New Research Models –

Understanding how myelin is formed, how the process is disrupted by MS and how to stimulate repair requires disease models that mimic human biology. MRF researchers are actively working to identify new models that will allow us to understand and control the underlying biological process critical to myelin repair.

1. Myelin formation process (myelination):

The Popko lab identified two mutant mice with abnormal myelin formation that can help advance our understanding of the myelin formation process. In the first mouse a genetic defect prevents proper myelin synthesis. In the second, oligodendrocytes fail to mature properly resulting in dramatically reduced production of many common myelin proteins and abnormal myelin formation. In each case the lab has identified the gene responsible for these mutations which may be useful in identifying failures in myelin formation during repair.

The S. Miller lab is designing and conducting time course experiments in a relapsing-remitting model of MS to measure the expression of molecules identified by other MRF labs to be important in the control of myelin formation. This is a complex investigation as over a dozen targets for molecular control are being evaluated and each may only be transiently expressed. We hope to gain significant insight as to how the molecular cues that drive remyelination are similar or different than those that drive early myelin formation during development.

The Colman lab has developed an elegant system for time lapse observation of myelin formation in both the central nervous system and the peripheral nervous system of transgenic zebra fish. Because the fish are transparent, by adding a gene that produces a fluorescent protein during myelin formation they can observe the process from start to finish. In the next series of experiments they will create demyelinating lesions and observe if and how myelin producing cells migrate into the damage area, and if myelin is repaired in these areas.

2. Disease models to test myelin repair therapies:

An antibody has been identified by the Barres lab that breaks down the blood brain barrier and appears to result in the formation of demyelinated lesions. Animals treated with this antibody are currently maturing to see if they develop MS like symptoms with adult onset. This could result in a powerful new animal model for MS research. We plan to examine serum samples from MS patients for the presence of this antibody.

The Popko lab has begun work on a new animal model that incorporates a fluorescent gene in oligodendrocyte precursor cells. Expression of this gene can be chemically controlled allowing it to be “turned on” at a specific time point. Using this model, demyelination can be induced by a variety of methods and the gene then “switched on” so that all of the newly formed myelin is distinct from the previously formed myelin. This critical tool for assessing the efficacy of myelin repair treatments should be ready by the end of 2006.
The S. Miller lab has completed time course studies for expression of major myelin proteins in the brain and spinal cord in three disease models of MS in order to develop a baseline of the course of myelin damage and repair. In the next phase of this research they will expand this analysis to include specific genes of interest identified by other MRF labs and conduct comprehensive gene array studies on specific regions of interest. To do so they are developing techniques to isolate and test individual lesions and areas within lesions to identify localized changes unique to this area.

**Target Identification and Evaluation –**

Investigations to discover the biological processes that stimulate or prevent myelin repair are the principle focus of the MRF research team and drive the development of new models. From these investigations come new therapeutic targets that must be evaluated in a variety of different cellular and animal disease models before they can be considered for human trials.

1. **Development, migration and differentiation of myelin producing cells (oligodendrocytes):**

   The Barres lab identified a cell cycle inhibitor that may play a critical role in linking proliferation of oligodendrocyte precursor cells with differentiation into oligodendrocytes. Inhibition of precursor differentiation in MS plaques may be responsible for the failure to repair myelin.

   The R. Miller lab is expanding previous studies of the interactions between axons and neural stem cells that induce formation of oligodendrocyte precursor cells. Work previously conducted in chick models is now being conducted with rodent neurons from various locations to confirm the effects in mammals.

   The R. Miller lab has been investigating whether oligodendrocyte precursor cells must expand in number in order to migrate to the site of maturation and myelin formation, or vice versa. They have demonstrated that in order for oligodendrocyte precursor cells to multiply they must first migrate to regions deficient in these cells. These studies have shown that simply increasing the concentration of survival factors in the area of myelin damage will not stimulate repair. The chemical signals that drive cellular migration are also critical for expansion and ultimately myelin repair.

   The R. Miller lab has also shown that the presence of signals in demyelinated lesions that promote oligodendrocyte precursor cells to become type 2 astrocytes rather than oligodendrocytes. These may be critical factors in the failure of myelin repair in MS.

   The S. Miller lab is using fluorescently labeled neurospheres (as a source of oligodendrocyte precursor cells), both delivered directly into the CNS and delivered intravenously, to determine their ability to home to demyelinated areas and to differentiate into adult oligodendrocytes in mouse models of relapsing-remitting MS.
Myelin formation process (myelination):

Gene expression studies in the Barres lab have revealed a series of cell surface molecules that are dramatically increased when precursor cells differentiate into myelin producing oligodendrocytes. Studies are underway to selectively block the production of each molecule to determine which may control myelin formation or repair.

Experiments in the Barres lab using a culture system of retinal ganglia neurons and oligodendrocyte precursor cells for controlled myelination were expanded to include neurons from the dorsal root ganglia and the cerebellum. This demonstrates that the ubiquity of the underlying mechanisms. Further studies using interfering RNA to block expression of specific proteins are being conducted to identify additional therapeutic targets.

Additional progress has been made by the Barres lab on identifying the role of structural proteins unique to the blood brain barrier. The cellular source of the signaling molecule that induces production of key proteins has been identified. Additional studies are underway to isolate this signaling molecule in order to test its ability to repair the blood brain barrier and prevent migration of immune cells into the brain.

The Colman lab has completed a catalog of proteins found in compacted myelin from rats and a similar catalog has been generated using human myelin so the results can be compared. By comparing this list with data from other MRF labs, and by using a variety of screening methods, this list of over 700 proteins has been analyzed to identify a subset with functional or structural roles unique to myelin. Antibodies to these candidates will enable further examination of the location and concentration of these proteins during myelin formation and stabilization and may provide insight into which processes are being prevented in MS lesions.

2. Myelin repair:

Interferon gamma is an inflammatory molecule produced by reactive T cells. Previously the Popko lab demonstrated that even small amounts of Interferon gamma added sufficient stress during new myelin production to kill oligodendrocytes. The Popko lab has identified two potential approaches to protecting myelinating oligodendrocytes from the detrimental effect of Interferon gamma. The first involves stimulating a receptor gene that protects developing oligodendrocytes from Interferon gamma. The second involves modifying the stress response pathway to block the cell from effects of Interferon gamma. Both findings have potential therapeutic application in promoting myelin repair inside MS lesions.

The R. Miller lab has identified a chemical signaling molecule, and its associated receptor molecule, that inhibits migration and promotes expansion of oligodendrocyte precursor cells, and may play a key role in myelin repair. Experiments in an animal model where this receptor was eliminated showed protection from demyelination or enhanced repair, thus making it an attractive target for myelin repair therapies.

The S. Miller lab is testing the agent found to stimulate myelin formation in culture by the Barres lab, in a relapsing – remitting animal model of MS. Whether this agent increases myelin repair in animals models of MS is not clear at this time, preliminary data indicates the treatment does have a positive effect in blocking differentiation or precursor T cells into the pro-inflammatory Th1 pathway and lowering the number of immune cells entering the brain that cause inflammation. Further investigations of this effect in conjunction with other potential therapeutic agents are currently under study.