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Basic research in immunology and molecular neurobiology is revealing why the immune system goes into attack mode in MS patients.

By **LOIS WINGERSON**
Illustration By **JILLIAN TAMAKI**

In 1860, Margaret Gatty, a popular children’s author and nature writer, finally saw a doctor in London about the weakness and trembling in her right hand, which had been getting worse for 12 years. When he could find nothing wrong with her, she sought out Dr. Thomas King Chambers, who later described her case in *The Lancet*. Gatty’s muscle fibers were degenerating from overuse, he theorized, because she spent her leisure time “furiously digging in her garden with a masculine spade” and mowing her lawn with a scythe.

Gatty visited numerous other doctors as her disabilities increased. They disagreed, and ridiculed one another’s opinions. “Still,” she wrote in her diary, “one must believe that the Drs. know something.” They didn’t. She continued to deteriorate, eventually dying of a respiratory infection.

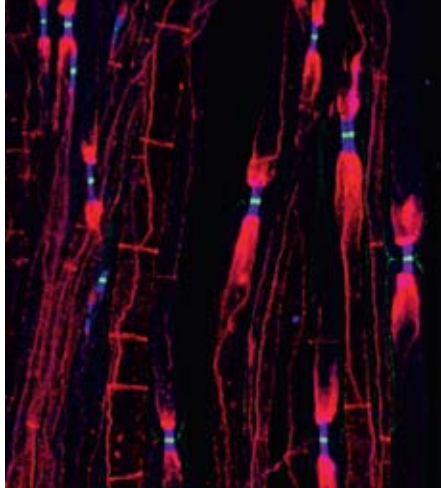
Six years after her first doctor's visit, the legendary French neurologist Jean-Martin Charcot gave the first definitive description of what probably affected Gatty. He described patients who had progressive movement and vision disorders, and plaques disseminated throughout their nervous systems at autopsy. He called the disorder *sclerose en plaque disseminée* (disseminated sclerotic plaques).

Thanks in part to Charcot's groundbreaking work, multiple sclerosis soon gained worldwide recognition as a distinct disease. But for more than a century afterward, even as researchers worked to unravel its biology, MS patients had little to rely on other than the hope that "the Drs. know something."

Fortunately, that has changed in recent decades. Doctors now know, for instance, that if Margaret Gatty did indeed have MS, it was her immune system's T lymphocytes, or T-cells, (not gardening) that damaged the nerves in her brain and spinal cord by chewing away at their sheath of myelin, the fatty substance that coats and insulates the body's nerve fibers, much like the insulation around a lamp cord. This loss of myelin ruins the ability of nerves to send impulses, resulting in a wide range of debilitating symptoms.

Scientists have also developed a handful of medications that slow the progress of MS somewhat. Still, these medications are far from a cure. "The available therapies are not satisfactory. Every physician will tell you that," observes Juan J. Lafaille, Ph.D., associate professor of pathology and medicine. "There's big room for immunologists to come up with something."

One key question is what sets off this immune system attack, and how to prevent it. But that isn't the whole story: MS also involves the failure of two protective factors—cells that should restrain the immune system (but don't), and another group of cells that could replace myelin



▲ Myelinated nerve fibers stained in different colors. Information flow along myelinated nerves depends on molecular pores, called sodium channels (green), that are concentrated at specific sites along the fiber. When myelin is lost in MS, this site-specific localization is also lost.

and rebuild nerve fibers (but don't).

These failures are the mysteries that captivate Dr. Lafaille and neurologist James L. Salzer, M.D., Ph.D., professor of cell biology and neurology, who together founded the Collaborative MS Research Center at NYU with support from the National Multiple Sclerosis Society. Their recent discoveries about the interactions of nerve cells and immune cells could lead to a whole new group of MS therapies.

▼ **A CLEAN SLATE** For Dr. Lafaille, the quest to understand how the immune system misfires began with a Ph.D. in Brazil, and continued at the Massachusetts Institute of Technology in the laboratory of geneticist Susumu Tonegawa, who won the Nobel Prize for his research on the immune system. Dr. Tonegawa was also one of the first to study the immune and nervous systems using the knockout mouse—a term referring not to pugilistic rodents, but rather mice that have had individual genes

eliminated through genetic engineering. This technique lets researchers define a gene's normal function by observing what happens when that gene is missing.

In Dr. Tonegawa's lab, Dr. Lafaille started by asking why we don't *all* develop multiple sclerosis. He focused on the immune system's T-cells that recognize and attack the molecule myelin basic protein (MBP), known to be a target of immune attack in MS. All normal animals have some T-cells that recognize MBP, but, it was hypothesized, not enough of them to damage myelin. To his surprise, however, when Dr. Lafaille bred mice supercharged with T-cells that can attack myelin, the mice remained completely normal.

Puzzled, Dr. Lafaille decided to clean the slate by creating mice that had only T-cells primed to attack myelin, and no other T-cells at all. These knockout mice invariably developed a condition similar to MS, known as experimental autoimmune encephalitis (EAE). Clearly, they lacked something that had protected the first set of mice from developing autoimmune nerve damage, despite their abundance of anti-myelin T cells.

Dr. Lafaille called these protective factors, which work to suppress the body's immune response, regulatory T-cells. Today, hundreds of laboratories are studying the effects of different types of regulatory T-cells on allergies, autoimmune diseases, inflammation, and other disorders. Dr. Lafaille's team is focusing on their interactions with other immune cells and with nerve cells, using the new live imaging technique intravital microscopy (see sidebar) to watch what happens when mice are injected with the regulatory T-cell that blocks demyelination of nerve fibers.

"So far, we've found that injecting regulatory T-cells prevents EAE in mice—and, even more important, ameliorates its symptoms in mice who already have EAE," says Dr. Lafaille. "We can't say that yet

REVOLUTION IN MICROSCOPY

● FOR DRS. JUAN Lafaille and James Salzer, the prospect of actually seeing the cellular actors in the very complicated drama of MS is now a reality, thanks to intravital microscopy, a breakthrough technology advanced at NYU by two Skirball Institute investigators, Wen-Biao Gan, Ph.D., associate professor of physiology and neuroscience and

Michael Dustin, Ph.D., the Irene Diamond Professor of Immunology and professor of pathology.

Until recently, even the most sophisticated neural imaging has been able to capture only single moments in time. This extraordinary new approach lets doctors see live, moving pictures of cellular events inside the nervous system. The difference is

profound. "Journals publish countless still images of interactions between immune cells and nerve cells, made using tissue slices and microscopes," explains Dr. Dustin. "It's difficult to figure out from these 'snapshots' just what's happening over time." The action is much clearer in the movies he is making with intravital microscopy. "There have been lots of ideas about the dynamics

of those interactions between lymphocytes and glial cells," adds Dr. Dustin. "Now we may be able to deduce or even examine what's happening."

Working together, teams led by Dr. Dustin and Dr. Gan have been perfecting this technology, which involves tagging neurons with a green fluorescent protein, then using a sophisticated optical technique called two-photon

microscopy to safely penetrate biological tissue and capture these cells in action.

Researchers are currently imaging cellular events both in tissue culture and in the brains and spinal cords of live, anesthetized mice, by peeking through a surgically created, ultrathin window about 0.2 millimeters in diameter (roughly the width of a human hair) in the mouse's skull. The method is safe enough to

continue studying the same mouse for up to two years (its normal lifespan), notes Dr. Gan.

He has begun imaging nerve cells as they interact with microglia, the first cells to react to a brain infection. Dr. Dustin is imaging immune cells in the spinal cords of mice with EAE. Drs. Lafaille and Salzer will be watching the footage to see if it sheds new light on what they have already learned. ●



Neurologist James L. Salzer, M.D., Ph.D., (left) and immunologist Juan J. Lafaille, Ph.D.

about MS, because it hasn't been tested yet in patients. We hope someone will be inspired to try it." In the long term, adds Dr. Lafaille, their research could lead to immunosuppressive medications that target only the part of the immune system that affects myelin, a key step toward curing MS.

▼ **WRONG SIGNALS** Dr. Lafaille's colleague, Dr. Salzer, is studying another part of the demyelination puzzle—why it is that nerves damaged by MS fail to grow new myelin coats. The *plaques disseminées* that Dr. Charcot observed are not merely evidence of a destructive process. They are scars from the body's repeated, futile attempts to repair the damage from the disease's attacks on myelin.

"In MS, the damaged nerves don't remyelinate completely," says Dr. Salzer, "and some never remyelinate at all." Precursors to glia, the cells that normally nourish nerve fibers and wrap them in myelin, have been seen lining the edge of MS plaques, but they don't make the repair.

"The cells exist in the brain, but they're not effective," says Dr. Salzer. Understanding them, and ideally finding ways to make them effective, is his next agenda.

As a graduate student, Dr. Salzer developed a method of growing nerve cells and glia in tissue culture. He learned that nerve cells emit a signal that stimulates glia to begin the myelin-wrapping process. Rather than

focus solely on discovering the source of this signal, however, he decided to learn firsthand what happens when this process fails by becoming a clinical neurologist. During his residency at New York Hospital and later as an attending neurologist at Bellevue Medical Center, Dr. Salzer treated many patients with MS, while also continuing his research.

"We knew almost nothing about the signals that drove glial cells to make myelin, or how myelin itself formed," he recalls. "I thought that understanding those questions would help in developing therapies for myelin repair."

In 2005, Dr. Salzer finally identified the molecule that signals glia in the peripheral nervous system (known as Schwann cells) to begin the myelination process. It turned out to be a subtype of a molecular signal prominent in the nervous system, called neuregulin. Like Dr. Lafaille, Dr. Salzer made his discovery with the help of knockout mice—engineered, in this case, to eliminate certain subtypes of neuregulin. Working with cell cultures, he and his team found that while Schwann cells normally myelinate certain nerve cells, they fail to myelinate nerve fibers taken from mice lacking a specific subtype of neuregulin 1.

Next, Dr. Salzer's team found that when they turned on production of neuregulin 1 in nerve cells, using a genetically modified virus to drive the process, they not only induced Schwann cells to initiate myelination around nerves taken from their

knockout mice, but even stimulated myelin growth around nerves that normally are *never* myelinated.

The discovery was significant. Whether it will lead to a treatment for MS remains to be seen. Schwann cells don't exist naturally in the brain and spinal cord. Although neuregulin 1 does have some effect on oligodendrocytes—the glia that myelinate nerve cells in the brain and spinal cord—they require other signals to carry out the process. When myelin is damaged in the brain, it's as if oligodendrocytes are waiting for an order to do their job, but never get the paperwork. Scientists have yet to discover the molecular identity of that work order.

"When an oligodendrocyte meets up with an axon during normal development, it's almost preprogrammed to make a myelin sheath," says Dr. Salzer. "However, in MS that's not the case. There are a surprising number of oligodendrocyte precursors scattered around the brain. Why aren't these cells able to migrate in and remyelinate nerve fibers damaged by MS?"

Dr. Salzer and his team are currently focused on identifying other molecules that may potentially be involved in triggering—or inhibiting—myelin formation in the brain. "To learn why remyelination doesn't happen well in MS, we're first trying to understand how remyelination occurs in a normal brain," he explains.

If Dr. Salzer can answer this riddle, "the Drs." will know a great deal more. ●