

A Summary of Scientific Findings

RESULTS

2004-2009

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Myelin 
Repair
Foundation

MYELIN REPAIR FOUNDATION

FIVE YEARS OF SCIENTIFIC PROGRESS

2004-2009

Message from the Chief Operating Officer

Dear Friend of MRF,

For the last five years, we have done our best to keep supporters and others interested in our research programs informed about our progress—on our web site, as well as in briefings and scientific progress reports such as this. In that process, we have found MS patients to be extraordinarily knowledgeable about their disease and current research initiatives across the board. To that end, we are providing this more detailed summary of the scientific achievements resulting from the first five years of our search to find myelin repair treatments.

In 2004, we promised to identify, validate and license the first of many myelin repair drug targets by 2009, and to bring an effective myelin repair treatment for MS to market by 2019. As this report testifies, we have made great progress toward that goal. First and foremost, all evidence continues to point to the genuine possibility that myelin can be repaired in MS and that the reality of myelin repair is within sight for many living with MS today.

We hope the information shared here will add to your deeper understanding of the biological systems that influence remyelination in MS and that you will continue to advocate for the potential of myelin repair treatments as the next generation of MS therapies.

We are grateful to all who have made this groundbreaking work possible.

Sincerely,



Russell “Rusty” Bromley
Chief Operating Officer
Myelin Repair Foundation
October, 2009

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FIVE YEARS OF PROGRESS: AN OVERVIEW

The major accomplishments of the MRF can be measured in a number of different ways. While traditionally some organizations have measured their progress based on the number of grants made or the amount of money given to research, the MRF feels that it is the outcomes from the research that matter most to people living with MS.

In 2004, the MRF initiated an ambitious research plan with focused investment in six main areas of research that we believed would be most directly applicable to finding new therapeutic targets for myelin repair. The six areas are:

- Understanding how the cells responsible for producing myelin (oligodendrocytes) are normally produced by the body, in the right quantities and locations, to replace missing myelin, if and how this process is perturbed by MS
- Understanding the biological mechanisms that result in axons being myelinated, if and how this process is perturbed by MS
- Understanding the normal structure and function of the myelin sheath, if and how it is perturbed in MS
- Understanding how the immune response in MS and the resulting inflammation affects myelin repair
- Understanding the role of the blood vessels in myelination, if and how this role is perturbed in MS
- Developing better animal models for the study of remyelination and MS

Here we will highlight several therapeutic approaches identified by the MRF research team in our first five years.

FIVE THERAPEUTIC APPROACHES TO MYELIN REPAIR IN MS

Therapeutic Approach #1

Promoting myelin repair, in the right place at the right time

Since it appears that the myelin repair process eventually fails in people with MS, resulting in chronically demyelinated areas known as lesions or plaques, one of the primary goals of the MRF team is finding ways to revive the repair process. It appears that oligodendrocytes, the cells that normally produce myelin, are eventually either depleted or lose their ability to repair damage. Therefore it is necessary to stimulate the generation of new cells capable of repair, get them to move to the site of the damage, reproduce until there is a sufficient population for repair, and then rapidly produce new myelin.

Work by Dr. Robert Miller and his team at Case Western Reserve University has identified many of the factors that promote the migration of repair cells and that may be preventing them from entering the lesions and replacing lost myelin. Dr. Miller was selected for the MRF because of his expertise in how the central nervous system develops and in particular the factors that drive neural stem cells to become myelin-producing oligodendrocytes.

This process begins with a pool of neural stem cells that is concentrated in a region near where the brain is joined to the spinal cord. Based on MRF research we believe that signaling molecules are produced in areas where damage takes place that will attract these neural stem cells and stimulate them to develop into cells capable of producing myelin. However, once these cells reach the edge of the lesion there appears to be a barrier that prevents them from entering the lesion and completing the job of myelin repair.

Dr. Miller's team has identified two therapeutic approaches enhancing myelination in chronically demyelinated areas.

The first is to control a signaling mechanism that appears to play a critical role in this process. By blocking the receptor for this signal, called CXCR2, they have been successful, in promoting rapid migration of repair cells into lesions in animal models. One of the most exciting aspects of this work is that a similar signaling pathway has already been identified for another, unrelated disease and drug compounds have already been developed by pharmaceutical companies that could be useful for this target. This allowed the MRF team to test the impact of actual drug compounds in cell cultures and animal models with positive results. While these results are exciting we know that delivering drug compounds into the brain is much more challenging than to other parts of the body. We are now working on target validation studies that are necessary to convince companies, already working in this area, to expand their interest to include myelin repair in MS.

The second approach developed by Dr. Miller's team is based on experimental use of stem cells derived from human bone marrow. Bone marrow transplants have been used for years to treat leukemia. Bone marrow contains two primary types of stem cells, one that replenishes the blood and another that produces bone, cartilage and other connective tissues. This second type of stem cell is called a mesenchymal stem cell or MSC. For more than 15 years, marrow-derived stem cells (MSC) have been removed from a patient, grown in culture and delivered back to the same patient by IV infusion, to successfully treat graft vs. host disease in transplant patients.

Since techniques for harvesting, growing and infusing MSC were developed at University Hospitals Case, Dr. Miller had ready access to human MSCs from collaborators outside the MRF. These cells were used to treat animals with an immune-induced demyelinating disease similar to MS. The results were remarkable, the animals recovered from the disease rapidly and completely. The results were so surprising that the experiment was repeated several times with the same results. The big question was "how were these cells promoting repair?" By adding a fluorescent label to the MSC cells they could be tracked in the brain. These cells migrated to the site of injury as would be expected but surprisingly the MSCs remained in the blood vessels and did not enter the brain or spinal cord tissue. The administering of these cells appears to both suppress the immune response and resulting inflammation, and promote myelin repair, in essence providing a combination therapy.

Transplantation of MSCs is a potential myelin repair therapy. However, the extraction of bone marrow, separation of the MSCs, culturing the MSCs in order to grow a therapeutic dose and making sure they are compatible from donor to patient is a complicated and likely expensive process. While we hope to see a proof of concept study in humans in the near future, Dr. Miller's team will continue to investigate how MSCs are promoting repair and if their effect can be duplicated with one or more drug compounds.

Therapeutic Approach #2

Turning on the myelination program in repair cells

Pathological studies have shown that even in chronic MS lesions there is a small population of immature oligodendrocytes near bare axons that should be able to produce myelin, but don't. Why this is not happening is a critical question for the MRF. What is preventing these cells from performing their normal function?

Understanding what controls the myelination process has been the primary focus of the MRF team at Stanford University led by Dr. Ben Barres. Dr. Barres was selected for the MRF team for his pioneering work in developmental neurobiology including the development of neurons, axons and synaptic formation along with his expertise in myelin biology. When Dr. Barres' team joined the MRF they brought longstanding skills in the purification of different types of neural cells and the ability to culture these cells in ways that controlled their development.

By applying these techniques, they have been able to study the transition from an immature, oligodendrocyte precursor cell to a mature cell that produces myelin and wraps bare axons. Understanding the genetic program that drives this process is critical to identifying potential drug targets and developing drugs that could stimulate myelin repair. Through extensive gene expression studies they have shown that there are several distinct stages in this process that must occur sequentially. They have also shown that in the absence of appropriate queues the oligodendrocyte precursor cells can become mature oligodendrocytes that do not produce myelin.

Now that several of the key steps in the oligodendrocyte development process have been identified, the search for ways to stimulate each stage has intensified. Thus far two potential drug targets have emerged.

The first target is a complex protein woven into the cell wall of oligodendrocytes called gamma secretase. Gamma secretase is of great interest in the treatment of Alzheimer's disease and a number of experimental drug compounds that inhibit its function have already been developed for this target. The MRF has evaluated some of these compounds in cell cultures and in animal models, with varying degrees of success. This tells us that the specific mechanism of action required for myelin repair may be considerably different than what is needed to treat Alzheimer's disease. This is a great opportunity for a pharmaceutical company to find a new use for a drug that has already been developed and tested for safety but may not be effective in Alzheimer's disease. Through a combined effort between Dr. Steve Miller's lab and Dr. Barres' lab we have found that the compounds that promote myelination work best in animal models of MS when combined with therapies that reduce inflammation by controlling the immune response (described in more detail below).

Dr. Barres' team also found that while inhibiting gamma secretase could initiate the myelination program, it was not sufficient to drive the process to completion. They began a search for other potential targets that could drive the entire myelination process. The second potential target they discovered was a gene with a previously unknown function that they named Myelin gene Regulatory Factor or MRF. The MRF gene appears to be responsible for driving the differentiation of immature oligodendrocytes and promotes the activation of many of the genes responsible for producing structural proteins that are critical to the formation of myelin. They have also found that turning off the MRF gene results in the breakdown of existing myelin demonstrating it is critical to maintaining stable, fully functional myelin.

Finally, work in Dr. Brian Popko's lab at the University of Chicago has led to the discovery of a gene defect that plays a key role in the late stages of myelin formation. The Popko lab specializes in identification and analysis of genetic mutations that affect the CNS and PNS, a process called forward genetics, and using genetic modifications to create new animal models of CNS and PNS diseases. In their forward genetics studies the Popko lab identified a mouse with a genetic mutation that resulted in late-stage disruption of myelination on most axons. The defect was traced to a single nucleic acid in a very long DNA sequence that resulted in premature termination of a critical protein, ZFP191. This seemingly minor gene defect results in oligodendrocytes that initially produce myelin membrane, and extend it to bare axons, but do not wrap the axons or produce normal myelin segments. This is especially interesting to the MRF as similar defects have been reported in MS lesions. Now the Popko lab is searching for potential drug targets, downstream of this defective gene, that can "rescue" the myelination process and allow the genetic program identified in the Barres lab to proceed normally.

Therapeutic Approach #3

Controlling the immune response to promote myelin repair

Traditionally MS has been viewed strictly as an autoimmune disease where the body's immune system turns against itself, in this case damaging the myelin that protects the nerves in the brain and spinal cord. This view is not surprising since MS disrupts the integrity of blood vessels in the CNS allowing immune cells, like T cells to enter areas they would not normally be able to access. As a result the vast majority of MS research has been focused on controlling the immune response in order to prevent relapses and limit damage. Research has shown that immune cells that react to myelin proteins, called activated T cells, are a major contributor to the inflammation associated with exacerbations, in the early stages of the disease. MRF research has shown that in MS disease models, T cells that are normally circulating in the blood stream, can migrate through the compromised blood vessels, enter the brain, and be activated locally within the brain. These myelin reactive T cells produce inflammatory chemicals that can prevent myelin repair. Since there is good evidence that spontaneous myelin repair is taking place when inflammation subsides during remissions in MS, one approach to myelin repair is to find new ways to prevent T cells from entering the brain and another is prevent to prevent them from reacting to myelin proteins and creating additional damage.

Drugs that help reduce the immune response, beta interferons, glitamer acetate and now Tysabri have been the standard of care in MS. Recently a number of anti-cancer drugs have also be studied in MS to deplete myelin-activated T cells. However none of these drugs are specifically targeting the activated T cells that cause damage in MS.

Three new methods to prevent T cell activation

Therefore the MRF has chosen to focus on new approaches that prevent T cells from reacting to myelin proteins. These efforts have been led by immunologist Dr. Steve Miller at Northwestern University. Dr. Miller was selected for the MRF team based on his work in understanding the immune response in the brain and how the immune response expands when myelin is damaged in MS through a process called epitope spreading. Dr. Miller is an expert in the use of the leading animal models of MS and other autoimmune diseases, including extensive research in diabetes.

Under Dr. Miller's direction, MRF researchers have identified three approaches controlling the activation of T cells by myelin proteins:

1. First, they identified cells called myeloid dendritic cells that enter the CNS and use fragments of myelin proteins to activate T cells. Previously it had been thought that the damaged fragments of myelin protein had to leak into the blood stream and reach the lymph nodes or spleen in order to activate T cells. Now that we know that T cells can be activated within the brain, a new therapeutic approach is to either prevent the generation of these myelin-presenting myeloid dendritic cells, or prevent them from passing from the blood stream, into the CNS. Both approaches are active areas of continued investigation for the MRF.
2. Second, for a T cell to become activated it requires both a fragment of a myelin protein along with a co-stimulatory signal. Just like a door with two locks, both keys must be inserted and turned to unlock the door (i.e. activate the T cell). Blocking the co-stimulatory signal is like plugging one of the two key holes — it prevents the door from being opened. Dr. Miller's lab has been working on two techniques for selectively blocking the co-stimulatory signals that activate T cells. These techniques have proven effective in several animal models of MS.
3. Third, we may be able to trick the immune system into recognizing that myelin proteins are not foreign materials. Fragments of myelin proteins can be chemically linked to blood cells and transferred back into the body, where they are digested and delivered to the spleen. Since new T cells are exposed to myelin proteins they no longer react to them as a foreign material. This pre-conditioning process to prevent T cells from reacting to myelin proteins is referred to as induced tolerance. We expect a human proof of concept study for this approach to begin in the spring of 2010. This therapeutic approach may have broad applications for other autoimmune diseases.

Success of any of these three approaches would reduce inflammation in the CNS and remove impediments to the myelin repair process.

Therapeutic Approach #4

Protecting the myelin repair process from inflammation

When the myelination process begins, the myelin-producing cells (oligodendrocytes) start to synthesize tremendous amounts of myelin proteins. In fact, this process increases myelin protein production 1000 times over the normal levels of protein synthesis in these cells. This is not surprising since the oligodendrocyte has to extend a sheet of myelin to several bare axons simultaneously and wrap the axon in multiple layers of myelin. These proteins are very large three dimensional molecules that provide the structure for the myelin that wraps around the axon to protect it.

As mentioned above, myelin reactive T cells create an inflammation that prevents myelin repair. By releasing inflammatory molecules the T cell can disrupt the process of producing myelin proteins. Defective protein molecules begin to accumulate in the protein synthesis machinery until finally they cause the cell to die. One approach to this problem is to control the immune response to eliminate inflammation as mentioned above. The second is to find ways to protect the myelin production process in these cells from the inflammation.

For the last five years, Dr. Brian Popko at the University of Chicago has led a team that has been looking for ways to protect myelin-producing cells from the deleterious affects of the inflammatory molecules released by activated T cells. Dr. Popko was selected for the MRF team based on his ability to develop innovative animal models using advanced genetic techniques and for his pioneering work in how myelinating cells are damaged by inflammation. Once his team understood how these molecules were damaging the myelin synthesis process they could begin the search for ways to intervene therapeutically. In this effort they have identified two places where the myelin synthesis process can be controlled so defective proteins no longer accumulate to toxic levels, protecting the oligodendrocytes. The search for potential drug compounds has already resulted in a candidate for one target and is being initiated for the other.

Therapeutic Approach #5

Restoring the integrity of the Blood-Brain Barrier

Blood vessels in the brain and spinal cord are different than in other major organs in the body. Most blood vessels are permeable to allow oxygen and nutrients to move freely from the blood into the tissue. However, in the CNS the cells lining the blood vessels are tightly connected to protect the brain from any toxic chemicals or infectious agents that would damage it. The cells that line the blood vessels in the brain also contain special proteins that actively remove potentially dangerous chemicals that do make it into the brain. In addition, the cells lining the blood vessels in the brain also have mechanisms to actively move oxygen and nutrients from the blood into the brain. What results is a complex system that protects the brain and spinal cord. This system is called the blood-brain barrier.

When the blood-brain barrier is compromised in MS it begins a cascade of events. Cells lining the blood vessels produce proteins that attract T cells and help them enter the CNS where they can be locally activated causing myelin damage and inflammation that can prevent remyelination.

One method to promote myelin repair is to restore the integrity of the blood-brain barrier. MRF researchers have shown that the blood vessels in the CNS are surrounded by specialized cells called pericytes that appear to play a critical role in maintaining the blood-brain barrier. Processes controlled by the pericytes may help to prevent T cells from penetrating the blood-brain barrier. Additional research is underway to find out how.

RESEARCH TOOLS

None of the investigations and promising results described in this report would be possible without the necessary research tools. When most people think of research tools they often think of expensive pieces of equipment like microscope or imaging devices. While in specialized laboratories this full, of state-of-the-art equipment is important, new animal models of disease, new cell and tissue culture methods and new databases of information on biological processes are critical to the identification of new drug targets and the development of new therapies.

Why does the MRF invest in the development of new research tools? When we developed our strategic research plan in 2003, MRF scientists pointed out that their current “tool box” was limiting the speed with which they could conduct research and identify potential new, myelin repair treatment targets. Since the MRF is committed to accelerating research this was a problem that had to be addressed. Over our first five years the MRF supported the development of advanced research tools in three areas:

- Gene expression databases
- Methods for evaluating myelin repair therapies at the cellular level
- New animal models of demyelinating disease

1. **Catalog of changes in gene expression during myelin damage and repair**

In 2004, MRF researchers did not understand the cellular changes that must take place in order for a cell to go from being a stem cell to a myelin-producing cell. This is an incredibly complex process requiring chemical triggers that stimulate some genes to be turned on and others to be turned off, in a carefully coordinated sequence, in order to produce proteins that provide the structure for the new myelin membrane. Without this knowledge it was difficult to identify opportunities for therapeutic intervention known as targets. MRF researchers, using state of the art technology were able to build a comprehensive database and identify several previously unknown but distinct steps cells go through to produce myelin.

Using this database has enabled the MRF research team to compare the normal process of embryonic and post- natal myelin production with what happens when specific genes are turned on or turned off in cell cultures or in various animal models in order to mimic specific aspects of MS. The MRF has also developed an extensive database of changes in gene expression in the most widely studied animal model of MS, the EAE model. In the past, scientists using the EAE model measure therapeutic response by subjective observation of the animal's behavior through the disease course. This information makes it possible to measure changes in gene expression to evaluate and compare different therapeutic approaches at a molecular level.

2. Cellular and tissue-based models for direct examination of myelin damage and repair

Measuring demyelination and remyelination in animals is difficult and time consuming. It requires that the animal be sacrificed and their tissues be carefully stained and examined under a microscope. One of the main drawbacks of this method is that it only provides a snapshot at a single time point. Therefore, multiple animals are used and sacrificed at different times in a disease course in an effort to monitor myelin damage and repair. The MRF has been developing techniques that use living tissue slices or purified cells under culture conditions that enable prolonged and direct examination of myelin damage and repair over time.

Trying to replicate the physiological conditions in the brain and spinal cord is a complex challenge. It requires the correct balance of nutrients, oxygen and numerous proteins, lipids, etc. to maintain healthy cells and tissue. It is easy to understand why this would be a difficult technique to develop and perfect. MRF researchers must duplicate exactly the same physiological conditions for each experiment in order for the results to be meaningful. This is a process fraught with opportunities for failure as finding exactly the right conditions is often a process of trial and error. In the end it is critically important that the final methods are robust and reproducible so that they can be used to evaluate and compare the ability of various drugs to stimulate myelination.

MRF researchers are continuing to develop and refine these methods with cells and tissue from laboratory animals. Hopefully in the near future these methods can be applied to human cells and tissue. Sources of viable cells and tissue are extremely limited so the MRF is very interested in the use of human neural stem cells. These cells can be maintained in culture indefinitely and treated to create neurons, astrocytes and oligodendrocytes, the three primary cell types needed for MRF research and validation studies. The MRF is actively seeking partners with expertise in producing human neural stem cells for this research.

3. New animal models to measure myelin damage and repair

When the MRF began research there were three dominant animal models of MS, all of which relied on the immune response to develop a MS-like disease. Studying demyelination and remyelination in these models can be confounded by the effects of inflammation. In order to study demyelination and remyelination directly without significant inflammation chemicals could be added to the animal's food or injected into the brain or spinal cord to create a lesion. The damage that resulted was not limited to the myelin-producing cells and was spontaneously repaired fairly quickly.

One method for studying the effect of a particular target on the myelination or demyelination process is to genetically engineer mice or rats so that the gene for the target of interest can be induced to "turn on or turn off." There are a variety of ways to do this and the MRF team has created several of these animals to study the affects of target specific genes on myelination.

While all of these methods have been successfully employed by MRF researchers in identifying new treatment targets, none proved to be the ideal compliment to the immune models for studying demyelination and myelin repair. Recently, MRF researchers have developed two methods for selectively killing oligodendrocytes, the cells that produce myelin so that the effects of demyelination and repair can be measured.

In one case mice are genetically engineered so myelin-producing cells can be killed throughout the brain and spinal cord. It then takes 30 days for the axons to become completely demyelinated and another 30 days for repair to reach a maximum level. During this process animals exhibit clinical symptoms that can be quantified to track progress of the myelin damage and repair. Since the damage and recovery process is slow, new candidate therapies for myelin repair can be tested in this model and the impact can be easily measured and compared.

In a second model, genes can be transferred into oligodendrocytes that react with a chemical compound to drive death of these cells in a specific location. This method enables MRF researchers to create myelin damage in various areas of the brain and spinal cord allowing them to compare the effect of potential therapies in different regions of the CNS. These two techniques allow for much more precise experimental control and easier measurement of myelin damage and repair than the methods that were available when the MRF began research in 2004.

Developing new animal models, especially those that require genetic modification, is difficult and time consuming. The model of systemic demyelination described above required four attempts before the right combination of genes was achieved and selective elimination of oligodendrocytes was possible. Each attempt required a tedious process of transferring genes and breeding animals before the mice could be evaluated.

The benefits of success are well worth the investment as these models can now be combined with the traditional EAE models to evaluate new therapeutic approaches and may provide unique insight into the myelin repair process that would not be evident with EAE alone.