



# Biology of Demyelinating Disease

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## Chapter 1: Introduction

**Saud Sadiq:** Thank you, Lucien. The next talk is going to be given by Tim Pedley, chair of neurology, so I have to be done by 11, I'm not that important. So we'll rush through.

What I'm going to do is I want to emphasize a few slides which I'll take more time over, but the first few slides I'll really rush through. But if I go too fast you can wave your hands and stop me. I would prefer if questions are left to the end, unless they're very slight[ly] pertinent.

We're going to talk about multiple sclerosis essentially, and how we think demyelination results in MS. There is a lot of interest at present in repair and regeneration of myelin, and that has led us back to how myelin is formed and how it functions, and what happens in development. I've got a few slides of that, and it is in that context that I'll cover myelin structure and function. I'll mention a little bit of the pathophysiology of MS as we understand it, and the emerging pathological patterns, which help explain why patients respond differently to treatment. And I'll just kind of mention one or two slides on the biological basis of treatment, and on certain repair and regeneration strategies that are being introduced at present.



## Chapter 2: CNS Demyelinating Disorders

Just for the nonclinicians, multiple sclerosis is just one of several demyelinating disorders, but it's the most common so I'll just limit my comments to it. But [a] closely related disorder, acute disseminated encephalomyelitis, progressive multifocal leukoencephalopathy [PML], which received a lot of publicity in two main . . . with the AIDS epidemic and also subsequently when there was a new medication for multiple sclerosis, the two patients died with PML, so that got a lot of publicity with that. But there are rarer disorders like Marchiafava-Bignami disease, central pontine myelinosis, and B12 deficiency and lipoprotein deficiencies, and so on. The reason I brought that up is just to say that if you see white matter lesions in the brain on MRI it doesn't necessarily always mean that you have multiple sclerosis. There's also a huge differential of multiple sclerosis that I won't get into.

I don't think I provided slides, but the organizers of the talk have my talk on PowerPoint, so if you want it later I'm sure it can be arranged. And this just completes the list, prolonged cerebral enema, and several toxins, and demyelination can also be secondary to axonal degeneration, and there are a whole host of leukodystrophies and metabolic disorders mostly affecting the pediatric age group that can superficially, very superficially, resemble multiple sclerosis.

Just for again the nonclinicians, two or three slides to explain multiple sclerosis. It's the most common demyelinating disorder. It usually affects young adults. Women are affected about two to three times more than men, and that's worldwide. Pathologically, multiple lesions are seen, or scars, and these are typically of different age, so some new lesions; this is from autopsy material, you'll see that some lesions are newer than others, and some of the lesions are chronic, so there are lesions of different age; and there's both spatial and temporal separation, hence the title multiple sclerosis. Clinically most patients present with a relapsing remitting course, which now correlates with an inflammatory phase of the disease; and then later it becomes more of a neurodegenerative phase, and that's called secondary progressive. Less commonly we have patients who start with progressive phase, and in this population, which affects about 15% of all



MS patients, it's a 50-50 distribution of male to female, and usually starts a little later in age.

The diagnosis is based on the typical clinical picture, which I won't go into in detail, imaging and laboratory findings. The pathology, like I mentioned, is usually limited to the white matter, although the gray matter can be affected, but it's usually periventricular white-matter disease; and even if you look at the lesions here and there, you can determine that one is an older lesion than this one, which has more of a fleshy appearance. The pathological lesions that you just saw are exactly what you see on brain MRI in the live patient, with several lesions in the periventricular region.

On spinal fluid examination, 90% of patients will have distinct bands on electrophoresis, which are present in spinal fluid and not in serum, and these represent oligoclonal bands that first led people to postulate that immune interactions or immune hyperactivity or immune aberrations were important in the causation of disease. Now this is what you see in a normal individual, this is what you would see in an MS patient; but it's important to recognize that this is not limited to MS, and in other diseases, like in this instance SSPE [subacute sclerosing panencephalitis], you do get oligoclonal bands. The difference between this and MS is that these bands are IgG against the measles virus, whereas in MS the specificity of the IgG bands is not yet known. And in addition, in MS these are intrathecal and not present in serum, whereas in some of the systemic conditions you see bands in serum as well.

So that explains, this gives you a hint, that immune factors may be involved in the pathogenesis of the disease. Another factor that has to be taken into account is the genetic basis, and if you see the worldwide distribution of multiple sclerosis, the high areas are typically North American, western Europe—actually Europe—and in areas where people have settled from the British Isles, and in some areas are less affected. Now this map's not absolutely accurate because there's been underreporting from certain areas, especially in Southeast Asia where more and more MS is being seen, but nevertheless it's clear that there's a significant difference between genetic populations. There are several qualifica-



tions to this. Africans have a very low incidence or prevalence of MS, and when they migrated over with the slave trade to North America, patients who are born here have the same risk factor as people who migrated from Western Europe.

### Chapter 3: Myelin Structure and Function

Now going on, myelin and the anatomy and so on, myelin basically is one of the highest lipid containing membranes in the body: 75% of myelin is lipid, about 25 to 30% is protein. And if you look at the whole brain, this ratio is reversed in the brain, so it's two-thirds protein, one-third lipid; but myelin is two-thirds lipid and one-third protein. The major glycosphingolipids are seen here, lysophospholipid, glycosphingolipid, and cholesterol in myelin. The major proteins are myelin basic protein and PLP and DM20, which is the same as a homologue of PLP.

If you look at the proteins on a gel and separate it out there's a difference between the peripheral nervous system and central nervous system myelin proteins. Now when I first started doing this course about 10 years ago or longer, Lucien told me to stop worrying about the peripheral nervous system, so that's the only thing I'm going to say about the peripheral system. There is a lot of demyelinating disease, but the lecture just became too unwieldy so we just concentrate on MS right now. But if you separate the proteins there are some proteins that are shared, like MAG, myelin-associated glycoprotein; but some proteins are very distinct, and they are CNS-specific, like CNPase. Myelin basic protein is seen in both PLPs, only seen in the central nervous system, P0 is seen in the peripheral nervous system. And so there is a difference between the proteins in peripheral and central, and that explains clinically why certain diseases do not have clinical or central or peripheral correlates. The differences are in some part due to the different cells that myelinate the peripheral nerve, which is the Schwann cell, which myelinates a single axon, and the abundant proteins that I mentioned are P0 and BPP2 and PNP22; whereas in the central nervous system, it's the oligodendroglial cell which is a different type of cell from the Schwann cell. It myelinates several internodes and the protein content is different. Therefore when it comes to repair strategies in the peripheral nervous system and central nervous system those cells cannot interchange, and it would be



much easier if they weren't different cells from a repair strategies, because access to Schwann cells is much easier than getting oligodendroglial cells.

Just to see the development, and we'll cover this later as well, from stem cells you get basically astrocytes, neurons or a cell that shares—the precursor cell that the default setting of this cell is to do development of oligodendroglial cell. When it's exposed to serum, it becomes a type 2 astrocyte. There is a reason for that, the astrocyte and the oligodendroglial cell, at least the type 2 astrocyte, has a very close relationship with the oligodendroglial cell; and probably to some extent controls myelination and how oligodendroglial cell knows how many internodes it needs. Similarly in pathological terms there may be astrocytic pathology that has an impact on MS, and there is a greater interest developing in that regard. But then if the right signals come about, which we'll talk about later, this develops into a pro-oligodendroglial site and then an immature leukodendrocyte, and then a mature dendrocyte, which you can see here myelinating several internodes.

Normal CNS myelin the cytoplasmic extensions form the myelin, and a single cell may myelinate several internodes and axons. The number of internodes is axon diameter dependent, so that means the greater the diameter the more internodes will be myelinated. As I've said, the lipid content is 70%. Myelin participates not only in the conduction, which everybody knows about, but also in metabolism and ion transport, and it modulates the maturation and survival of axons. So in demyelinating the Schwanns, at least early in development, you can have an arrest, and in fact loss of axonal growth as well. So they interact in that fashion. Myelin facilitates rapid neuronal communication, as we all know, by saltatory conduction. I'll show you a couple of slides. It's an energy-dependent process. Just to see that, and you'll see that in the next several slides, unmyelinated fibers conduct electricity at 1 millimeter per second, usually depending on diameter, and this increases for the largest myelinated fibers by a hundredfold, which is important if we were going to evolve into large organisms, for the speed and for the diameter of axons would have had to be fairly enormous and unwieldy to transmit the same amount of information.



Myelin is also very compact, and that is important for its function. As you can see the oligodendroglial cell forming several dense compact myelin, and this is what normal myelin looks like. So when you see demyelinating disorders you'll see the unraveling of this structure.

Here you see from a rat, this is actually a rat embryo histogram which shows on the histopathological picture the oligodendroglial cell myelinating more than one axon. Again here you see that multiple internodes are myelinated, and then when you take a picture from here, from the node side, you can see the nodes of Ranvier are naked, and this allows for the conduction. The rest of insulin is really insulating against electrical transmission, so the current has to jump from node to node.

Sodium channels are found on this side, and potassium channels here, for repolarization, and that allows for the saltatory conduction. I'm sure all of you know this from school, in an unmyelinated fiber that's how fast the electricity goes, it's very pedantic and slow, and if you look at the myelinated fiber, it jumps so it's much faster. For those of you who missed it I've got two slides on that, I think.

Now this jumping of electrons is achieved by the sodium going in, potassium coming in, and both are important. It's energy-dependent, so the sodium concentration is important. This may actually be abnormal in conduction when patients get conduction block. So it may not just be just a purely demyelinating problem with loss of conduction, there may be also nodal pathology. And also, for those of you who are clinicians and have ever used things like thoramine or pyridine and patients then get better, that actually probably helps repolarization of demyelinated fibers by increasing the potassium concentration that's in the paranodal myelinated region. If you look at this one, that's what happens when you demyelinate, the sodium goes in, potassium changes, and then that goes out and allows for myelination and remyelination.

Essentially this is the summary slide of what I've just said. At less than 0.2 micrometers in diameter, there's no difference between myelinated and unmyelinated fibers, so the very small fibers will not be myelinated—there's no functional



advantage to that. But as the fibers get larger for the nerves in the legs, and it's going through the entire length of the spinal cord, the myelination has a clearly significant advantage for the same diameter. The reason why that is important is these are some of the factors we have to look into as you plan remyelination and repair strategies in patients who have had neurodegenerative phases of the disease.

#### **Chapter 4: Pathophysiology of Demyelination**

We think that basically there is a genetic predisposition based on the world map that I showed you, and several other factors which I'll go into the next time; and then there's primary triggers of inflammatory that result in auto-reactive cells being made; and then the disease can go into a quiescent phase until secondary triggers come about. I will go into details of each of these. There's increased blood-brain barrier permeability after secondary triggers have acted. Then when encountering the CNS site, vulnerability or antigen, there is amplification of the immune response, which goes into the inflammatory phase of the disease. If the inflammatory phase of the disease has not halted after several relapses and phases of that, it goes into a degenerative which results after several repeat relapses. With demyelination there's also axonal loss with scarring, and that probably is the most significant factor in disability.

The genetic susceptibility, there's racial, geographic differences, and epidemiological differences that support that; 20% of affected patients will have a positive family history, which is a fairly high number compared to diseases that do not have a genetic susceptibility. There's increased risk of MS in first degree relatives and offspring of affected patients, which is about the same thing as the second point. The concordance rate for monozygotic twins is about 25 to 30%, which implies that it's not a pure genetic disease and that environmental factors also play a role. But compared to the dizygotic twin rate it's about 2 to 5%. There's association and linkage to the class II haplotype listed there.

We don't know what happens after that, and the current general agreement is summarized in this slide. Basically you're genetically predisposed, and let me not



read the slide, I'll just tell you we think that there's some virus or some trigger—maybe herpes 6, maybe EBV—that everybody, 90% of the population in this room has. But if you are genetically predetermined to have an aberrant immune response to that very virus, then you may start a whole cascade of events that may make you at even greater risk to get MS. If you look at MS patients, for example, 100% of them have EBV antibodies, compared to 90% of the general population. That's why this comes about. When you see that your initial impulse is to say well, everybody gets EBV. That's true, but it's slightly different. And this was studies based on thousands of patients from military recruits when everybody had to go to the military and get tested for bloods; and they went back to those blood banks and see who developed MS, and each one of them had developed Epstein-Barr. What is curious is if you develop in a pediatric population they will also have Epstein-Barr antibodies positive, so it's not just at college age. If you are 14 and you develop MS and you test for EBV antibodies, you'll have EBV antibodies, provided you do it in a good lab, there'll be some exposure to EBV.

But herpes 6, there are several people who think herpes 6 is the virus, and there's equally persuasive arguments for that. It could also be that we are barking up the wrong tree and that's just a coincidental issue and there could be a bacterial super-antigen. But anyway, whatever happens there's processing of the foreign antigen and that was presented to naive T cells in the context of the genetic background of the patient; and that leads to a population of activated T cells that are formed, as well as probably auto-reactive B cells that involve CD40 ligand and CD40 interactions that further prime the antigen presenting cell. This population then, falling resolution of this initial trigger which may occur anytime in adolescence probably, there are memory B cells and T cells that exist in the body outside the CNS. At the time of this whole event, the patient may be, is usually in fact, undiagnosed and untreated and doesn't know that they have developed at least a significant susceptibility to developing MS. This probably is the greatest hurdle to developing new treatments for MS for researchers, because as you perform experiment[s] with the EAE [experimental allergic encephalitis] model you can actually start the disease and then start the treatments; whereas in MS clinically we just don't have any idea when the disease



started, so it's very hard to really plan treatment effectively because the time you get to the patient and plan those experiments, they've already developed multiple scars and so on, so it's a very different disease in the human being from EAE. Secondary triggers which may occur at several years, some of them remain undefined, but they include a whole host of very sort of vague, and almost one would say nonscientific, but clearly they're there. Stressor events lead to alterations in the immune system, and usually we're talking about chronic stress that the patients will also say that they're going through a terrible divorce and it's been going on for months, or some sort of thing, or medical student doing exams, or hasn't been doing well in school or whatever. Several patients will have secondary stressor events. They could have an infection, there could be hormonal turbulence changes, pregnancy is a time for increased susceptibility to developing demyelinating events. For patients who develop primary progressive MS or patients who have had a very good course of MS in that they will have had the disease diagnosed at 28 with optic neuritis—they'll probably not even go on any treatment, at least in the old days—will then have 10 years when they're completely well, 15 years will develop and go into menopause and subsequently will become severely affected. Clearly hormonal influences do have a role. There's been recent interest, and I think we'll be showing an abstract at the academy next week, that even in males and in females estriol levels may be correlating with disease worsening. Many patients will have nonspecific infections leading to relapses in the disease or starting the disease initially, and all these factors probably have an effect on the immune system, and that's how degeneration of escape of tolerance and development of disease. Certain vaccinations may be associated and have been associated in the past.

I was looking through some things for this talk and there's a very good article that I'm sure none of you can read this, so I'll just tell you that there's an article in the March 2 issue of the *New England Journal of Medicine* reviewed by Fromann on demyelinating disease—so for those of you who are neurology residents I would almost make it compulsory reading—that really summarizes most of the things that are going on in MS. This slide we'll repeat later and we'll go over the slides.



It's actually interesting. John Noseworthy did a review in 2000 which seemed like state of the art and we understood everything about MS. If you read that article and you read the article now in 2006 you'll see certain dogmatic things that were said in 2000 now are just complete bullshit. And so while I present this, I do it with some humility because it's not my slide anyway, but even though I present it, it seems very perfect right now, but I'm sure all of it is crap, or at least it may be crap. I mean we don't know that yet.

Anyway, going on, the next event that happens in disease, and this we have pretty good evidence for, so you can take it and say this is true—there is evidence of blood-brain barrier disruption in the inflammatory stage of the disease. Once you get the peripheral initiation of disease and the secondary triggers coming in, you do get activation and disruption of blood-brain barrier and the immune cells upregulate cell adhesion molecules of the blood-brain barrier expediting cell entry and activated lymphocytes from trafficking in. We know that because we have now had medications that were designed to stop this, and do so so effectively that even normal immune surveillance was affected. We also know this from MRIs, that you can see this patient with active disease, and this is an MRI with gadolinium enhancement, and you can see a very clear gadolinium enhancing lesion that's just indicating blood-brain barrier breakdown. In fact in this patient you can tell there's another periventricular lesion there that's enhancing as well. The blood-brain barrier, for those of you who haven't looked at it recently, is basically just made of those specialized endothelial cells with tight junctions and is under the control of several adhesion cell molecules that allow it either trafficking of certain cells and controls, even solute trafficking of most substances, and that provides for the privileged site for the central nervous system. The process of lymphocyte trafficking through the blood-brain barrier involves lymphocytes first rolling around the endothelial membrane; then getting arrested, usually with upregulation of the alpha-4, beta-1 integrin molecule or VCAM interactions with its ligand, and then firm adhesion involves other adhesion molecules, and then the transmigration follows from that. If this process is upregulated, that results in the increased trafficking of lymphocytes.

Now again mechanisms of the disruption are not completely elucidated, but



many of the inflammatory cytokines or chemokines at least in vitro models, such as an interferon gamma, interleukin 1-beta and TNF-alpha mediating this disruption, and the disorganized cell junctions decreases the solute barrier. There's also increased inflammatory cell adhesion and migration, there's increased expression of certain class II MHC antigens, there's also shedding of endothelial micropeptides to protect the blood-brain barrier integrity. And also MMP proteins, the metalloproteinases can be increased, and they're involved in dissolution of the blood-brain barrier.

All I said is summarized in this very busy slide, and I wish I could remember the reference for this, but it'll be in your PowerPoint and you can look at it and project it; it doesn't project very well. But basically it states everything that was stated in the last slide except in picture form, so you're not doing too badly if you just read the previous slide.

The central molecule that's involved in the immune hyperactivity is the T cell in MS, even though antibodies clearly play a role and microglial cells are involved in the final destruction of myelin, it's really the CD4 cell which in the murine model has been shown to be undifferentiated and then becomes a proinflammatory T cell or anti-inflammatory T cell. If IL4 is high, it prevents this. If IL12 and its family, IL23, 17, and so on then it results in a proinflammatory cytokine and the biggest cytokine that's involved in that differentiation of a proinflammatory CD4 cell is gamma interferon. Then that leads to further production of these inflammatory cytokines, and there's a positive feedback for that; whereas if IL4 levels are high that leads to anti-inflammatory T cell, that's a TH2 profile cell, and then all the interleukins that are anti-inflammatory get produced.

That is the basis of the next group of events that lead to the inflammatory phase of multiple sclerosis, which involves a high number of TH1 cells, and if TH2 or thyroid regulatory cells are deficient, then you get focal white matter lesions that lead to the relapsing remitting form of MS. What kind of pattern that leads to is dependent on what further interactions take place. Lucchinetti's work and Lassman's work in Austria have shown that there are four main patterns of MS that I'll come back to in the next slide.



The pivotal role of T cells in demyelination should be mentioned, and in the last slide I mentioned it, but this kind of summarizes the events. If IL12 levels are increased it promotes TH1 responses. Inflammatory T cells also help B cells to become autoreactive, and antibody production takes place which activates complement and further leads to the immune cascade activation. IL23, which leads to T cells to produce IL17 in the brain, and that promotes inflammatory demyelination. This is brain-specific. This has definitely been shown in EAE, may not be absolutely proven in human MS. And, as I mentioned in the last slide, once you get activated inflammatory T cells that leads to gamma interferon production, as well as lymphotoxin and TNFL and other cytokines activate microglial cells and complement. Complement then opsonizes further oligodendroglial cells that lead it to susceptibility to macrophage ingestion. The battles one sees with this are basically either a macrophage T cell dependent phase of disease, or a pattern of antibody mediated demyelination, but both of them are controlled by T cell activation as the initial response.

Once you get the cells coming across, and with T cell activation, you get CNS autoreactive T and B cells that were previously in the peripheral circulation now encountering the CNS autoantigen, and I have a list of possible antigens in the next slide. There's clonal expansion and secretion of the proinflammatory cytokines, like I've just mentioned, but the TH1 cells, and that then leads to a circular cascade of immune amplification of all the lymphokines and proinflammatory cytokines. Ultimately the event that's most damaging is macrophage activation and demyelination in most of the inflammatory phases of the disease. In certain of the degenerative phases that I'll show some slides about in the next few slides there's oligodendroglial pathology and also apoptosis, but that doesn't play a large role in the inflammatory phase of the disease.

The antigens that have so far eluded us to identification, but possible antigens include almost all the myelin proteins that are associated with the oligodendroglial cell that I mentioned. But we may be actually missing the target, because there's been increased interest in the type 2 astrocyte and in the molecules that are associated with that cell rather than the oligodendroglial cell.



There may be a bystander effect of oligodendroglial cell loss, if the pathology really is in astrocytes.

Microglial cell activation and demyelination is probably the key event that takes place in the final destruction of myelin. Proinflammatory cytokines that I mentioned in the previous slides activate the microglial cells, these release free oxygen, free radicals, and proteases that actually eat up the myelin, and cause the actual myelin loss. If it's unchecked it almost always leads to axonal loss, and that's what ultimately causes irreversible damage in MS. This again is a positive feedback loop of activation. This was taken from the Compson's book published this year, and T cell activation leads to activation of microglial cells that have a respiratory burst of energy and release of several noxious substances that activate and opsonize complement, further activate T cells by releasing cytokines that would further amplify this response, and then lead to antibodies being activated and antimyelin antibodies being formed, and then attack oligodendroglial cell. Ultimately the destruction of myelin requires T cell activation, then leads both complement and microglial activation with antibody formation in certain patients. This is a scanning microgram showing basically these activated microglial cells almost destroying this oligodendroglial cell.

## Chapter 5: Patterns of Disease

I talked about patterns of disease, and I'm going to go through this very quickly because it's received a lot of press for the last four or five years, and also for treatment purposes it may help you understand why some patients respond well to beta-interferons, and others respond well to plasma exchange or [inaudible] and so on. The point of the next several slides is that there are patterns of disease that appear in individual patients tend to hold true. Now there have been criticisms of this study, but I won't get into that today, because there were biopsy specimens, and by definition when you're picking those type of patients you're actually picking out patients with a different kind of disease, because the standard MS patient doesn't have a biopsy taken. The acute lesions in pattern one have CD4 positive cells. Pattern one and two you both see this acute infiltration with lymphocytes and macrophages that is very vascular, around vessels and venules. In the second pattern, which is related to the first pattern, you get anti-



body formation and plasma cells are around the active lesions. Complement activation takes place—and this is all slides from Lucchinetti's work and Lassman's lab in Austria and at the Mayo Clinic. You get complement activation, and then you result in the very sharp demarcation around the vessel wall of demyelination. This is a myelin stain, looks all blue, and this is demyelinated fibers.

The important factor in the first two patterns of disease is that remyelination is taking place at the same time. But if you look at proteolipid protein it's increased in the same areas where demyelination is occurring, which explains why you get recovery from acute events in the early phase of the disease. This is proteolipid in a dividing cell in the cytoplasm of oligodendroglial cell. Now if this disease is unchecked in the inflammatory phase and in certain patients that start with primary progressive that I mentioned initially, you may go on to just develop the persistent microglial activation, which results ultimately to diffuse demyelination and the neurodegenerative phase of the disease. In this phase what is important is that the treatments that we commonly use in the inflammatory phase, like beta-interferons and glatiramer acetate may not be as effective. In fact, steroids are also less effective in this phase of the disease. If you look at the pathology that's associated with the degenerative phase, that will explain some of that, because there's less inflammation, there's a distal oligodendroglialopathy, lesions do not surround blood vessels; so it's not an inflammation coming out of the venules, there's a loss of myelin associated glycoprotein; importantly there's an absence of complement, there's limited if any remyelination, and there is also apoptosis; all features that you associate more with the degenerative phase of the disease. The disease looks fairly different. If you look at myelin, we're staining here for MAG actually, there's a loss of MAG around the lesions, and it's an indistinct pattern, and it's not perivenular in demyelination in this phase.

This just features apoptosis and the things that are listed in the last slide. I'm trying to rush through because the first speaker was late and the next speaker is here, so I want to keep moving to some slides that I want to get into.

The fourth pattern of disease which is most commonly seen in primary progressive MS is associated with lesions that have sharp macrophage edges, so there



is macrophage activation, but there's a lack of complement, and in this phase of the disease there's never any remyelination and there's a normal myelin-associated glycoproteins. The difference between the third and the fourth is that there's no remyelination in the fourth pattern, and there is normal myelin-associated glycoprotein. It's not oligodendroglial disease as much as the third pattern is. Again the demyelination is almost like a cut picture, it's not perivenular, it's just this area is demyelinated and this area is not.

Both forms, the inflammatory and the progressive degenerative forms, are associated with axonal loss, and you can see this evidence of bulbing, which is associated with transected nerve, which is work by Bruce Trapp at the Cleveland Clinic. The amyloid precursor protein staining is positive confirming that. So when you come to chronic lesions of multiple sclerosis there is a lack of inflammation, there's demyelination, there's axonal loss, astrogliosis, and oligodendroglial cell loss. The node of Ranvier which I showed you earlier in the normal myelin is a completely distorted structure, and there's little if any normal myelin left. There are some areas of compact myelin but it's very little. This would not result in any functional myelinated conduction that you saw in the earlier slides.

The reason I keep bringing this up is it's very important if you can to arrest the disease in the inflammatory phase of the disease. When one is said that you're active in controlling the disease or you're somewhat aggressive, actually you've lost the battle if you let the patients go from the inflammatory phase of the disease into the degenerative phase, because once they get into this phase of the disease there is no possibility of getting them better or reversing, until we get some remyelination strategies working.

## **Chapter 6: Repair and Regeneration Studies**

I want to mention natalizumab [brand name Tysabri] because it's a new medication that was released and FDA approved at Thanksgiving of 2004, that in February 2005 was taken off the market, and now the FDA's expert panel, this week I think, has recommended that it be released onto the market. Basically it's the first molecule, it's a wonderful antibody that's directed against alpha-4, beta-1



integrin receptor molecule, that's expressed on activated white cells as they try to cross the blood-brain barrier and lymphocytes. It blocks adhesion of these cells and inhibits transmigration. Several trials, and these were published this week or last week in the *New England Journal*, four trials were published, and so I thought I'd put [it] in this slide just to tell you about it. It's given as a monthly infusion at 300 milligrams, and if it's prescribed right and there are no more cases of PML associated with it, because all the cases of PML were actually associated with combination treatment, it has the potential to become a first line treatment.

There are several genes that I'm going to go very quickly over in development that would have to be looked at in neural stem cell development if repair and regeneration systems have to be looked for. The most important probably would be the [inaudible] protein molecule here, Shh, which is involved in neural stem. Without that initiation of oligodendroglial cell, development doesn't take place, so some trials are looking at that, as well as blocking or dis-blocking NOTCH and PDAGF growth factors. That leads to oligodendroglial cell repair and regeneration. Now the reason why you focus on this is there is some evidence in multiple sclerosis that oligodendroglial progenitor cells are unable to mature past the progenitor phase. There are progenitor cells at the edge of myelin lesions, but they don't develop into mature oligodendroglial cells for some reason. We feel that looking at what transcription factors to turn on may provide an answer.

What are the strategies that we are looking at at many centers including my lab in animals models at least. One is to stop the disease activity. That is pretty obvious. And with beta-interferons and if we can use natalizumab and also other immunotherapeutic agents I think that can be accomplished in at least 80% patients with inflammatory disease currently. How long that lasts is still open to question. But then there's areas where you can to block neurite outgrowth inhibitors that would increase axonal sprouting. That's one strategy being used. There are several transcription and gene factors that block oligodendroglial cell differentiation, and if you can block these, like LINGO-1 and NOTCH they can all be used to promote oligodendroglial cell differentiation. Olig-1 activation, and there's also olig-2 which may play a role, but olig-1 activation, which is a tran-



scription factor for oligodendroglial differentiation, is also being looked at in animal studies to increase oligodendroglial cell differentiation. Certain stem cell growth factors that I just listed in the last slide are being looked at to increase neuronal survival in general, and oligodendroglial cell survival. There is some work going on with embryonic stem cell research. In our laboratory we are looking at whether autologous adult stem cell research is a feasibility. We are using a marmoset model and trying to see if that can be extended to human disease. Then the strategy that I think Eliot Frommann suggested in his *New England Journal* article last week was that probably a combination will be needed to provide for growth factors, inhibition of glial cells, scar tissue, as well as stem cell infusions or some sort of repair treatments.