Myelin Repair Foundation

Discovery through Collaboration

Myelin Repair Foundation
Research Progress Report – Fall 2005

As part of the Myelin Repair Foundation Research Plan, the principle investigators cataloged critical gaps in current knowledge about the fundamental biological processes that control myelin formation and that could prevent myelin repair in multiple sclerosis (MS). Each year the MRF Research Team selects objectives that will lead to answers for two key questions:

- **What is preventing remyelination in MS?**
- **How can we stimulate myelin repair?**

These research objectives must fall into six principal areas of investigation.

1. **How are oligodendrocytes, the cells that produce myelin, generated and how does MS disrupt this process?**

2. **What stimulates oligodendrocytes to produce myelin, how does this process take place and how does MS affect this process?**

3. **What is the structure of myelin, and the nodes of Ranvier, and how are these structures and their functions affected by MS?**

4. **How does the immune response and related inflammation in MS affect myelin repair?**

5. **What is unique about the structure of the blood brain barrier (BBB), how is it disrupted in MS and how does this affect myelin repair?**

6. **What are the cellular and animal models necessary to understand these questions, and evaluate potential therapeutic approaches to myelin repair?**

This report provides an overview of progress towards the Myelin Repair Foundation's overall goals as well as detailed information on each area of investigation as reported at the MRF annual research meeting September 29 – 30, 2005.

Building tools and searching for targets

Answering the key questions posed by this process involves breaking new ground. In some cases, the methods and tools necessary to perform MRF studies do not yet exist and must be created by the MRF Research Team. These include:

- Creating new purification and culture techniques for neural cells,
- Making new antibodies to visualize protein distribution or control signaling between cells,
- Constructing new interfering RNAs and breeding transgenic animals to control gene expression and protein production
- Developing new models to study biological mechanisms and evaluate therapeutic effects in cell culture, animal models and ultimately with human tissue.

Creating and scientifically validating new research tools is a painstaking, time consuming process that is critical to MRF's success. The MRF Research Team is committed to accelerating this process by continuously searching for the necessary tools from commercial and academic sources, and by assisting each other in creating these tools when existing sources cannot be identified.

The search for new therapeutic targets depends on these tools. In several cases, MRF has benefited from materials and methods developed by the participating labs over many years. Much of MRF's progress in identifying new areas for therapeutic intervention described in this report is the result of those efforts.

The principal accomplishments of the MRF Research Team in its first year can be divided into two principal categories; new tools to further our investigations, and discoveries that provide insight into potential therapeutic targets.

New Tools:

- Cell culture systems of neurons and oligodendrocyte precursor cells where MRF researchers can stimulate myelin formation and observe the process over several days
- A method for purifying adult oligodendrocyte precursor cells to expand experiments using these new culture systems.
- A catalog of proteins unique to myelin.
- Profiles of gene expression for principal myelin genes in animal models of immune demyelinating diseases.
- Profiles of gene expression during developmental myelination in animals.

Discoveries with Therapeutic Potential:

- Identification of a new oligodendrocyte like cell that surrounds the nodes of Ranvier.
- Identification of an experimental drug that may promote proliferation of new oligodendrocyte precursor cells in areas of demyelination.
- Discovery of a stress pathway in oligodendrocytes, which is stimulated by an inflammatory molecule released by T-cells, which results in cell death during early stages of myelin production.
- Studies of an experimental drug that stimulates myelin formation and enhances recovery in culture, and in animal models of immune demyelinating disease.
- Identification of proteins unique to the structure and function of the blood brain barrier that may be responsible for the breakdown of this barrier in MS.

Each of the six main areas of investigation in the MRF research plan is currently under investigation by members of the MRF Research Team. In many cases investigators from multiple labs are conducting parallel studies in order to accelerate a comprehensive understanding of the processes involved and potential areas of therapeutic intervention.

1. How are oligodendrocytes normally generated and how this process is perturbed in multiple sclerosis?

The chain of events that directs stem cells to become myelin producing oligodendrocytes is a critical area of study. Stimulating myelin repair, whether by existing neural stem cells within the brain and spinal column (the central nervous system or CNS), or by transplanted stem cells, will rely on the appropriate chemical signals. These signals control four processes: differentiation into the right type of cell, proliferation into a sufficient quantity of cells to do the job, migration to the location where the cells are needed, and maturation to the stage where they can produce new myelin. To that end, MRF scientists are making steady progress in identifying signals that control these processes during development so we can determine which of these signals are necessary to stimulate remyelination in MS.

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Over the last few years, it has become increasingly clear that the signals that control differentiation of stem cells into the three principal cell types in the CNS include many different chemicals. These signaling molecules may work against each other, in some cases resulting in processes that are reversible. Thus it is not sufficient to simply increase the signals that drive differentiation of oligodendrocytes; we must also reduce or block the signals that retard or reverse this process. Similarly, migration of these cells to the point of injury is controlled by both chemo attractants and chemo repellents. These cues must be time coordinated in order to drive the desired cellular migration within the CNS. Finally, once these cells have reached the appropriate destination, they must receive the appropriate signals to multiply, mature and produce myelin.

Since the body frequently reuses of the same chemical signaling molecule for multiple purposes in different parts of the body. We must be careful not to inadvertently affect other important biological processes when developing methods for controlling signals that stimulate oligodendrocytes to produce myelin. Similarly, evolution has often resulted in redundant signaling and control mechanisms for the same biological processes. Therefore we cannot assume that manipulating a single biological pathway will result in the desired therapeutic outcome.

> "Remyelination failure is unlikely to be the fault of a single factor but instead arises because of disturbances in the controlled regulation of the many factors required to orchestrate remyelination" "Therefore the most effective strategies will be those that target the least redundant pathways." (R. Franklin, et.al., Neuron, 2005)

In the first year the MRF Research Team made substantial progress in identifying some of the signaling processes that result in:

- Differentiation of neural stem cells into different types of brain cells (neurons, astrocytes and oligodendrocytes) during embryonic and neonatal development. Now these signals can be tested on mature neural stem cells, both in culture and in animals.

- Migration of neural cells within the developing spinal cord and brain. The next step is to express or suppress these signals in areas of myelin loss in animals and measure the migration of new oligodendrocyte precursors.

- Maturation of oligodendrocyte precursor cells into mature cells capable of producing myelin. The MRF team has shown that some of the markers typically used to measure this process are not unique to oligodendrocytes, and have identified new markers that appear to be indicative of these cells maturing and becoming ready to produce myelin.

Understanding which signaling molecules block oligodendrocyte development and are produced in conjunction with demyelinated lesions will provide MRF researchers with potential therapeutic targets for MS.

2. What causes oligodendrocytes to produce myelin, how does this process take place and how does MS affect this process?

Since generally direct observation of the myelination process in animals is not possible, for years myelin researchers have theorized about what triggers myelin formation and how the process works.

In the last year MRF team members have:

- Refined cell culture techniques to allow direct stimulation of myelin formation in culture, allowing the introduction of agents suspected of disrupting this process, so that their effects can be directly observed.

- Refined imaging techniques that, when combined with these cell culture techniques, allows direct observation of myelin formation over prolonged periods. This new technology creates the opportunity to select cells at specific time points in the myelination process in order to analyze normal stages of gene expression and protein production.

- Demonstrated support for a novel theory that myelin is produced by extension of a spiral process down a bare axon, followed by anchoring to the axon and extensive myelin sheet spreading, to ultimately form stable and compacted myelin.

With these new tools MRF researchers can begin to dissect the signals that control this process, within oligodendrocytes, between oligodendrocytes and axons and within the CNS environment. As new signals are identified, they can be blocked via antibodies, interfering RNAs or by modifying the genes in model animals. Each new signal to be tested can take years of effort. The broad expertise of the MRF team allows more rapid identification, testing and validation via the technique that is most appropriate.

Molecules used by MRF researchers to stimulate myelination in cell culture and animal models are providing insight into therapeutic targets for stimulating remyelination.

3. What is the structure of myelin, and the nodes of Ranvier, and how are these structures and their function affected by MS?

Members of the MRF Research Team have undertaken an extensive study of the structural proteins found in myelin. Understanding the underlying architecture of myelin may provide insight into how myelin is damaged by MS, and releases the antigens that stimulate the autoimmune response in MS.

It is also important to understand the distribution of these proteins within the myelin as they may perform unique functions critical to successful myelin formation and stability. One of the principal areas of interest is the proteins that are found in the areas surrounding the nodes of Ranvier, the paranodal region. The nodes of Ranvier are the gaps between the myelin segments that cover the axon. The paranodes are the region where the myelin attaches to the axon and maintenance of the paranodal structures are critical to proper nerve conduction. Since the paranodes constitute a very small fraction of the overall myelin structure, the most critical proteins may only appear in very small amounts. Because of the large number of proteins found in myelin, identifying critical paranodal proteins has required the development of new isolation approaches by members of the MRF team.

Thus far, the search has revealed twenty two new proteins with unknown function. One of the first steps in understanding the importance of these new proteins is to identify the location of these proteins within the structure of the myelin.

MRF researchers identified one such protein that appeared to be associated with the paranodal region and created an antibody that would allow them to visualize its distribution within the myelin sheath. They were astonished to find that this protein was not in the myelin but in a completely different, oligodendrocyte-like cell that surrounds the node of Ranvier. This discovery has opened a broad new series of questions about the formation of this cell, its function and its role in the disease process of MS.

By understanding the unique role of these newly discovered myelin proteins, MRF researchers expect to gain insight into several critical areas:

- What are the critical interactions between the myelin and the axon that promotes survival and stability?
- How does the disease process in MS break down the myelin, releasing antigens that stimulate the immune response?
- Do new therapeutic approaches to stimulating remyelination result in normal composition and distribution of critical myelin proteins, and formation of stable myelin that restores conduction?

4. How does the immune response and related inflammation in MS affect myelin repair?

Multiple sclerosis is commonly viewed as an autoimmune disease whereby reactive immune cells (T-cells) in the circulatory system, which are normally prevented from entering the CNS by the blood brain barrier, are able to reach this protected environment and attack the myelin. These immune cells react to fragments of myelin proteins resulting in focal inflammation, increased myelin damage and ultimately axon loss. Since it is extremely difficult, if not impossible to study the underlying disease process in human patients, animal models are used by researchers to simulate various aspects of MS.

Understanding the specifics of how the T-cells may be responsible for preventing remyelination is critical to the success of MRF. It is similarly important to understand how oligodendrocytes can be protected from these effects. Simulating the role of the immune system in MS in animal models can be accomplished in two ways.

Immune disease models - A fragment of human myelin peptide, or a virus, is introduced into laboratory animals to stimulate an immune response. In some cases the resulting disease course is a series of episodes of rapid myelin loss followed by a period of remyelination and recovery. In other cases the loss of myelin is a single, continuous process. In either case, these models provide researchers with an opportunity to study how reactive T-cells affect oligodendrocytes, and develop strategies for protecting them from attack.

To that end, MRF researchers have been studying how these simulated diseases affect the production of common myelin proteins throughout the course of the disease. By developing this baseline understanding they will be able to monitor the effects of various therapeutic techniques. The first therapeutic agent tested is a compound that was first demonstrated to stimulate myelination in cell culture by MRF researchers. Preliminary studies in animal models with immune induced demyelination show a significant protective effect.

Whether this effect is preventing myelin destruction or enhancing remyelination is not yet known. Since the biological processes that are being affected may not be unique to oligodendrocytes or to the CNS, it is important to understand the specific pathway being affected and the underlying mechanisms of protection or repair. Once the relevant mechanisms are understood, it may be possible to exploit those mechanisms with more highly targeted therapeutic agents.

From immune disease models MRF researchers have demonstrated that inflammatory responses can be highly localized within the CNS. Until now the prevailing theory was that myelin fragments had to exit the CNS and find their way to the thymus or lymph nodes in order to stimulate activation of inflammatory T-cells, and that these activated T-cells had to migrate back through the blood brain barrier. Using animal models of disease, MRF researchers have shown that non reactive T-cells can move into the CNS, along with a special class of dendritic cells that use myelin protein fragments to activate them, resulting in local stimulation of the immune response and corresponding inflammation. Preventing the migration of these unique dendritic cells into the CNS is another possible therapeutic approach to minimizing the immune response in MS, which could contribute to myelin repair.

Models using inflammatory agents - In MS, and various immune induced animal models of the disease, reactive T-cells produce a repertoire of chemical signals during inflammation. The second way to study the role of the immune system in myelin repair is to independently test the effect of individual chemical signaling molecules produced by T-cells during the inflammation process. This can be accomplished by creating genetically engineered animals that produce a specific chemical that would ordinarily be released by T-cells during the inflammatory process, without creating an inflammatory disease. This is a critical tool in evaluating not only the effect of these molecules on oligodendrocytes, but for evaluating therapeutic agents to block their effects.

The first example of how this technique is helping the MRF Research Team identify new therapeutic targets is with a chemical called Interferon-gamma. Years ago Interferon- gamma was tested as a potential treatment for MS with very negative results. It is now known that T-cells produce Interferon–gamma as part of the inflammation process. By creating an animal model with controllable genes for producing Interferon- gamma in the CNS, MRF researchers have shown that after inducing demyelination, Interferon-gamma prevents remyelination by triggering the death of oligodendrocytes as they begin to produce new myelin.

By identifying the underlying molecular pathways and mechanisms behind this process it may be possible to develop targeted therapies to protect oligodendrocytes from the effects of Interferon –gamma.

5. What is unique about the structure of the Blood Brain Barrier, how is it disrupted in MS and how does this affect myelin repair?

The blood vessels in the CNS are unique within the body. Ordinarily the cells that line these vessels are tightly connected, preventing the flow of antibodies and T-cells from the blood stream into the brain and spinal cord. In MS, the tight junctions between the cells lining the blood vessels are selectively disrupted, allowing antibodies and T-cells to reach and attack the protective myelin. Understanding the structure of the blood brain barrier and how its integrity can be restored may be critical to preventing future myelin damage and allowing effective repair.

Members of the MRF Research Team have identified structural characteristics unique to the blood brain barrier. Based on those characteristics, they have isolated and identified adhesion proteins that are unique to the tight junctions between the cells that line the blood vessels. Armed with this knowledge they are working to identify mechanisms that cause the breakdown of these tight junctions and how they can be repaired. Repairing the blood brain barrier in MS could have a dramatic impact on promoting myelin repair.

6. What are the cellular and animal models necessary to understand these questions and evaluate potential therapeutic approaches to myelin repair?

Cellular models are important for studying biological interactions because they allow the study of specific cells outside the body without the environmental influences of the surrounding tissue. In these models individual genes can be controlled by the introduction of interfering RNAs or signaling proteins, in order to observe the direct effect on specific cells. The limitation of these models is that they do not represent the complexity of whole tissue or of the entire body. Tissue culture can be used to study the effects of manipulating biological interactions within a given tissue. This gives more insight into the systemic effects of a treatment and how its effectiveness is influenced by the surrounding tissue. This form of study allows outside control of biological processes that may not be possible or practical to control in animals.

Researchers also use animal models to test the effect of potential therapeutic agents in order to understand their effect on the entire body. In addition, animals are widely used to create imitations of human diseases. These models have the benefit of rapid development and progression of disease and allow detailed analysis of diseased tissues during the course of the disease. Unfortunately, no single animal model has yet been developed that is truly representative of MS. Therefore the MRF Research Team employs several existing animal models and is actively developing new models in order to gain a comprehensive understanding of the disease processes which promote or inhibit myelin repair.

Critical steps in the development of new models include:

1. Developing methods to purify and culture cells of interest at the appropriate levels of maturity, or during a disease course. In many cases, research has relied on the use of neonatal cells to study developmental cues and myelin formation. Since to repair myelin damage in MS we hope to stimulate adult cells already present in the CNS to produce new myelin, we must be able to evaluate the effect of any potential therapy using mature cells. New purification techniques developed by MRF researchers for adult oligodendrocyte precursor cells are critical to this process.

2. Creation of culture systems that allow prolonged observation of cellular interactions. Two MRF labs now have the capability of creating cultures that allow the observation of the myelin formation process over several days. This allows them to extract sample cells to analyze the underlying molecular process during myelination and to observe the effects of various agents on those processes.

3. Creation of multiple models of demyelinating disease. Since the underlying causes of myelin damage in MS are not well understood, it is important to expand our repertoire of inducible disease models to mimic multiple pathways. In addition, it would be beneficial to be able to distinguish new myelin from old when evaluating the effect of new therapeutic agents. MRF researchers are both expanding the number of methods for inducing demyelination in animals, and evaluating methods for labeling new myelin formation. One promising avenue for a new animal model comes from MRF's blood brain barrier research. If the blood brain barrier can be artificially compromised, will animals develop an MS-like disease? This is currently under investigation.

4. Creation of human cell and tissue culture systems. Ultimately any potential therapeutic target for MS must be evaluated using human tissue. Unfortunately, human tissue suitable for culture, from the CNS, is not readily available, so the conditions for maintaining it in culture are not well developed. MRF researchers are working to find new culture techniques suitable for human tissue, and we are starting to look for sources for human cells and tissue to support this future effort. This is a critical tool for validating potential therapeutic targets and evaluating potential therapeutic agents.