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INSIDE:

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What we learn in the lab

by Sara Bernstein

Multiple sclerosis presents scientists with great mysteries: What causes the immune system to attack the brain and spinal cord? What early, crucial signs are we missing that could predict disease course? What can we do to stop the progression of disease?

If we could take a better look inside people with MS, we might find the answers, but technology does not yet allow it. The human body is a remarkable collection of intricate systems that cannot be artificially replicated. Computer modeling and tissue culture studies cannot yet replace research in living organisms.

One tool scientists use to augment what can be studied in people who have MS is the study of aspects of the disease in animal models—most often rodents. This responsible animal research has led to the approval of treatments for MS, has offered new possibilities for repairing damage to the central nervous system, and promises to bring us novel MS treatment strategies in the future.

The original MS model

The first evidence of immune cells attacking the brain was discovered in 1933 by Thomas Rivers, MD, and colleagues

(Rockefeller University, New York). They were investigating why some people have neurological reactions to vaccinations by injecting monkeys with samples of brain tissue from rabbits. The result was an MS-like disease, which they labeled "experimental allergic encephalomyelitis." (**Journal of Experimental Medicine** 1933;58:39–56) Now also known as experimental **autoimmune** encephalomyelitis, EAE is the predominant model of MS-like disease.

What have we learned from EAE? Well, let's start with this—all six therapies approved to treat MS were first tested in this model. For example, mitoxantrone (Novantrone, EMD Serono) was administered to rats with EAE because of growing interest in using cancer treatments for MS. When paralysis was reversed and tissue damage reduced in these models, investigators began the clinical trials in people that led to this drug's approval for worsening MS. (**Clinical Immunology and Immunopathology** 1985;35:35–42)

Experimental drugs now in the pipeline for MS have shown their mettle in EAE. In a study funded by the Society, the oral cholesterol-lowering drug atorvastatin (Lipitor, Pfizer) prevented or reversed EAE in mice. (**Nature** 2002;420:78–84) Atorvastatin is now being tested in several clinical trials in people with MS and those at high risk for developing definite MS.

Studying MS models such as EAE has also helped to point out failures. The growth factor IGF-1 had shown some success in promoting myelin formation, so a Society-funded team led by Stéphane Genoud, PhD (The Salk Institute, La Jolla, Calif.), injected it into mice with EAE. The injections actually worsened the disease. (**Journal of Neuroimmunology** 2005;168:40–5) Such failures are important to pinpoint before they affect people with MS in clinical trials.

Putting us on the right track

Studies in models such as EAE directed our understanding of MS in humans. In 1986, Dr. René Lebar and colleagues (Hôpital de la Salpêtriére, Paris, France) looked at guinea pigs with EAE and described a protein later called myelin oligodendrocyte glycoprotein, or MOG. Although only a small component of the myelin insulation of nerve fibers, the team found it to be a key target of the immune response that damages myelin, spurring on much further research on MOG in EAE and MS. (**Clinical and Experimental Immunology** 1986;66:423–34)

Researchers have turned to other models as well, such as an MS-like autoimmune disease induced by Theiler's murine encephalomyelitis virus (TMEV). Long-time Society grantee Stephen D. Miller, PhD, and colleagues (Northwestern University, Chicago) have used this model to investigate how a virus might trigger the MS immune attack, possibly through "molecular mimicry."

In this scenario, because of molecular similarities between myelin and proteins on the viruses or bactera, immune cells generated against the invader also launch an attack against myelin. By altering TMEV to contain bacterial proteins resembling myelin, this team showed how T cells responding to the virus would initiate, or exacerbate, MS-like disease. (**Journal of Virology** 2005;79:8581–90)

As the tools for scientific research have advanced, the directions provided by disease models have become even more fine-tuned. One example is "knockout" models, made when investigators insert artificial DNA that instructs cells to produce too little of a certain protein, to determine the exact role of that protein in disease.

Knockout studies are now a staple of MS research. Recently, Dennis Bourdette, MD (Oregon Health and Science University), and his team induced EAE in mice in which they had knocked out the gene for cyclophilin D—a key protein in generating energy in cells. The mice got the disease, but a striking number of nerve fibers were protected from damage compared with mice that had the normal gene for cyclophilin D, despite similar levels of inflammation. (**Proceedings of**

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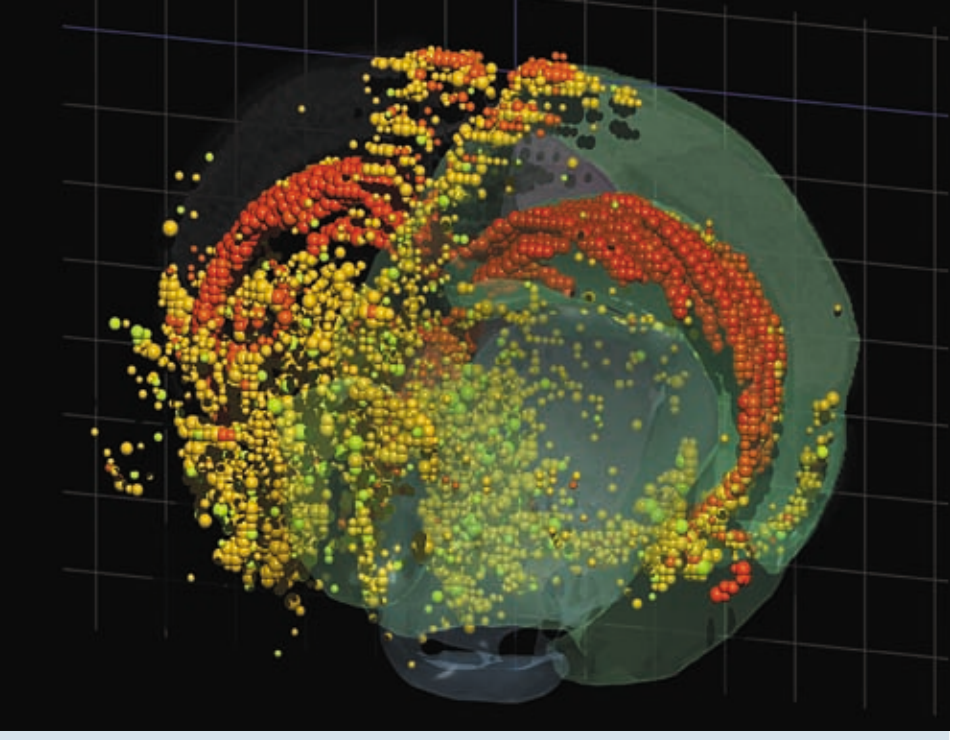

A NEW LOOK AT AN OLD MODEL: This is a picture of the mouse brain showing where one gene (*Man1a*) is expressed, or turned on. The large red arc indicates that this gene is turned on strongly in the hippocampus, a part of the brain known to be involved in learning and memory.

This image was generated from the Allen Brain Atlas—a map of all genes active in the mouse brain—completed by Ralph Puchalski, PhD, and colleagues. With funding from a National MS Society pilot research award—and a consortium of public and private entities—the Allen Institute for Brain Science is now creating a similarly comprehensive map of genes that are active in the mouse spinal cord. This atlas will map an estimated 20,000 genes in the spinal cord and present the information in a publicly-accessible, Web-based application. These atlases provide key baseline information on normal brain and spinal cord function, and will help investigators compare biological processes involved in disease models that mimic many aspects of MS.

the National Academy of Sciences USA 2007;104:7558–63) Dr. Bourdette is now funded by a National MS Society Collaborative MS Research Center Award to explore what effects dysfunction in cellular energy production might have on MS.

DNA "microarrays" are another advance being applied to MS-like disease models; these are created by robotic machines that arrange thousands of gene sequences on a single microscope slide. Larry Steinman, MD, and colleagues (Stanford University, CA) conducted a microarray study of lipids—fatlike molecules—in mice with EAE. The study identified sulfatide as a possible target of the immune attack. The team followed up this study by immunizing mice with sulfatide and inducing EAE, resulting in more severe disease. (**Nature Medicine** 2006;12:138–43)

Dr. Steinman also has received a Center Award to continue these microarray studies. He is testing the resulting targets in blood samples from people with MS enrolled in clinical trials of experimental therapies, to determine the effects of these treatments on targets identified by microarrays.

Here come the zebrafish

Scientific advances come in all shapes and sizes—even zebrafish. This small fish shares many of the same genes as humans, reproduces rapidly, and its genes are easily manipulated. It is also transparent, making it easy to observe biological processes such as myelin growth. MS researchers are using this model to delve deeper into the mysteries of MS.

Bruce Appel, PhD (Vanderbilt University, Nashville), is using zebrafish models to investigate the genes and signals that lead immature myelin-making cells (or oligodendrocytes) to mature and wrap myelin around nerve fibers. His team has found that signals from a molecule called "Notch" are continuously required during the development of a zebrafish embryo to permit myelin-making cell development. (**Developmental Dynamics** 2008;237:2081–9) Dr. Appel has identified six genes in zebrafish that may regulate the migration and wrapping abilities of oligodendrocytes. He is now studying them further with a Society research grant.

William S. Talbot, PhD (Stanford University), and colleagues

have developed methods of studying the nodes of Ranvier in zebrafish. These unmyelinated gaps on nerve fibers allow for rapid transmission of nerve impulses. In MS, the nodes of Ranvier are disrupted, causing neurological symptoms. Dr. Talbot's group did a massive screening of proteins in zebrafish, and identified "nsf" as a protein that might be important in node formation.

The team then knocked out nsf in zebrafish, and showed that this protein is indeed required for the formation of the nodes of Ranvier and, ultimately, the myelination of nerve fibers. This study was funded by the Society. (**Current Biology** 2006;16:636–48) Identifying such genes and signals is crucial to developing repair strategies in people with MS.

Modeling MS progression

The puzzle of why MS progresses and how to stop it is a tough one for researchers and they are devising models specifically to mimic these later stages of MS.

One of the international teams funded by the National MS Society's Promise: 2010 initiative—led by Gavin Giovannoni, PhD (Queen Mary University of London)—recently published exciting findings relating to this effort. Previous studies have indicated that inducing EAE in the "Biozzi" strain of mice causes clinical symptoms that mimic secondary-progressive MS, in which the disease starts with a relapsing course and then worsens with or without occasional flare-ups.

Dr. Giovannoni's team studied the underlying damage to tissues in these models and found that the pathology simulated secondary-progressive MS as well. There was widespread myelin damage, proliferation of certain types of brain cells, nerve cell loss, and nerve fiber loss. The team concludes that this mouse represents an excellent model of secondary-progressive MS and could be used to test therapies aimed at stopping or reversing MS progression. (**Journal of Neuroimmunology**, July 29, 2008, Epub ahead of print)

Laboratory studies of MS models are the backbone of MS research, providing scientists with views of the development of MS-like disease and strategies for stopping it. As technology advances, these views are moving us closer to a world free of MS.

The National MS Society requires a humane approach to all necessary animal research related to MS. Every grant request to the Society must detail any procedures involving animals, and the Society monitors compliance with federal, state and local regulations related to humane treatment of lab animals. The Nuremberg Code, as well as U.S. FDA regulations, prohibit testing drugs on humans until they've first been tested and shown to be safe in animal models. This testing is essential to efforts to develop new treatments for MS.

Investing in the future

We don't know when the cure for MS will come, but we know we need a continuous stream of highly trained scientists and physicians to search for it and to make sure it gets to people with MS. The National MS Society funds nine different fellowship programs that allow young men and women to train with seasoned MS scientists and physicians in laboratories and MS clinics, and ease their transitions into independent careers. Often these are the hands doing the experiments and providing the first line of care for patients.

Committing to MS research

From its humble beginnings—funding six fellows for a total of $13,000 in 1955—the research fellowship program is now funding 76 fellows with about $50,550 each per year. These relatively small awards pay off well: Prominent researchers making MS breakthroughs today began their careers as Society trainees, and the Society's investment in fellowship awards has leveraged at least $400 million over the years in MS grant funding from all sources.

Do Society-funded fellows **stay** in MS research? A new survey of former Society research fellows revealed that 56% of survey respondents are still involved in basic and/or clinical MS research. Of those, a majority have written

ANTHONY D. POMICTER

Former fellow Jeffrey Dupree, PhD, explores myelin damage on nerve fibers using a transmission electron microscope.

more than 10 research articles on MS and 78% have themselves trained at least one fellow in MS research or clinical care.

"The fellowship was critical in allowing me to pursue my interest in myelin repair," said Victor Friedrich, PhD, associate professor at Mount Sinai School of Medicine in New York, and a Society fellow from 1976–1980. "Had that funding not been available I would undoubtedly have done research in another area. Instead, I pursued research on myelin formation and repair during the following two decades."

Although the majority of the former fellows have remained in academia, some find other roads to continuing their commitment to MS. For example, some go on to establish or work for companies involved in MS drug development.

"This was an important phase in my career," said David Wraith, PhD, chief scientific officer and founder of Apitope Technology (Bristol) Ltd., referring to his Society fellowship from 1986–1989. "My company recently completed its first clinical trial in people with MS, and this new therapeutic vaccine is the culmination of work that started when I was a Society fellow." Dr. Wraith is referring to Apitope's experimental ATX-MS-1467, in which synthetic peptides are used to alter abnormal immune responses such as those that occur in MS.

DAVID SUPER—THOMAS JEFFERSON UNIVERSITY

Annette Okai, MD (l), former clinical fellow, learned how to manage MS at Thomas Jefferson University.

Meina Tang, PhD, is director of Pharmacokinetics at PDL Biopharma, Inc., a company that is developing the monoclonal antibody daclizumab along with Biogen Idec. She was a fellow from 1996–2000. "We enrolled our first patient in a daclizumab monotherapy study in February of this year! I am very proud to be able to continue my contribution toward fighting MS," she said.

Nearly half of the respondents have also volunteered for the Society in some capacity, from participating in Walk MS to serving as a peer reviewer of research applications. "Meeting people with MS puts the research in a new light," said Jeffrey Dupree, PhD, assistant professor at Virginia Commonwealth University. Dr. Dupree was a Society fellow from 1996–2000, and has spoken at events for his chapter. "Also, getting involved with local researchers provides hope for people who have the disease."

Fostering MS care

Similar commitment is shown by physicians who complete the MS Clinical Care Physician Fellowships Program, which since 2003 has trained 25 neurologists or physiatrists in specialized MS care. The John Dystel Fellowship in MS has trained 21 nurses in MS care.

"My clinical fellowship allowed me to spend extensive time caring for people with MS," said John Rinker, MD, assistant professor of Neurology at the University of Alabama. "I feel

very comfortable now in diagnosing and treating MS."

"I now approach the management of the person with MS from various angles—both therapeutically and symptomatically," said Annette Okai, MD, who trained at the Comprehensive MS Clinic at Thomas Jefferson University Hospital. "I also became comfortable with managing advanced stages of MS."

Coming together

In early November, the Society is holding the first-ever Tykeson Fellows Conference on MS in Chicago, intersecting with the Society's National Conference. It is funded through a generous contribution from Donald E. Tykeson, an active volunteer and member of the Society's National Board of Directors. Several distinguished scientists are contributing their expertise as keynote speakers, offering advice and support to the next generation of scientists focused on MS.

The importance of fellowships to the goals of the National MS Society cannot be overestimated. "The Society has been a leader in training future generations of scientists," said William Karpus, PhD, Marie A. Fleming Professor of Pathology at Northwestern University and a Society fellow from 1991–1995. "It has done a great job at developing innovative career development tracks. Keep the bar high on these mechanisms and you will continue to develop the very best scientists and clinicians."

Stepping up to the plate for MS research

by John R. Richert, MD

The approval of a new round of research grants is an exciting time: Will one of these grants hold the key to unraveling the mysteries of MS? But for each one of the men and women who applies to us for funding, it's a tense and uncertain time. If a grant proposal is rejected—or if it is approved and cannot be funded—it could signal the end of a line of research, or worse, a career.

Building laboratory research

When we look at the budget section of any grant, it's easy to see what is at stake for the investigator who is applying. From Petri dishes to laboratory technicians, research grants are the key to a scientist establishing and maintaining his or her own laboratory.

People — A grant usually pays at least a portion of the salaries of all personnel involved in the research project, from technicians and young postdoctoral fellows to research faculty members. Fellows, for example, are paid between $40,000 and $51,000, depending on years of experience. The grant also may cover the cost of health insurance and retirement plans. If a grant is not funded, many of these people are out of a job.

Equipment/supplies — As technology advances, so does its price. The process of analyzing thousands of proteins at once with a mass spectrometer is thrilling, but the equipment can cost as much as $750,000. Maintaining a colony of mice for biomedical research—which includes feeding, housing and cleaning—can cost as much as $50,000. MS genetics research is speeding along—but not for free! Genotyping, the process of examining the genetic make-up of a person by studying DNA samples taken from the individual's blood or cheek cells, can cost up to $10 million for a major study such as the ones that are necessary to nail down the MS genes.

Patient costs — We strive to bring MS drugs from the laboratory to the clinic, where clinical trials incur some of the biggest bills in MS research—often in the millions of dollars. Patient costs include blood tests, x-rays, and other imaging scans (one MRI can cost on the order of $1,000), and trials usually require more salaries for staff to coordinate the study.

Other costs — Research grants also cover what are called "indirect costs." These are costs that are not just for that specific project, but are incurred by the researcher's institution. Indirect costs may include telephone, heat, light, and air conditioning,

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Research funding ensures that researchers can get the people and the equipment they need to keep the science going. Here Cheryl Bergman, lab manager for Society grantee Nancy Ruddle, PhD (Yale University), prepares a reagent to be used in an experiment designed to study the structure and function of myelin.

or secretarial salaries. The Society pays an additional 10% on top of a grant's direct costs to help cover indirect costs—but indirects actually cost the institution much more. NIH grants, for example, generally pay about 60% above the cost of the actual grant for indirect costs.

Our research grant programs range from $44,000—for a pilot project that tests a new innovative idea—to $5.6 million, to fund four international teams of top scientists working on therapeutic strategies to repair nerve damage in MS through the **Promise: 2010** initiative. If we don't provide these funds, equipment can't be purchased, animal models can't be tested, patients can't be recruited for clinical trials, and salaries can't be paid. A researcher might have to abandon his or her ideas, change fields so that he/she can study other disease areas where funding is available, or even look for a livelihood outside of the laboratory.

Now more than ever

The role of the National MS Society in funding MS research is more crucial than ever. Since 2003, the National Institutes of Health budget has not kept up with inflation and has lost ground. According to the NIH, investment in MS-related research in FY 2006 was $110 million. In FY 2007 and 2008, that figure dropped to $98 million. They expect the investment in MS to drop by another $1 million in 2009.

The effects of these funding decreases are disastrous. "In competition for limited resources, scientists at every point along the academic research pipeline are feeling the destructive effects," according to a statement from concerned universities and research institutions published in March 2008, and entitled "A Broken Pipeline?" "The system is backlogged with proposals and too few are being funded—impeding scientific progress."

This is the real tragedy of unfunded research. When the money is tight, researchers become more conservative, taking fewer risks on applications that might be rejected because of bold and possibly expensive visions.

Now more than ever, we need to maintain our stance as a driving force of MS research, relentlessly pursuing prevention, treatment and cure. We need to step up to the plate and fund the novel, innovative ideas that just might be the key to MS, as well as the international collaborations that are so necessary to speed research along. So many people depend on us to be this force—the men, women and children who have MS—and the researchers themselves, the men and women who are leading the way to the cure.

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Dr. John Richert is executive vice president for the Society's Research & Clinical Programs.

In the news and on our Web site

Read more news and details of these stories on our Web site: **nationalMSsociety.org/bulletins**.

● **Tysabri: Two new cases of PML—and a label change**
Two new cases of PML were reported in individuals who were taking Tysabri (natalizumab, Biogen Idec and Elan Pharmaceuticals) as a monotherapy. PML (progressive multifocal leukoencephalopathy) is a severe viral infection of the brain. This highlights the need for people taking the drug to be sensitive to any new or worsening symptoms and to contact their prescribing physician immediately if they experience any changes in thinking, eyesight, balance, strength and other functions.

The FDA recently approved new labels for Tysabri that not only update warnings, but also clarify indications, suggesting that the drug is generally recommended for patients who have had an inadequate response to, or inability to tolerate, a single alternate MS therapy. Because previous wording had suggested that a patient would generally need to do poorly on more than one alternate therapy, this change should improve its coverage by health plans.

● **Researchers reprogram adult stem cells in mice to become myelin-making cells**
Salk Institute scientists reported that adult stem cells in mice that were on their way to developing into nerve cells could be redirected to turn into cells that make myelin, the nerve insulation that is a key target of the immune attack in MS. The result was achieved by changing a single gene. Further research is needed to translate these findings in people and to determine their significance to myelin repair in MS.

● **High-dose regimen of cancer drug reduces MS disease activity**
A small, uncontrolled clinical trial in nine people with relapsing-remitting MS suggests that a regimen of high-dose chemotherapy (IV cyclophosphamide) significantly reduced disability and MRI-detected disease activity in most, and resulted in sustained remission in some; they had been followed for an average of 23 months. Larger, controlled trials are needed. The study, by Johns Hopkins University researchers, was funded in part by the National MS Society.

● **Study yields clues for predicting future course of MS**
A long-term study that has been tracking individuals for over 20 years provides important clues that may help doctors predict the clinical course of MS, a notoriously unpredictable disease. Among their findings, the University College London investigators reported that those with relatively rapid increases in brain lesion volume during the first five years after their initial neurological episode, as detected with MRI scans, were more likely to develop long-term disability than those with slower rates of lesion accumulation.

● **Molecule may be key to autoimmune attack in MS**
Harvard researchers funded by the National MS Society report that a molecule called the aryl hydrocarbon receptor—which helps the immune system respond to environmental toxins—seems to regulate the balance between inflammatory and anti-inflammatory cells in MS-like disease in mice. The results may eventually help tease out the effects of environmental factors that may trigger the launch of autoimmune attacks against the nervous system in MS. ■

Researchers need your help:

MS Trial Alerts were posted on new clinical trials recruiting volunteers across the country and in Europe to test the next generation of MS therapies, including ones taken by mouth. Go to **nationalMSsociety.org/trialsrecruiting** to find ongoing studies in your area.

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