Myelin Repair Foundation

Collaboration. Acceleration. Results.

Myelin Repair Foundation
Research Progress Report
June 2006-July 2007

In July 2004, the Myelin Repair Foundation initiated a five-year research plan with the mission of discovering and validating new therapeutic targets for myelin repair in multiple sclerosis. Our goal was to have the first target validated and ready for drug development by a pharmaceutical or biotechnology Company by July 1, 2009. This report summarizes several of the most exciting and important discoveries made by the MRF research team over the last three years.

In addition, at the end of the progress report is a review of some of the major challenges that lie ahead for the MRF as we move these targets forward into drug discovery.

How do we unlock the secrets of myelin repair?

When the MRF was founded in 2002, little was known about the underlying biological processes that resulted in myelin formation and how these processes were affected by multiple sclerosis. What was becoming apparent, however, was that the brain and spinal cord had a capacity for repair and that myelin repair seemed to be taking place spontaneously in the early stages of multiple sclerosis. The MRF research team understood that a number of critical questions would have to be answered before effective myelin repair therapies could be developed.

The MRF developed a five-year research plan to answer the following critical questions:

What causes neural stem cells to (1) become precursor for myelin-producing cells (oligodendrocytes), (2) migrate to the right place, (3) multiply and (4) mature in order to repair myelin damage? How does multiple sclerosis block this process?

What are the processes that drive immature oligodendrocytes to differentiate into myelin-producing cells?

How does myelin form? What is the structure that maintains properly formed myelin? How does it interact with the axon (nerve fiber)? And how is this structure and function affected by multiple sclerosis?

What is the role of the immune response in damaging myelin or preventing its repair?

What role does the breakdown of the blood brain barrier play in multiple sclerosis and how can it be repaired?

Since it is difficult to study these processes in humans, what new or improved animal or cellular models do we need to ensure our research provides answers applicable to multiple sclerosis in humans?

Beginning with the end in mind

Answering these questions is not simple. In many cases the MRF research team needed new scientific strategies, tools and methods to identify and study the relevant processes before these questions could be answered and new myelin repair treatment targets discovered.

A target is like a "lock" that controls a biological process. Just like a lock, it can be opened to allow the process to proceed or closed to prevent it. Drugs (or other therapeutic agents) act as "keys" to open or close the "locks" that control a specific biological process in order to provide a therapeutic benefit. The **MRF considers a discovery a target when we can show that affecting (locking or unlocking) this step in the biological process**, **in cell culture (*in vitro)* or in animal models of disease *(in vivo),* has a positive effect on myelin repair.**

In order to find these new targets or "locks" the MRF research team identified the need for a variety of new tools and techniques. The MRF first looks for state-of -the-art resources both within, and beyond, the labs in our core target discovery team. When the necessary tools and techniques are not available, the MRF invests in developing them and will make them available to the neuroscience research community. These new tools and techniques have enhanced and accelerated the ability of the MRF target discovery team to make significant progress in answering the critical questions outlined above. In the last three years we have identified more than a dozen new targets with therapeutic potential and are beginning a rigorous evaluation process to determine which of these targets should move forward into the drug discovery process.

The search to "unlock" myelin repair

Multiple sclerosis is not a simple disease and there are still a great number of unknowns about the disease process. For example, how is the myelin sheath damaged by multiple sclerosis? Does the immune system attack the sheath itself? Does the cell body die leaving the myelin to deteriorate over time? How does the disease attack the myelin or the cell body? Until these and many other questions are answered, we must try to find repair processes that address multiple possibilities. This is one reason why the MRF uses a broad range of animal and cellular models to evaluate possible targets and therapeutic strategies.

This review describes five different therapeutic approaches where the MRF research team has identified targets or "locks" and has begun the process of identifying the appropriate keys for stimulating myelin repair.

1. Repairing the blood brain barrier to promote repair

The blood brain barrier is a very important factor in any treatment of CNS diseases. The blood vessels and capillaries of the CNS are different from any other tissue in the body. The cells that line the vessel walls are surrounded by pericytes and astrocytes rather than muscle. While most vessels are "leaky" to allow diffusion of nutrients, oxygen and other molecules to pass in and out of tissues, cells that line the vessels of the brain are tightly connected to protect the brain. These tight connections form early in fetal development and provide a highly selective filter that protects the

brain from foreign materials. This filter is very effective at preventing most drugs from entering the brain.

In multiple sclerosis, early in the disease process, the tight connections between the cells that line the blood vessels are disrupted allowing antibodies, proteins and cells to enter the CNS and myelin debris to exit the CNS and stimulate an immune response. Thus, breakdown of the blood brain barrier contributes to the disease processes that are damaging the myelin and axons.

Closing the blood brain barrier should retard these disease processes and help allow normal repair to take place. MRF researchers have begun to tease apart some of the main features of this vascular architecture. They have identified two target molecules that appear at relatively high levels in the vessels of the CNS but are absent, or at very low levels, elsewhere in the body. This enables us to begin the search for drugs that will act as a key to restore the blood brain barrier and to develop new methods to measure their therapeutic potential.

2. Promoting myelin repair by cells that are already in the lesion

For several years neuropathologists have been puzzled by the fact that within persistent multiple sclerosis lesions there appear to be normal, mature oligodendrocytes that did not myelinate bare axons nearby. Why would these cells not respond and repair the areas where myelin had been damaged?

Based on recent studies by the MRF research team it appears likely that there is only a brief window, early in the process of oligodendrocyte precursor cells becoming mature oligodendrocytes, when myelination or remyelination is possible. If the correct cues to stimulate myelin formation are not present at that time, then the cell continues to mature into a non-myelinating state.

How did the MRF research team build a more comprehensive understanding of this process over the last three years? First we needed a new tool, a method of studying the myelination process over time. MRF researchers developed a method for culturing neurons and oligodendrocyte precursor cells together and found an experimental compound that stimulated myelin formation in this culture system. Critical to the value of this new culture system is that the myelin segments that are formed appear to be identical to those found in the animal and develop over the same time course. Now MRF researchers can directly observe the myelin formation process and extract cells at any point in the process.

Using state-of-the-art gene chip technology to measure changes in gene expression, they discovered that the oligodendrocyte precursors matured in two distinct stages with one major group of myelin genes being “unlocked” early in the process, and a second major group being “unlocked” later in the process. Each of these stages appears to be controlled by a different “key” and these keys must function in the proper sequence if the cell is to interact with the axon and produce myelin segments. Not only is the sequence of unlocking these genes important, but there also appears to be a “master lock” that regulates the entire process.

It is likely that the keys that control this process are normally provided by the axon during development but may be absent or blocked once the axon has been demyelinated or damaged. By identifying drug compounds that unlock these control genes, in the proper sequence, it may be possible to affect repair using the oligodendrocyte precursor cells that are already present.

3. Protecting the myelin repair process from the immune response

MRF scientists have shown that in addition to damaging myelin and axons, the immune response may also be preventing myelin repair. The inflammation created in the CNS by the immune system in multiple sclerosis causes T cells, astrocytes and microglial cells to release chemicals that can destroy myelin-producing cells. When oligodendrocytes are stimulated to produce myelin, the rate of synthesis for myelin proteins increases up to a thousand times the normal rate. This creates tremendous stress on the metabolism of the cell. If the cell undergoes additional stress from inflammation during this process, it begins to accumulate misfolded proteins, overloading the cell and ultimately causing its destruction.

MRF scientists have been investigating ways to protect oligodendrocytes from these inflammation-induced chemicals. By regulating the synthesis of myelin proteins, they can prevent the cell from becoming "overloaded." It appears that there are several steps in this biological process that could be used to "lock out" the inflammatory stress produced by the immune response. For the last year MRF scientists have been working with the Laboratory for Drug Discovery in Neurodegeneration at Harvard to identify drug compounds that would act as keys to protect oligodendrocytes. Today there are two promising leads and additional tests are being conducted to confirm the effects of these compounds *in vivo and in vitro.*

4. Disrupting the immune response and stimulating myelin repair

MRF scientists have found that in animal models of multiple sclerosis the immune response can be stimulated locally within the CNS which could explain why persistent, demyelinated lesions develop in a few locations but not throughout the CNS. In areas where the blood brain barrier has been compromised, circulating T cells can enter the CNS along with a special group of dendritic cells that pick up fragments of proteins from damaged myelin and use it to stimulate the T cells. Once stimulated these activated T cells produce additional myelin damage creating an expanding lesion. However it requires more than just a fragment of myelin protein for a dendritic cell to activate a T cell. It must also produce a co-stimulatory factor. The myelin protein fragment and the co-stimulatory factor are both "locks" and the T cell must have the right "keys."

Since in multiple sclerosis there is preexisting myelin damage it is unlikely that first "lock" can be controlled by a drug, but it is possible to find a key to "lock up" the co-stimulatory factor and prevent further T cell activation by dendritic cells. This same "key" may also reduce the inflammatory response by astrocytes and microglia. Once this local immune response is suppressed the environment is much more conducive to myelin repair. This approach can be combined with Key #2 to more effectively drive the repair process.

By using this combination strategy to simultaneously control the immune response and promote remyelination, MRF researchers have achieved dramatic recovery in an animal model for relapsing–remitting demyelination. The results achieved by this combination therapy were significantly better than results of experiments using either therapy alone. MRF investigations are continuing to evaluate this combination of therapies in other animal models, and in culture, to optimize this synergistic effect.

5. Stimulating neural stem cells to repair myelin damage.

Today, MRF scientists have a much better understanding of the biological processes that control how and why neural stem cells become oligodendrocyte precursor cells. These cells must migrate to the site of a demyelinated lesion, then stop, multiply, mature and produce new myelin. This complex process is controlled by different chemical signals and cellular interactions at each step. By developing new techniques for creating controlled, demyelinated lesions in new animal models, and following the progress of these lesions and how they repair over time, we have gained considerable insight into this process. What we have learned is that:

Myelin damage appears to stimulate neural stem cells to become oligodendrocyte precursor cells and migrate to the site of injury.

Some of the molecular clues that are part of this process are not unique to the CNS and thus may not be good targets for stimulating myelin repair.

For normal repair to occur it must be initiated rapidly after inflammation subsides and lesion size affects the success of this process.

Two new targets that appear to play a major role in blocking the migration of new oligodendrocytes into a persistent lesion and preventing repair have been identified.

In recent studies, MRF scientists successfully stimulated myelin repair in a persistent lesion by blocking, or "locking", one of these targets with an antibody. Since antibodies are normally excluded from the CNS by the blood brain barrier, this is not an ideal therapeutic agent. However, based on the success of this experiment, we are now looking for small molecule drug compounds that will similarly enable oligodendrocyte precursor cells to move rapidly into the lesion, multiply and initiate the repair process.

As an additional complication, through the natural process of evolution, the body has used the same molecules to perform different functions in the CNS than they do in other parts of the body. Thus, it is critical to ensure that by locking or unlocking a gene in the CNS, tissues in other parts of the body are not being damaged. "Off target" effects which may result in toxicity are a major source of failure in drug development so selectivity for a specific target in a specific cell or tissue is highly desirable.

In addition to the critical role played by neural stem cells to myelin repair, we have demonstrated that another type of stem cell may play a beneficial role in promoting remyelination. Mesenchymal stem cells (MSCs) are derived from bone marrow and form many types of connective tissue in the body. Experiments by MRF and other labs administering human MSCs in animal models of demyelinating disease have shown dramatic improvement. In experiments designed to track these cells, it appears that they do not actually perform the repair, but instead appear to induce other resident neural stem cells to do so. We will continue to investigate the mechanisms by which these cells are promoting repair in our ongoing effort to identify new targets and therapeutic agents.

Conclusion

While the results of our target discovery effort have exceeded our expectations, we know that many of the most daunting challenges lie ahead. Even the most promising discoveries may never result in new treatments because of the challenges in finding or creating drugs (keys) for the targets (locks) that have been found. The gap between target discovery and drug development is referred to by industry as the "Valley of Death". Many of these challenges are described in the next section.

The Road Ahead: Preparing to Cross the "Valley of Death"

According to a recent study by the Boston Consulting Group over 99% of potential therapeutic targets identified by scientists fail to result in a new drug reaching the clinic. The majority of these failures (90+% occur early in the process, either because the target has not been adequately characterized, or there is insufficient data to justify a drug development program. The Myelin Repair Foundation is committed to overcoming this attrition rate by improving the target discovery process and by initiating a target validation and drug discovery process that reduces the risk of failure and optimizes our targets for drug development by industry.

Continuing Challenges in Target Discovery

Need for better *in vitro* models of the blood brain barrier

While there are a number of models that are used for testing the ability of a drug to pass through the blood brain barrier, none are designed to test targets for repair. The MRF team is working on developing a culture system that will imitate the *in vivo* system and allow comparative studies with animal models. This platform may also be extended to using human cells to study efficacy for new therapies.

Need for target specific animal models to test each target

Even the most profound effects of locking or unlocking a gene switch in culture must be confirmed in animal models (*in vivo)* in order to take into account other biological processes that could affect that lock or be affected by that key. To do this MRF researchers created transgenic animals in which specific genes are eliminated (knocked out) or added (knocked in) from oligodendrocytes to determine what effect it will have when the animal is given a demyelinating disease. Creating these transgenic animals is as much an art as it is a science. The process is very time consuming and may require several attempts, each taking six months to a year to achieve success.

Need for additional animal models

While there are several good models for stimulating an multiple sclerosis-like immune response, most have at least some level of spontaneous recovery which can make it difficult to measure the impact of therapy. In addition, disease course in these animal models is monitored by a clinical score that must be assessed by a well-trained investigator in order to achieve consistency. To make these models more robust, MRF researchers began measuring changes in gene expression during the course of the disease. By examining areas within lesions, near lesions and in unaffected tissue by using microscopic laser dissection, they were able to compare how thousands of genes behave throughout the disease course. This process has provided a new method for measuring changes in the disease course at a molecular level. In the future, to provide a more complete picture of this process, these studies will be validated by measuring changes in protein and lipid levels within the lesion tissue.

Even this new, more comprehensive process for evaluating the immune-induced disease models cannot give us a complete picture. There is compelling evidence that in later stages of multiple sclerosis, myelin damage occurs even in the absence of an active immune response. Since all the ways that multiple sclerosis can damage myelin are still not understood we must use other models also.

One approach is to chemically induce demyelination. Historically these methods have also resulted in spontaneous repair making it difficult to measure therapeutic effect. MRF scientists now have methods for creating lesions, controlling their size, and blocking repair to create persistent lesions. Repair of these persistent lesions is another way to measure therapeutic effect.

In addition, the MRF research team has recently developed two methods for selectively eliminating oligodendrocyte cell bodies in animal models. This can be done locally to create a specific lesion area or systemically to create widespread demyelination throughout the CNS. Both approaches are valuable in studying repair strategies and comparing those results to the immune- and chemically-induced models. If a myelin repair treatment proves effective in all three types of models there is a much greater chance that it could be effective against multiple sclerosis in humans.

These newest models give MRF a unique opportunity to study how myelin debris may affect myelin repair. In other parts of the body, when cells die, the resulting debris is typically cleared by cells called macrophages. Because of the blood brain barrier, macrophages cannot normally enter the CNS. Though under some conditions microglia can be activated to play this role, debris from damaged myelin often seems to persist long after the damage occurs and may be blocking the repair process. Myelin debris in the proximity of the axon may prevent it from providing the signals to initiate myelin repair. Identifying a drug compound that will artificially stimulate the myelination process will be critical to myelin repair.

Need for new *in vitro* tools

The blood vessels in the brain and spinal cord (central nervous system or CNS) are designed to prevent foreign materials from entering the CNS and causing disease. Unfortunately this blood brain barrier also blocks delivery of many types of therapies including large molecule drugs, proteins and antibodies. As these studies continue, it is critical for MRF researchers to be able to assess the therapeutic value of various compounds without interference from the blood brain barrier. Recently we have started using brain tissue, in culture, to directly observe the effects of various compounds on lesions and surrounding tissue. By combining results from animal models (*in vivo)* and tissue culture *(in vitro)* it should be possible to compare the direct and systemic effects of different therapies and thereby identify the ideal combination of potency and selectivity for the target.

Demonstrate the applicability to human disease

Since MRF research is predominantly performed using animal cells and animal models of disease, we must always be aware that many therapies that once looked promising in animal models were not successful in humans. It is important to know how closely the targets in humans match those in our animal models in order to predict if human cells and tissues will respond in the same way. As these new *in vitro* techniques using tissue slices continue to be refined they will be expanded to duplicate experiments using human neural cells and tissue.

The Next Big Challenge – Target Validation and Drug Discovery

Discovery of new treatment targets is only valuable to the MRF and to multiple sclerosis patients if it results in the development of new drugs or therapies for myelin repair. While the MRF target discovery team has done a magnificent job of identifying important biological mechanisms and potential targets within those mechanisms, target identification is just the first step.

Today fewer that one percent of all the drug discovery and development programs that are initiated by biotechnology and pharmaceutical companies ever reach the market. The cost of this amazing attrition rate is staggering. For every new drug brought to market, industry spends about $1 billion in research and development. Although the high failure rate of new projects contributes significantly to this cost, industry must be prepared to spend at least $100 million to complete a drug development program.

Developing and testing drugs for CNS diseases is among the most difficult and therefore most costly.

The biological processes responsible for many CNS diseases are poorly understood.

The brain is a protected organ that has a barrier designed to protect it from foreign substances. Because of this blood brain barrier, compounds that may be effective in other tissues cannot reach the brain.

The brain and spinal cord are a complex system made up of many different tissues, each of which may respond differently to a given drug.

The limited availability of human brain and spinal cord tissue makes it difficult to get diseased tissues for research and to measure therapeutic effect.

Clinical trials for CNS drugs tend to be lengthy and require a large number of patients in order to evaluate therapeutic outcomes.

This problem is compounded by the fact that being first in any new therapy involves significantly more scientific and financial risk than being second or third in a well established market.

For all of these reasons any new target proposed by MRF has to meet stringent qualification criteria before it will be accepted for development by industry. Typically the research done during the target discovery phase does not provide enough information for industry to commit to drug discovery and development. Industry is looking for multiple studies with comparable results before making a significant financial commitment to a new therapy.

There are few incentives for academic scientists to develop new target qualification assays or perform the repetitive studies necessary to confirm findings made by their lab. Without high-quality, reproducible data it is impossible to convince industry of the merits of a new mechanism or target. This is one reason why many promising discoveries made in the academic world move slowly, if at all, towards the clinic. This gap between target identification and drug development is referred to by both academics and industry as “the Valley of Death”

MRF’s next steps - Motivating pharmaceutical companies to work on MRF’s targets

Now that promising targets have been identified, we must demonstrate that there are “keys” with the potential to be converted into therapeutics, in order to attract companies to work on them. Targets are considered “drugable” when, using existing technologies, there is a reasonable chance that a drug or therapeutic agent can be developed that will act on the target.

A recent study by the Boston Consulting Group for the MRF shows that 71% of the cost of failure in pharmaceutical research and development is incurred in target identification, qualification and early-stage drug discovery. Navigating potential targets into drug discovery has traditionally been challenging and time consuming, however we believe that expanding MRF’s ARC model will significantly accelerate this process. Collaboration between MRF and pharmaceutical or biotech companies can significantly reduce these costs and the risk associated with novel targets.

Step 1: Drug Discovery - Target qualification

Running a variety of experiments and tests, in both animal and cellular models, and showing a beneficial effect is a way to reduce the risk associated with a new target for industry. These experiments and tests must establish that “locking or unlocking” of this target has the potential to improve the disease condition and must provide insight into biological pathways, mechanism of action, and a link to vivo models. Insight into the experimental designs necessary to qualify these targets will insure that we can provide industry with the motivation to partner with and support the MRF. Although pharmaceutical companies and commercial testing laboratories have the expertise to perform these studies, they lack the insight into the emerging biological experiments being conducted by the MRF researchers. The expansion of the ARC model into drug discovery will allow a synergistic relationship between the expertise of the MRF target discovery team and industry experts in drug discovery.

Step 2: Drug Discovery Assay Development, High Throughput Screening and Medicinal Chemistry to identify hits and establish leads.

Large libraries of potential drug compounds, or "keys", exist in most pharmaceutical companies. If the preliminary assays identified in target discovery or qualification processes can be modified to screen these existing libraries and reveal "hits" this makes the target more attractive. When an assay responds favorably to compounds in the screening library, in a dose-dependent, reproducible manner, it further increases the value of the target. Multiple hits enable medicinal chemists to build an understanding of the relationship between the structure and function of the target and the "hits". By refining this relationship it is often possible to design a lead compound with significantly improved activity, selectivity, more favorable pharmacological properties for *in vivo* testing. Having a well characterized lead, or series of leads, reduces the risk of failure and can increase the value of the target dramatically. This process of "hits" to leads is best performed by companies with sophisticated equipment and expertise in this area but the expertise of the MRF target discovery team in understanding the biological processes and their relationships to the assays is a critical element.

Step 3: Drug Discovery – Lead refinement

Once lead compounds have been identified, they must be refined to address the criteria needed to deliver a preclinical candidate compound. These criteria include:

Amount of compound necessary to achieve therapeutic benefit (acute and long term) in animal models
Ability of compounds to reach target tissues
Selectivity of compound for the target
Acute and long term toxicity of the compound

Again, these tests are best performed by companies that specialize in these methods but may rely heavily on understanding of the biological processes, animal models and experimental techniques developed by MRF scientists during the target discovery process.

Achieving our goal – new myelin repair treatments

How far down this path will the MRF have to go in order to interest development partners from industry is unclear and is likely to vary from target to target. For each step we take along this path the costs increase dramatically. Completing this process for a single target can cost in excess of $3 million Clearly it is in the best interest of multiple sclerosis patients for the MRF to partner as early as possible with pharmaceutical or biotechnology companies that can contribute their expertise and experience in target qualification and drug discovery.

In the meantime the MRF is recruiting industry advisors with a broad range of expertise, and qualifying contract research organizations, and industry partners that can perform target qualification, high throughput screening and pre-clinical testing needed to accelerate the MRF's drug discovery process. By mid-2008 we expect to have all of the necessary components in place to accelerate the progress of our current targets and maintain a stream of new targets based on our ongoing target discovery research. Even with these pieces in place it is likely to take 3-4 years to evaluate all of the current MRF targets.

More importantly there is no guarantee that we will find the key to unlocking myelin repair using any of the current targets. The process of evaluation and drug discovery can have many outcomes.

For some targets we may not be successful in finding an existing key or creating a new one
For others, finding the key will prove that this is not the right target or "lock"

And in many cases finding a key leads to identifying new targets or “locks” and the search for a new key begins

In addition, as we described above, it may take a combination of several keys to affect myelin repair or different keys at different stages of the disease.

Target discovery remains a top priority for the MRF research team. While results from our target evaluation and drug discovery efforts will move our existing targets toward the clinic, our target discovery team continues to advance the frontiers of myelin biology to uncover additional new targets. Linking the MRF target discovery team with best in class industry partners in target evaluation, drug discovery, drug development and clinical trials is a powerful next step in the evolution of the MRF’s Accelerated Research Collaboration model. By expanding the model we can achieve the goal of finding new myelin repair treatments better and faster than is possible today.

We remain confident that by expanding the ARC model the MRF will achieve its initial five-year goal of licensing its first target to a commercial partner by July 2009.