



Joined at the Bench

By [Jason Socrates Bardi](#)

Last summer, when Professor Hugh Rosen came to The Scripps Research Institute (TSRI) from Merck & Co., where he was the executive director of Immunology and Rheumatology, he found himself looking over rows of empty shelves and cabinets in his laboratory.

Coming from industry, he says, he did not have chemicals and reagents to bring with him. He had no cell lines and no major laboratory equipment. Most importantly, he had no students or postdocs with whom to work. His lab was something of a blank slate—he didn't even have his own office table or chair (fortunately, he was able to borrow these).

"It was an interesting moment," he reflects.

At that time, he was writing grants full-time to get his research up and running. To clear his head, he would take frequent breaks. It was during one of these breaks that he struck up a conversation with his colleague Associate Professor Michael McHeyzer-Williams, who was also a then-new recruit to TSRI's Department of Immunology. Rosen and McHeyzer-Williams share one contiguous laboratory space in a TSRI building that houses the Institute for Childhood and Neglected Diseases.

McHeyzer-Williams was also setting up his laboratory, having arrived near the end of 2001 with a core group of two postdocs and one technician. They were just finishing the calibration of his new, dedicated flow cytometer machine, getting it running at peak performance.

After several conversations, McHeyzer-Williams recalls, he suggested to Rosen that they do an experiment together. They designed a "pilot" experiment looking at the late stages of T cell selection in the thymus that was intended to combine Rosen's expertise in chemical biology with McHeyzer-Williams's expertise in flow cytometry.

But after they completed their pilot experiment and analyzed the results, they saw that they had something completely new.

Out of the Thymus

The thymus is the organ that supplies the body with helper and killer T



Professor Hugh Rosen's laboratory is now focused on the study of a mechanism relevant to autoimmune conditions. Photo by Kevin Fung.



Associate Professor Michael McHeyzer-Williams notes that, because of their collaboration, both he and Rosen "have started a whole new direction of research that we wouldn't

immune cells, crucial mediators of the adaptive immune response. T cells circulate through the blood to secondary lymphoid organs—lymph nodes, Peyer's patch, the spleen. If they encounter antigen, usually viruses or bacteria, in the secondary lymphoid organ, they begin to proliferate and to undergo clonal expansion, producing various effector cells. These effector cells remove what they recognize as non-self.

have had." Photo by Michael Balderas.

A single T cell, one of two key players in the adaptive immune response, can proliferate into a million cells in a matter of days once it has been activated, helping to clear the body of invading bacterial or viral threats.

Crucial to this effort is the maintenance of the T cells in the periphery by the continual development and release of mature T cells from the thymus.

The thymus is constantly generating new T cells, but each day only a small percentage survive. Well over 95 percent of the T cells that are made in the thymus are destroyed there. The small number of survivor T cells are selected to replenish the peripheral T cell pool.

The maturation of T cells in the thymus occurs through a highly sophisticated mechanism whereby the thymus sorts out those cells that are potentially useful in the periphery from those that are not. This is achieved by screening the cells for their binding affinity for major histocompatibility complex (MHC) molecules, the receptors that are present on antigen-presenting cells recognized by the T cells' own receptors.

For mature T cells in the bloodstream, antigen-presenting cells display pieces of pathogenic invaders (antigens) in their MHC receptors, and this leads to the activation of T cells that have the right receptor—one that binds antigen-loaded MHC tightly. In the thymus, MHC molecules also play a crucial role, and they must be recognized by the T cells. This positive selection takes place in one part of the thymus, called the cortex,, and the organ selects out all the T cells that are unable to recognize MHC molecules.

Of the remaining cells, those that have been positively selected for their ability to recognize self antigen, a further selection takes place. Those that have been positively selected in the cortex pass into the "medulla" portion of the thymus where a negative selection awaits them.

While much is known about the positive thymic selection, less is known of the mechanisms regulating the negative selection that occurs in the medulla as the T cells undergo their final maturation before they are released into the blood.

In the medulla, T cells that are highly reactive are selected to die. This negative selection is an important complement to the positive selection. If these cells were allowed to get out of the thymus, some would attack our

own tissue. We would suffer from autoimmunity and might even reject our own organs.

Now the paper by Rosen, McHeyzer-Williams, and their TSRI colleagues Associate Professor Charles Surh and Research Associate Christopher Alfonso may shed some light on this mechanism.

In their paper, they describe a new mechanism in which the thymus may sense peripheral inflammation and modulate the T cell response.

The S1P Receptors

The TSRI team focused on a family of receptors for a fatty lipid molecule called sphingosine 1-phosphate (S1P) that is produced by platelets—those flat, circulating, molecule-filled protoplasmic disks in the blood that are necessary for clotting—and by a variety of tissue cells.

Sphingosine 1-phosphate acts on a family of receptors called the S1P receptors or the "edg receptors," originally defined as endothelial differentiation genes. S1P is produced by endothelial cells and other cells at sites in the body where there is inflammation—and where there are inflammatory cytokines like tumor necrosis factor-alpha, for instance. S1P lipids activate the S1P receptors and regulate a range of physiological functions that include cardiovascular function and blood pressure.

Rosen and his colleagues previously showed that S1P receptors can also control the recirculation of lymphocytes, a mechanism which was never understood before. Furthermore, they found that either S1P lipids or synthetic chemical agonists of the S1P receptors are able to alter the trafficking of lymphocytes in a reversible way. These synthetic chemicals, they found, are very potent and bind to S1P receptors in the low nanomolar and sub-nanomolar range.

They have now shown that when S1P agonists interact with thymus, they cause the T cells to lose a receptor on their surface called CD69, promoting their maturation in the medulla. In addition, a biological switch is activated that shuts off emigration of mature T cells from thymus, preventing T cells from reaching the periphery.

These small molecules also interrupt antigen responses by misdirecting peripheral T cells to the wrong lymph nodes by interrupting recirculation. Regulating the release of new, mature T cells from the thymus while inhibiting the expansion of the T cells that are already in the periphery synergistically combine to create a strong immunosuppressive effect.

This immunosuppressive effect could potentially be used to prevent the rejection of organ transplants or the effects of autoimmune disease.

"What we have," says Rosen, "is a biological toggle switch—an on-off switch—that is regulated as you activate these receptors. You essentially shut off a switch and, as you activate these receptors, the lymphocytes disappear in a reversible way from peripheral blood and you can protect an animal or a person from transplant rejection and from autoimmune-mediated tissue damage."

Now, Rosen adds, his laboratory is focused on the study of this mechanism under normal and autoimmune conditions.

"We're very excited about [the results]," says McHeyzer-Williams. "Both of us have started a whole new direction of research that we wouldn't have had. We have uncovered a new phase in late thymic development using these powerful chemical tools focused on complex biological processes. Our laboratory has a wealth of experience in the analysis of T cells, their antigen receptors and cell fate in vivo. We can now directly interrogate the process of negative selection with increased cellular and molecular resolution to provide insight into this fundamental process."

To read the article, "Rapid induction of medullary thymocyte phenotypic maturation and egress inhibition by nanomolar sphingosine 1-phosphate receptor agonist" by Hugh Rosen, Christopher Alfonso, Charles D. Surh, and Michael G. McHeyzer-Williams, which was published online by the *Proceedings of the National Academy of Sciences* on September 3, 2003, please see: <http://www.pnas.org/cgi/content/abstract/1832725100v1>.

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