Myelin Repair Foundation

Collaboration. Acceleration. Results.

Myelin Repair Foundation
Research Summary
February 2007

This report on research progress by the Myelin Repair Foundation research team is divided into the six major areas of investigation included in the MRF Strategic Research Plan.

Section I – What causes neural stem cells to become oligodendrocytes, migrate to the right place, divide and mature in order to repair myelin damage? Does MS block this process?

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

Section III – How does myelin form, what is the structure that maintains properly formed myelin, how does it interact with the axon, and how is this structure and function affected by MS?

Section IV – How is the immune response involved in damaging myelin or preventing its repair?

Section V – What role does blood brain barrier breakdown play in MS and how can it be repaired?

Section VI – Since it is difficult to study these processes in humans, what new or improved animal models do we need to ensure our research provides answers applicable to MS?

A list of recent publications from the MRF research team can be found at the end of this report.

Section I – What causes neural stem cells to become oligodendrocyte precursor cells (OPCs) , migrate to demyelinated areas of the brain and spinal cord (CNS), divide and expand to provide a sufficient number of cells for repair, and then mature to the point where they become myelin-producing oligodendrocytes?

Section Leader – Robert Miller

The team continues to make good progress in understanding the principal steps in this complex process. The fate of neural stem cells in the CNS seems to be driven by the relative abundance of each type of the four major neural cells: neurons, astrocytes, microglia and oligodendrocytes. However, new oligodendrocyte precursor cells (OPCs) will not be formed unless the previously formed OPCs have moved away from the source in the spinal cord, and towards the areas of myelin damage. Thus, controlling migration has a profound impact on the generation of new OPCs. The migratory process is affected by both chemoattractants and chemorepellents. In order for repair to occur chemoattractants must be released at the site of injury and chemo-repellants must be suppressed.

In addition, once OPCs migrate to the site of injury, they must divide to provide an adequate population to repair the injured area and finally undergo terminal differentiation into myelin-producing oligodendrocytes. While the critical chemical signals for controlling these processes remains a challenge, several early candidates have been eliminated. An improved method for creating a single, controlled lesion in the spinal cord, and monitoring its repair, is being perfected that will allow the evaluation of several new possibilities. One thing is clear, if the repair process does not begin within days of the initial damage; astrocytic scars will prevent the migration, proliferation and differentiation processes necessary for myelin repair.

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

Section leader - Ben Barres

The team made several critical observations in this area over the last few months. First, additional studies confirmed that inhibiting an enzyme, gamma secretase, in cultures of neurons and OPCs stimulates the myelination process and that this is a separate step from the initial differentiation of OPCs into early oligodendrocytes. This study also determined that there is a short time period during which maturing OPCs are receptive to this stimulus and that if the proper signals are absent, mature but non-myelinating oligodendrocytes will result. This could explain why this type of cell has been found in studies of MS lesions. Experiments were also performed to determine if the ability to form myelin was based on the age of the OPCs or the presence of this stimulation. This study found that OPCs from mature animals are just as capable of being stimulated to form myelin in culture as very early cells.

Second, the team continues to identify components of the system that drives OPC differentiation in mature oligodendrocytes that produce myelin. One of the first critical observations is a mechanism that controls the number of times an OPC will divide before it enters the two final differentiation steps necessary to produce myelin. It appears that this “clock” must run out before the cell stops dividing and starts to produce myelin. Thus anything that prevents OPC division at the site of myelin injury is critical to myelin repair. Several possible mechanisms for regulating the two terminal differentiation and myelination steps have come to light and are also being investigated.

Section III – How does myelin form, what is the structure that maintains properly formed myelin, how does the myelin interact with the surface of the axon, and how is this structure and function affected by MS?

Section leader – David Colman

Our understanding of the myelin wrapping process and the resulting protein-lipid structure continues to expand by observing the myelin formation process microscopically in live Zebra fish. Our recently completed analysis of myelin proteins is enabling the creation of protein-specific labels to trace the location and movement of myelin proteins in order to determine their function. This information will be coupled with our emerging ability to selectively eliminate oligodendrocytes *In Vivo* by laser dissection of the cell body, observing how the myelin breaks down and how it repairs. These observations can then be compared with studies of human MS tissue using similar reagents to see how the process of myelin damage occurs, either via attack on the myelin itself or by damage to the cell body.

This work is complemented by a new research effort to identify MS biomarkers in cerebrospinal fluid or circulating blood. Using the protein analysis of compacted human myelin that MRF completed last year, several proteins novel to CNS myelin were identified. Even though these proteins were found at very low concentrations, since they are unique to the CNS, it is possible that when myelin damage occurs their release would stimulate an immune response. Thus, finding fragments of these novel proteins, or antibodies to these protein fragments, in the cerebral spinal fluid or circulating blood could be an early diagnostic indicator of MS activity. Furthermore, if the level of these markers can be monitored, it could indicate the relative effectiveness of myelin repair therapies. The MRF is actively evaluating partners with state-of-the-art analytical techniques to assist us in our search for biomarkers and methods for measuring them. We are also developing active partnerships with other organizations who collect a broad range of patient samples to be used in these studies.

Section IV – How is the immune response involved in damaging myelin or preventing its repair?

Section leader – Steve Miller

In earlier reports we showed that one critical component of the inflammatory immune response, interferon-gamma, appears to prevent myelin repair by triggering the endoplasmic reticulum (ER) stress response in oligodendrocytes. Ordinarily this ER stress response protects cells from damage but when the oligodendrocytes begin to produce massive quantities of myelin proteins, even a little additional stress is sufficient to kill them. Therefore we have been investigating ways to protect oligodendrocytes from anything that triggers the adverse effects of the ER stress response. Additionally, we have found that in adults the ER stress response can actually provide protection to oligodendrocytes against subsequent stresses through the activation of the integrated stress response (ISR), which is a general stress response. We have demonstrated this in mice by controlling a key gene necessary for ISR activation, and we have used brain tissue sections from mice to show that an experimental drug compound that prolongs the effects of the ISR can provide protection to oligodendrocytes against the adverse effects of interferon gamma. This project has now advanced to the point where a high throughput drug screen is being conducted at the National Drug Discovery Laboratory at the Harvard Center for Neurodegeneration and Repair to look for possible drug candidates that will have similar effects.

Previous MRF research progress reports discuss how dendritic cells have been found to penetrate the CNS in animal models of MS. These cells, along with memory and naïve T cells from the circulating blood that enter the inflamed CNS, result in the local activation of T cells specific for released fragments of myelin proteins called epitopes. This process is called epitope spreading and plays a major role in disease progression. Continued efforts have shown that myeloid dendritic cells are the primary subset present within demyelinated lesions and are responsible for activation of pro-inflammatory T cells that secret interferon gamma. Before we begin investigations into possible therapeutic approaches for targeting this critical antigen-presenting cell type, our animal studies are being expanded to include examination of human tissue from the Rocky Mountain MS Center to see if a similar pattern of myeloid dendritic cells is present in, or around, MS lesions. This study could provide considerable insight into the nature of the immune response in MS. Furthermore, selectively controlling their generation or migration, or their ability to stimulate a localized immune response, offers a new therapeutic approach to MS.

Continued investigations into combinatorial therapeutic approaches targeting *both* the underlying autoimmune response (using specific immunotherapeutic approaches) and promoting myelin repair for the treatment of established EAE have shown great promise. Combined short-term therapy using antibody fragments to block delivery of critical costimulatory signals required for T cell activation, along with a gamma secretase inhibitor which has been shown to promote myelination *In Vitro*, have demonstrated an ability to work synergistically to prevent disease relapses and promote recovery in mice with relapsing-remitting EAE.

Section V – What role does blood brain barrier breakdown play in MS and how can it be repaired?

Section leader – Ben Barres

To understand the role of the blood brain barrier (BBB) in myelin repair, it is important to understand how and when it is formed, and the function of neural cells in this process. For many years the dominant theory was that astrocytes in the CNS interacted with the cells lining the blood vessels in the brain in a unique way to create the blood brain barrier. This theory was supported by studies that showed intimate contact between the extensions or “feet” of the astrocytes and the cellular junctions of the blood vessels. Members of the MRF team have now shown that cells lining the blood vessels begin to form the BBB early in development, before there are significant numbers of astrocytes present. This study has shown that there are some very significant differences between the cells lining the blood vessels in the brain and those in other parts of the body. This observation is leading to studies that will determine if this is a result of differences in the cells themselves or other factors present in the brain beyond the presence of astrocytes. MRF researchers have identified a receptor unique to the cells that form the BBB and are currently investigating several signaling molecules that may play a critical role.

As further progress is made, this will enable us to examine tissues from MS patients and healthy controls in order to determine how the cells and/ or the signals are different. By focusing on unique receptors or signaling molecules, it may be possible to repair the BBB to retard the progress of MS and enhance the environment for myelin repair.

Section VI – Since it is difficult to study these processes in humans, what new or improved animal models are needed to ensure our research provides answers applicable to MS?

Section leader – Brian Popko

There are two main types of models used to study disease inside an animal (*In Vivo)* and outside the animal (*In Vitro)*. Both play critical roles in understanding fundamental biological and disease processes. Animal models are widely used for many reasons including shorter life span; ease of controlling genetic variability, and in the case of MS, the inaccessibility and limited availability of human tissue. The MRF team continuously works to refine existing models to more closely represent human disease *In Vivo* and specific molecular processes and effects *In Vitro.* In this section we highlight some of the most exciting areas of recent progress.

Purifying and culturing neural cells is a difficult, costly and time-consuming process. Minor differences in technique, materials or conditions can all contribute to the failure of an experiment or the inability to compare data from one experiment to another. Eliminating experimental variability is one major way to improve results and accelerate progress. While the MRF team has made great strides in developing novel culture conditions for neural cells, using these methods to test new drugs is tedious and somewhat subjective, as someone has to observe and measure myelination under a microscope. A reproducible, automated system for growing and measuring myelin, would be invaluable. To that end the MRF team is currently testing various artificial microfibers that can be used to support myelin growth. These fibers are coated with chemicals to mimic the surface of an axon, and different chemicals can be added to determine their role in this process. Similarly various signaling molecules and drugs can be added to see if they promote or retard myelin formation. While development of this tool is still in its infancy, early attempts to get oligodendrocytes to coat fibers have been successful.

The most common *In Vivo* models of MS are the Experimental Autoimmune Encephalitis or EAE models. Several different models exist since no single form or EAE has exactly the same characteristics as human MS. The MRF team continues to refine the use of these models for our research. Studies over the last two years have been conducted to measure changes in myelin gene expression during the course of disease in different models. We have continued to refine the techniques by increasing the number of genes being measured, from 10s to 100s, and decreasing the size of the tissue sections being studied. Most recently this technique has been further refined to incorporate laser dissection that will allow even smaller tissue samples to be taken from the center, edge and outside of a single lesion so gene expression can be compared between these three samples. This technique provides us with a strong analytical tool to measure the effects of various therapies in these models and eliminates much of the subjective nature of scoring systems based on symptomatic observation. In the future by applying this technique to human lesion samples we may be able to identify critical similarities and differences between MS and the current models and use this knowledge to create new models that more closely mimic the biology of MS.

RECENT PUBLICATIONS – The following publications acknowledged support from the Myelin Repair Foundation and the other members of the MRF research team.

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