Myelin Repair Foundation

Discovery through Collaboration

Myelin Repair Foundation
Research Progress Report – Spring 2006

Plans for the 2006-2007 Research year

The Myelin Repair Foundation research team continues to make excellent progress in understanding the biological processes that result in the formation of myelin, both in early development of the central nervous system and in repair after damage by disease. By carefully examining key steps in these processes they have been able to identify promising areas to seek new therapeutic targets and methods to measure the effect of potential treatments on those targets. This report highlights ongoing progress that led to the selection of the projects that will begin in July as part of the MRF's 2006-2007 research plan.

1. Investigations into the development of myelin-producing cells (oligodendrocytes)

What causes adult neural stem cells to become myelin producing oligodendrocytes is an area of ongoing investigation for the MRF research team. Gene expression studies led by Dr. Barres' lab have shown that oligodendrocyte precursor cells go through several distinct stages in the process of maturing and producing myelin. They have identified specific signals that cause immature oligodendrocyte precursor cells to differentiate, proliferate and a genetic program, in two distinct stages, that drives them to myelinate. But not all oligodendrocyte precursor cells are needed to produce myelin and thus about 80% will instead mature to a non-myelinating state. This may explain why these cells are observed in demyelinated plaques in MS.

2. How do axons induce oligodendrocytes to myelinate them?

This question is being answered collectively by investigators in the Barres, Colman and Popko labs. They have created methods for stimulating myelin formation in cell culture that can be observed via time-lapse photography. Using myelinating culture systems they have been able to observe the unique mechanics of the myelin formation process. Dr. Colman's lab has completed an analysis of the proteins in compacted myelin, and in conjunction with Dr. Eliot Peles from the Weizmann Institute, is developing tools to label specific proteins of interest. With these new labeling tools they can now begin to study the location and movement of key signaling molecules that control the process of myelin wrapping, spreading and anchoring, and clustering of sodium and potassium channels, on the surface of both the axon and the myelin. Once the key signals and their sequence of expression is more clearly understood, we can look at axons in demyelinated plaques to see which key signals may be missing and must be restored to affect repair.

3. How does disease activity prevent remyelination?

Understanding how MS creates an environment that is hostile to remyelination is critical to the success of MRF. This has been an area of exceptionally productive collaboration between the labs of Drs. Steve Miller and Brian Popko. Dr. Popko's lab has shown that oligodendrocytes are especially sensitive to stress caused by MS inflammation during myelin production. They have also shown that drugs or cellular therapies that protect oligodendrocytes from this stress promote remyelination. Dr. Miller's lab has shown that drug compounds identified by Dr. Barres that promote myelination in

culture, can be combined with various approaches to immunotherapy to promote remyelination. Dr. Miller has also identified a role for dendritic cells in promoting chronic myelin destruction.

4. How do we monitor disease activity and repair?

Dr. Steve Miller's lab is monitoring the changes in gene expression during both demyelination and remyelination in animal models of MS in order to identify new ways to measure disease activity and therapeutic effect. Dr. Popko's lab is working on a new genetically engineered mouse that will enable MRF researchers to identify when new myelin is being formed in previously demyelinated areas. These tools will provide insight into potential markers of disease activity. These candidates can be combined with the efforts in Dr. Colman's lab to look for fragments of unique myelin proteins, and antibodies to those proteins, in the blood of MS patients. Collectively these efforts could lead to new blood-based diagnostics or new methods for measuring effectiveness of myelin repair therapies. If these new forms of measuring disease activity are accepted by the FDA, it could significantly reduce the time needed to complete future clinical trials of new MS treatments.

5. How do we determine the value of new therapeutic targets?

Since historically, viable therapeutic approaches to over 90% of all targets are never found, it remains critically important that the MRF research team continues the search for additional targets while rapidly validating those it has already found. In our third research year, the efforts of the MRF research team will be equally divided between the searching for new therapeutic targets and validating the targets we have already identified.

A key objective of the MRF is to have a battery of evaluation assays that will enable us to rapidly screen each new target. In order to focus our efforts on finding the most effective therapeutic approaches for those that appear most promising. Since the identification of novel treatment targets for myelin repair is so recent, new methods for validating these targets, and potential therapeutic agents, must be developed to supplement animal models like EAE that have been used for years. The MRF research team is continuing its aggressive effort to develop new test methods using neural cells, CNS tissue and new *in vivo* disease models. For instance, the two current culture models that combine neurons and oligodendrocyte precursor cells will be expanded to include the third major type of brain cells, astrocytes. More sophisticated techniques will be applied to removing specific cells from specific lesions in order to perform gene expression assays. Spinal cord slices will be combined with various activated T cells to simulate the disease process. In addition the team is expanding its repertoire of methods for creating controlled demyelination that can be coupled with the new labeling techniques mentioned above in order to study the speed and effectiveness of various therapeutic approaches. Finally we are developing new models of the blood brain barrier to evaluate the potential of reparative therapies in that area.

Finally, during the third research year the MRF research team will explore using human tissue (blood, CSF and CNS tissue) for development of new assays to confirm the results of animal cell assays and monitor the effects of new therapeutic candidates.

Myelin Repair Foundation

Discovery through Collaboration

6. How do we promote myelin repair?

.

The MRF research team is actively working on validating targets and therapeutic approaches in these areas:

- Drugs and cellular therapies that protect myelinating oligodendrocytes from inflammation
- Drugs that promote migration of neural stem cells or oligodendrocyte precursor cells into demyelinated areas
- Drugs that promote proliferation of oligodendrocyte precursor cells in demyelinated areas
- Drugs that stimulate oligodendrocyte precursors to mature and produce myelin
- Compounds that stimulate the production of signals on axons that promote remyelination or suppress signals that inhibit myelination
- Compounds that stimulate repair of the Blood Brain Barrier

Since many promising new therapies fail in the clinical trials, creating a successful new myelin repair therapy may require the identification of more than 100 potential targets in order to find and validate 10 that are suitable for therapeutic development and clinical trials. , success may require combining several of these approaches. Thus we are committed to continuing to accelerate the pace of both discovery and validation.

7. Results to date – Targets and Publications

The MRF measures success by the number of validated targets that can be developed into new treatments but the young scientists who are a critical part of our research team are judged by the publications that result from their efforts. Because of the structure of the MRF Accelerated Research Collaboration™ model these measures are completely compatible. The MRF is committed to sharing the results of our research with the scientific community for the benefit of all neuroscience research. Because MRF encourages rapid validation and publication of results, we believe that the MRF research plan is accelerating the pace of publication of important new scientific discoveries.

New Therapeutic targets discovered by MRF scientists:

1. Target for stimulation of myelin formation and a therapeutic candidate
2. Targeting developmental inhibitor of myelination to stimulate repair
3. Targets for protection of myelin-producing cells from inflammation-induced stress
4. Targeting antigen-presenting dendritic cells to control immune response
5. Target and therapeutic candidate for driving proliferation of precursors to myelin-producing cells
6. Targets for repairing the blood brain barrier
7. New genetic target for control of normal myelin protein expression
8. Second target for control of proliferation of precursors to myelin-producing cells
9. Third target for driving proliferation of myelin-producing cells to promote repair
10. Development of new immunoregulatory strategies for specifically regulating myelin-specific autoimmune responses

New research tools developed by MRF scientists:

A. Identification of low-abundance proteins unique to myelin
B. Genetically-controlled animal model of demyelination
C. Culture system for controlled stimulation of myelination
D. Profile of gene expression during developmental myelination
E. Culture systems for extended observation of myelin formation
F. Purification method for adult oligodendrocyte precursor cells
G. Profile of myelin gene expression in immune-demyelinating disease models
H. Method for imaging cells surrounding the nodes of Ranvier
I. Method for observing myelin formation process in situ
J. Method for disrupting the blood brain barrier in new animal models

Publications:

The MRF is committed to advancing research as rapidly as possible through prompt publication of results. The MRF acknowledges and appreciates supplemental support provided to MRF investigators from the National Institutes of Health, and other non-governmental organizations, that contributed to the advances described in the publications listed below.

Myelin Repair Foundation

Discovery through Collaboration

Publications by MRF Scientists:

Roth AD, Ivanova I, Colman DR (2005) New observations on the compact myelin proteome. *Neuron Glia Biology 2, 15-21*

Huang JK, Phillips GR, Roth AD, Pedraza L, Shan W, Belkaid W, Mi S, Fex-Svenningsen A, Florens L, Yates JR 3rd, Colman DR. (2005) Glial membranes at the node of Ranvier prevent neurite outgrowth. Science. 2005 Dec 16;310(5755):1813-7. Epub 2005 Nov 17.

Gao, L., Macklin, W.B., Gerson, J., Miller, R.H. Intrinsic and extrinsic inhibition of oligodendrocyte development by rat retina. *Developmental Biology* 290:277-286, 2006. (no acknowledgements listed)

Dupree JL, Mason JL, Marcus JR, Stull M, Levinson R, Matsushima GK and Popko B: Oligodendrocytes assist in the maintenance of sodium channel clusters independent of the myelin sheath. *Neuron Glia Biology* 1: 1-14. 2005

Balabanov R and Popko B: Myelin Repair: developmental myelination redux? *Nature Neuroscience,* 8:262-264. 2005

Lin W, Harding HP, Ron R and Popko B: ER Stress Modulates the Response of Myelinating Oligodendrocytes to the Immune Cytokine Interferon-γ. *J. Cell Biol.* 169:603-612 2005

Lin W, Kemper A, Dupree JL, Harding HP, Ron D and Popko B: Interferon-γ Inhibits Central Nervous System Remyelination through a Process Modulated by Endoplasmic Reticulum Stress. *Brain* (2006) in press.

Balabanov R, Strand K, Kemper A, Lee JY and Popko B: Suppressor of cytokine signaling 1 expression protects oligodendrocytes from the deleterious effects of interferon-gamma. *Journal of Neuroscience* (2006) in press.

McMahon, E. J., S. L. Bailey, C. L. Vanderlugt-Castenada, H. Waldner, and S. D. Miller. 2005. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nature Med. 11:335-339.* (*highlighted in Nature Med. News and Views 11:252-253)*

Minter, L. M., D. M. Turley, P. Das, H. M. Shin, I. Joshi, R. G. Lawlor, O. H. Cho, T. Palaga, J. C. Telfer, L. Kostura, A. H. Fauq, K. Simpson, K. A. Such, L. Miele, T. E. Golde, S. D. Miller, and B. A. Osborne. 2005. Inhibitors of γ-secretase block *in vivo* and *in vitro* T helper type 1 polarization by preventing Notch upregulation of *Tbx21* *Nature Immunol. 6:680-688.*

Bailey, S. L., P. A. Carpentier, E. J. McMahon, W.S. Begolka, and S. D. Miller. 2006. Innate and adaptive immune responses of the central nervous system. *Crit. Rev. Immunol. 26:149-188.* (*Co-first authors)

McMahon, E. J., S. L. Bailey, and S. D. Miller. 2006. CNS dendritic cells: critical participants in CNS inflammation. *Neurochem. Intl. 49:195-203.* (*Co-first authors)