are often treated with high doses of chemotherapy and radiation to kill the cancer. The treatment also destroys the patient’s bone marrow, where new red and white blood cells are made. This regimen is followed by the transplantation of healthy, blood-making stem cells and immune cells from a donor — creating a fresh pool of white blood cells to rove around the body and kill off lingering cancer cells.

Graft-versus-host disease — GVHD for short — occurs when the white blood cells from the transplant decide the recipient’s normal tissue is a foreign threat and attack it. The most severe, treatment-resistant forms of GVHD are almost always fatal.

Less severe cases can still lead to chronic skin, digestive and breathing problems.

“These are health concerns that these patients have to live with for their entire lives, even if they have been cured of their original cancer,” says Maillard, who also directs the leukemia program at the U-M Comprehensive Cancer Center.

Today, doctors can try to prevent GVHD by depleting specific immune cells, known as T cells, from the transplanted cells or by suppressing the patient’s immune system with powerful drugs. These efforts, however, make the transplant less effective against the cancer — and many patients still develop GVHD.

“What we have seen in our research in mice is that we can get very good, long-term protection against GVHD by inhibiting selected components of the Notch pathway for just a short period of time right after transplantation,” Maillard says. “This is very exciting because blocking all of the Notch signaling turns out to be quite toxic, and because Notch is such an important
pathway, shutting down even part of it for a long time could also cause unacceptable side effects.”

**LIGANDS AND T CELLS AND RECEPTORS, OH MY**

To understand the research developments, it’s helpful to know how the pathway works.

When a signal is transmitted, one cell acts as a sender and its neighbor as a receiver. A protein protruding from the surface of the sending cell, called a ligand, binds to a receptor protein sticking up out of the surface of the receiver — in this case a T cell. This sets off a molecular chain reaction that influences T cell activation and how the donor T cells will respond to the recipient's body.

In humans, the Notch pathway has five ligands and four receptors, which pair up depending on the context. Through a series of experiments, Maillard’s group found that just two of the ligands and two of the receptors were responsible for the effects seen in GVHD — with a dominant role played by just a single ligand-receptor pair.

Using antibodies developed by collaborators at Genentech, a biotechnology company based in San Francisco, the researchers were able to block the key individual Notch ligands in mice that received bone marrow transplants. Doing so prevented the mice from developing GVHD — without the severe gastrointestinal side effects that occur when Notch signaling is fully blocked throughout the body.

“Importantly, this limited blockade did not prevent the donor T cells from recognizing and destroying cancer cells, which is most often the ultimate goal of bone marrow transplantation in humans,” Maillard says. The findings appeared in the *Journal of Clinical Investigation*.

The discovery that these components of Notch signaling had to be blocked for only a short period of time after transplantation to confer long-term protection against GVHD was a welcome surprise for the researchers.

“GVHD is a sustained attack on a patient’s body by the new immune cells,” Maillard says. “If Notch turned out to be important in generating this response, we thought it might have to be inhibited indefinitely. But when we tested it, it turned out that there was a critical window of time in the days immediately following the transplant when the T cells get primed to have this damaging response.”

Work continues to explore precisely why this is the case, and what combination of factors and players are influencing...
the T cells’ response.

Collaborations with colleagues in the United States, Canada and Switzerland have been instrumental to the research, Maillard notes, providing specialized knowledge, reagents and mouse models that were not widely available. “Since the beginning of my career, I’ve strongly believed that building strong relationships is one of the best ways to move a scientific agenda forward,” he adds.

BEYOND GVHD

For the scientists, then, it was not a great leap to think that Notch signaling might also be important in other health problems caused by an aggressive immune response.

Maillard’s lab teamed up with D. Keith Bishop, Ph.D., a now-retired professor of surgery at the U-M Medical School, to look at Notch’s role in organ transplant rejection.

They found that, once again, blocking signals delivered by two of the Notch ligands shortly after transplantation significantly improved the survival of the transplanted organ. Their findings in a mouse model of heart transplantation were published in the Journal of Immunology, and Maillard and several colleagues recently authored a review in the American Journal of Transplantation on Notch as a potential target for preventing rejection.

“It makes sense,” says Maillard. “Transplant rejection is basically the reverse of GVHD — it’s essentially host-versus-graft, where the immune cells of the host attack the new organ.”

A similarly impressive improvement was seen in a model of multiple sclerosis, which was initiated by Ashley Sandy-Sloat, Ph.D., when she was a doctoral student in immunology working in Maillard’s lab.

“She had a family history of multiple sclerosis and was very interested in studying the disease,” Maillard says. “Before joining my lab, she had actually rotated in the lab of Benjamin Segal, the director of U-M’s Multiple Sclerosis Center. The idea turned into a great project and a great campus collaboration.”

NEXT STEPS

For many reasons, successes in the lab can be extremely challenging to translate into clinical interventions. “Even after animal studies suggest that a treatment will be safe and effective, more than 80 percent of potential therapeutics fail when tested in people,” a Nature commentary noted in 2014. But Maillard says two factors leave him optimistic about the Notch results: the rigor with which the experiments were designed and conducted, and the profound nature of the results.

“In the immune responses that we are studying, the effects are really black and white,” he says. “We are not looking at subtle little effects. We’re looking at mice that either live or die.

“It’s really striking,” he continues. “In graft-versus-host disease, it’s either death from bad GVHD or long-term survival with no GVHD. In transplant rejection, it’s very rapid rejection versus markedly delayed rejection. In the multiple sclerosis model, it’s either bad, life-threatening brain damage or near-complete protection.”

Maillard has started exploring industry partnerships to move the research toward the clinic, and meanwhile his lab continues to do additional research and validation in animal models.

“The bottom line is that we are diving deep into the mechanisms because we are discovering important new ways in which the immune system is regulated — so there is basic immunobiological significance to the research — and also because the more we understand about how all the different pieces work, the better we will be at designing a potential clinical intervention,” he says. ☝️

Notch signaling in T cells plays a critical role in the development of graft-versus-host disease and other disorders — making it a potential therapeutic target.