ISOLATION, PURIFICATION MYELIN BASIC PROTEIN (MBP) AND GENERATION SPECIFIC ANTIBODIES AGAINST ITS

Azeeza Kheder YOUSIF

Master Thesis

BIOLOGY Department

Supervisor: Prof. Dr. Viktor NEDZVETSKYI

2017

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REPUBLIC OF TURKEY
BINGOL UNIVERSITY
INSTITUTE OF SCIENCE

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Dedication

I would like to dedicate this thesis to my Husband Honar has been a constant source of support throughout the years of my postgraduate study. In addition, I would like to thank my family, especially my mother and Brothers for constant support and encouragement.
To my Father
To my Husband

Azeeza Kheder YOUSIF
Bingöl February 2017

CONTENTS

ACKNOWLEDGEMENT…… ii
CONTENTS…… iii
LIST OF ABBREVATIONS…… v
LIST OF FIGURES…… vii
ÖZET…… viii
ABSTRACT…… ix

1. INTRODUCTION…… 1

2. LITERATURE REVIEW…… 4
   - 2.1. Study Myelin and in Characteristic of Myelin…… 4
   - 2.2. General Biology of Myelin…… 6
   - 2.3. Myelin Function…… 13
   - 2.4. Mechanism of Myelin…… 15
     - 2.4.1. Characteristic Myelin Basic Protein…… 17
     - 2.4.2. Myelin Protolipid Protein…… 20
   - 2.5. Biochemistry of Myelin…… 21
   - 2.6. Myelin Biogenesis, Structure and Cell Function…… 21
   - 2.7. Salutatory Conduction in Myelinated Nerve…… 23
   - 2.8. Myelin Pathology…… 24
     - 2.8.1. Multiple Sclerosis…… 24
     - 2.8.2. Remyelination…… 25
     - 2.8.3. Demyelination…… 26
   - 2.9. Oligodendrocytes Development…… 29
   - 2.10. Schwann Cell Development…… 30
   - 2.11. Oligodendroglia are Critical for Axon Function…… 31

3. MATERIALS AND METHODS…… 33
3.1. Methods of Isolation Rat Brain and Cleaning White Matter…… 33
3.2. Method of Centrifugation in Difference Organic Solvents of Delipidation Myelin Proteins…… 34
3.3. Solubilization Myelin Proteins with Acid Buffer…… 34
3.4. Method of Solubilization Proteins with Acid Buffer, Solubilization with Ionic Detergent Contained Buffer…… 34
3.5. Method of the Proteins Purification with PAAG Electrophoresis…… 35
3.6. Isolation MBP Protein According Molecular Mass from PAAG Should made with Stained Molecular Weight Markers…… 35
3.7. Method the Immunization Rabbits with Purificated MBP Antigen Should Passes Accordingly the Requests of Ethical Committee…… 35
3.8. Method of Animals Bleeding from Ear Vien is Passes Accordingly the Requests of Ethical Committee…… 36
3.9. Isolation and Purification Specific Antibodies Against MBP Realizewith 30% Sulphate Ammonium Precipitation…… 36
3.10. Testing of Antibody Specifity with Immunoblot Method…… 37

4. RESULTS…… 39
4.1. Isolation Myelin Proteins from Rat Brain…… 39
4.2. Immunization with MBP…… 39
4.3. Testing the Affinity and Specify Recognizing of MBP Antigens with Anti-MBP Antibodies by Western Blot…… 39

5. DISSCUSION…… 43

CONCLUSION…… 45
REFERENCES…… 46
CURRICULUM VITAE…… 50

ABBREVIATIONS

MBP	: Myelin Basic Proteins
PLP	: Proteolipid Protein
MOG	: Myelin Oligodendrocyte Glycoprotein
GalC	: Glycosphingolipid.
PNS	: Peripheral Nervous System
CNS	: Central Nervous System
APs	: Action Potentials.
SDS/PAAG	: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
MAG	: Myelin-Associated Glycoprotein
IDP	: Intrinsically Disordered Protein
IDP	: Polyproline Type II
PK	: Protein Kinase
S-L	: Schmidt-Lanterman
NF155	: Neurofascin
PMD	: Pelizaeus-Merzbacher Syndrome
MLD	: Metachromatic Leukodystrophy
MS	: Multiple Sclerosis
BBB	: Blood-Brain-Barrier
EAE	: Encephalomyelitis
NMO	: Neuromyelitis Visual
AQP4	: Aquaporin 4
O-2A	: Oligodendrocyteastrocytetype-2
SVZ	: Sub Ventricular Zone
OPC	: Oligodendrocyte Progenitor Cells
Shh	: Sonic Hedgehog
PMP22	: Peripheral Myelin Protein 22

SNP : Cyclic-Nucleotide 3’-Phosphodiesterase

PMSF : Phenylmethylsulfonyl Fluoride

Sc : Schwann Cell

MLD : Metachromatic Leukodystrophy

SPG2 : Spastic Paraplegia 2

LIST OF FIGURES

Figure 2.1. Schematic of mechanisms of MBP………………………………..….. 12

Figure 2.2. Primary demyelination may be caused either by genetic abnormalities affecting OLs or by inflammatory damage affecting myelin and OLs,as is the case in MS……………………..……..... 28

Figure 4.1. Result of electrophoresis in 15% PAAG…………………………..…… 40

Figure 4.2. SDS/PAAG Isolated membranes were purified MBP…………………………………………………………........ 41

Figure 4.3. Result of western blot with antibodies against MBP Dilution of antibodies – 1:2 (1-4) and 1: 5500 (5)……………………………….. 42

TEMEL MİYELİN PROTEİNİNİN (MBP) İZOLASYONU PÜRİFİKASYONU VE BU PROTEİNE KARŞI SPESİFİK ANTİKORLARIN OLUŞTURULMASI

ÖZET

Temel miyelin proteinleri (MBP) miyelin kılıfının oluşumunda önemli rol oynayan dış membran proteinleridir. MBP izoformları Oligodendrosit (OLG) ailesinde bulunan Golli geninden 14-21,5 kDa arası değişen büyüklükte kalıtılan sorunlu proteinlerdir.. Miyelin proteinleri merkezi sinir sisteminde (CNS) çok tabakalı miyelin kılıfın konfigürasyonunda önemli bir yere sahiptir. MBP'nin 18,5 kDa'lik klasik izoformu ensefalitojenik belirteç olarak 50 yıldan fazla bir süre önce izole edildi. Bu protein merkezi sinir sistemi miyelininin yapısına katılan bir proteindir ve electron mikrograflarında gözlemlenen ana belirgin line oluşturmak için oligodendrosit tabakasının sitoplazmik leafletlarını biribirine bağlar. MBP'nin bu önemli rolü MBP geni susturulmuş, CNS miyelin oluşumu çok az olan ve yapısal olarak tam olmayan mutant farede ortaya çıkarılmıştır. Bu çalışmada MBP proteini rat beyninden 600 mg doku homojenize edilerek izole edilmiştir. Rat beyin homojenatı suda çözünebilen proteinleri ayırmak için santrifüj edilmiştir. Homojenat 14000 rpm'de 60 dk santrifüj edilerek pellet deneyde kullanılmıştır. Lipidleri ayırmak için pelete methanol-aseton solusyonu eklenmiştir. 45 dk +4 C^0'de inkübasyondan sonra pellet 14000 rpm'de 30 dk santrifüj edilip pellet elde edilerek asit tamponu (pH=2) kullanılarak MBP ekstraksiyonu yapılmıştır. Elde edilen ekstrakt 60 dk +4 C^0'de karıştırılmıştır. Ekstrakt 14000 rpm'de 30 dk boyunca santrifüj edildikten sonra MBP'nin SDS/PAAG yöntemi ile pürüfikasyonu için kullanılmıştır. Gel polipeptidinden 18 kDa olarak elde edilen protein tavşanda bağışıklık oluşturmak için antijen olarak kulanılmıştır. Western blotta antikor titresi, bir membran üzerinde boyanan ana MBP polipeptidlerinin yoğunluğunu azaltmaksızın görünür sonuçlar için maksimum seyreltme olarak belirlendi. Anti-MBP antikorları için maksimum seyreltme oranı 1:5500 olarak çalışılmıştır. IgG fraksiyonunun pürüfikasyonu ile 18,5 KDa izoformunu içeren rat beyninden MBP'nin spesifik antikorları elde edilmiştir. Elde edilen MBP'nin spesifik antikorları miyelin üretim bozukluğu ile alakalı hastalıklar ve oligodendrosit patolojisi çalışmak için başarılı bir şekilde kullanılabilir.

Anahtar kelimeler: Temel miyelin proteini, anti-MBP.

ISOLATION, PURIFICATION MIELIN BASIC PROTEIN (MBP) AND GENERATION SPECIFIC ANTIBODIES AGAINST ITS

ABSTRACT

Myelin basic proteins (MBP) are extraneous membrane proteins that play an essential role in the original organization of the myelin sheath. The classic (MBP isoforms are inherently disordered proteins of 14-21.5 kD gain size emerging from the Golli Gene in the Oligodendrocyte Lineage) gene combination, and are accountable for configuration of the multilayered myelin sheath in the central nervous system. The ‘classic’ 18.5-kDa isoform of myelin basic protein (MBP) was primary isolated more than 50 years ago as an encephalitogenic determinant. This protein is an integral part of central nervous system (CNS) myelin, adhering the cytoplasmic leaflets of the oligodendrocyte (OLG) layer to each other to form the major dense line observed in electron micrographs. This essential role is manifestation by mutant mouse which has an ablation in the MBP gene, and whose CNS myelin is rare and relatively unstructured. In this study MBP was isolated from rat brain 600 mg brain tissue and homogenized. Buffer for homogenates contain the cock tail of the inhibitors proteases benzamidine, leupeptine and PMSF. Rat brain homogenate was centrifuged for a separation of water-soluble proteins. The rat brain homogenate centrifuged for 14000 rpm 60 min and after the pellet collect. This pellet mixed and resuspend with methanol-aceton solution for delipidation. Later 45 min incubation in +4 C^0 this mix centrifuged for 30 min 14000 rpm and after the pellet collect. Obtained pellet was used for extraction MBP with acid buffer pH 2.0. The extraction was performed 60 min at +4 C^0 with permanent mixing. After centrifugation for 30 min 14000 rpm this extract was used for isolation and purification MBP by SDS/PAAG. Extracted from gel polypeptide with Mm 18 kDa was used as antigen for rabbit immunization. The titer of antibodies in western blot was determinate as maximum dilution for visible results without decreasing intensity of main MBP polypeptides staining on a membrane. This maximal dilution for anti-MBP antibodies was 1:5500. Purification of IgG fraction resulted to obtain specific antibodies against pure MBP from rat brain containing mainly the 18.5 KDa isoform. Obtained specific antibodies against MBP may be successfully used for study demyelinated diseases and olygodendrocyte pathology.

Key words: Myelin basic protein, Rabbit Anti-MBP.

1. INTRODUCTION

Myelin basic proteins (MBP) are extraneous membrane proteins that play an essential role in the original organization of the myelin sheath. The classic (MBP isoforms are inherently disordered proteins of 14-21.5 kD gain size emerging from the Golli Gene in the Oligodendrocyte Lineage) gene combination, and are accountable for configuration of the multilayered myelin sheath in the central nervous system. The transcendent membrane-associated isoform of MBP is not quite a structural component of compact myelin but is highly post-translation ally modified and multi-functional, having connections with various proteins such as Ca^{2+}-calmodulin, and with actin, tubulin, and proteins with SH3-domains, whichever it can bind to a lipid membrane in vitro [Boggs JM. 2014]. MBP plays primary function in protein-protein interactions during membrane and cytoskeletal expansion and remodeling into glial cells. In addition to membrane-association, the 18.5 kDa, and all other classic isoforms are capable of connecting with a multitude of proteins, including Ca^{2+}-calmodulin, actin, tubulin, and SH3-domain containing proteins, and thus may be signaling lingers amid myelin development and remodeling. A highly conserved central domain presents an amphipathic alpha-helix in association with a phospholipid layer and contains threnody sediment that is phosphorylated by MAP-kinases. In multiple sclerosis, this segment represents a first immune dominant epitope. Here the helical structure is adjacent to a proline-rich region that gives a classic SH3-ligand, comprises a second mitogen-activated protein MAP-kinase phosphorylation place, and forms a polyproline type II helix. This domain of the protein is so essential to proper positioning of a protein interaction motif, with the local structure and convenience existing regulated by MAP-kinase. What's increased, the C-terminus of 18.5 kDa MBP has been identified by NMR spectroscopy as a Ca^{2+}-calmodulin-restricting site and is of note for having a high thickness of post-translational adjustments (Harauz and Libich 2009).

There is an N-terminal binding domain in MBP for Ca^{2+}-calmodulin, in extension to the primary site located in the C-terminus. This MBP Ca^{2+} action is sufficient for calmodulin caused actin depolymerization. These three domains of MBP represent molecular fragments with multiple roles in both membrane and protein-association. Protease cleavage of MBP with calpain has been extensively considered, its physiological function is poorly 'understood. The role of MBP in the pathophysiology of different conditions has been included, containing that of test allergic encephalomyelitis (EAE), an animal model of the demyelinating ailment multiple sclerosis (MS). In EAE, calpain deteriorates myelin proteins, containing myelin basic protein (MBP), proposing a role for calpain in the disruption of myelin in this disease. Subsequent studies revealed increased calpain activity and expression in the glial and inflammatory cells concomitant with loss of axon and myelin proteins. Here submitted an urgent part for calpain in demyelinating disease (Schaecher, Shields et al. 2001). Myelin is a cholesterol-rich augmentation of oligodendrocytes and Schwann cells (SCs) the plasma membrane, which renders as a specialized isolation sheath for axons in the nervous system. Myelin facilitates axon signal conduction through enabling "saltatory conduction. However, the significance of myelin in the central nervous system (CNS) is beyond its role in rapid signal conduction along axons as its disturbance also cause other severe functional and neurobehavioral disabilities. Myelin is essential for axon maintenance and function Perturbations of myelin structure and function or "demyelination" is connected with a long list of CNS pathologies from congenital and immune system issue metabolic disturbances Progressive demyelination also results in axonal degeneration due to the disarrangement of axon-oligodendrocyte signaling. An excellent cross talk between axons and oligodendrocytes is required to preserve the proper metabolic function of axons, trophic support, cytoskeletal arrangement, ion channel organization, and axonal transport. Axons become dependent on myelinating glia as myelin appears during the development. This concept was pretending PLP/DM20 insufficient mice where the absence of these essential myelin proteolipids resulted in axonal swellings only in myelinated axons with no evidence of axonal pathology in natural unmyelinated fibers established (Alizadeh, Dyck et al. 2015). Most oligodendrocytes are situated in white matter where their essential part is to frame myelin. The myelin sheath is a fatty insulation formed of the qualified plasma membrane that circles axons and improves the rapid and efficient conduction of electrical

impulses along myelinated axons. An only oligodendrocyte is competent of myelinating up to 60 different axons depending on the individual axon field and axon breadth. Myelination is fundamental for the natural functioning of the mature CNS. Disruption of CNS myelin through injury, pathological degeneration, or genetic intervention leads to severe functional deficiencies and commonly a decrease in the lifetime measure. Even focal myelin loss, as occurs in demyelinating diseases such as multiple sclerosis, results in a rapid loss of neurologic function (Aggarwal, Snaidero et al. 2013).

The aim of this study is the design and probing of new method of isolation MBP and generation high affinity antibodies against this neurospecific antigen.

2. LITERATURE REVIEW

2.1. Study Myelin and Characteristic of Myelin

The word Myelin was coined by German pathologist Rudolf Ludwig Virchow (1821–1902), author of the fame Neuroglia term. There was much confusion mid- 19th century regarding the medullar substance, and Virchow expressed the need for a better terminology in Virchow's Archive, the journal he founded “das Bedürfniss, size it line Worte beechen Tzu können, earliest, so schlage rich vor, um jede Verwechselung mit and even school bezeichneten, Abler not problematical Substance Tzu vermin, sir Markoff, Myelin Zu Brenan. The necessity exists of being able to identify aword, so I suggest, to prevent any confusion already created by others and avoid more problematic substances, to name the marrow material myelin.” Myelin derives from the Greek myeloid after bone marrow color and texture. Nevertheless, Virchow article entitled “On the disseminated occurrence of a substance analogue to nerve marrow in animal tissues” suggested myelin was quite a floating term, and discussed a substance found in sicken lung and other tissues. In his landmark textbook on cellular pathology. gave myelin wide exposure, but did not strictly anchor it to the nervous system From blood cells, from pus-corpuscles, from the epithelial cells of the most various glandular parts, from the interior of the spleen and similar glands unprovided with excretory ducts, this substance can in every case be obtained by extraction. It is the same substance which forms the principal constituent of the yellow mass of yolk in the hen's egg, whence its taste and peculiarities, especially its peculiar tenacity and viscidity which are employed for the higher technical purposes of the kitchen, are familiar to everyone. It is this substance, for which I have proposed the name of modularly matter (Mark off), or myelin, that in extremely large quantity fills up the interval between.

The axis-cylinder and the sheath in primitive nerve-fibers. The following decade showed an occasional use of myelin for equally various attributions. The discovery a decade later of osmium stain changed everything. Max Schultze (1825–1874), Professor in Bonn and friend of Otto Deiters published in the first issue of his journal that a weak solution of prosaic acid was excellent to contrast and harden tissues "Next after the fats are the nerve marrow, which takes exceptionally quickly osmium staining…A nerve cord freshly taken from the animal turn's deep blue-black. Bundles of medullated fibers, which are embedded in gray matter, or individual fibers that were previously hard to see, come forth now with great sharpness It is obvious that with the help of this staining method some important questions will be solved, and that a great future lies ahead for the osmium staining in the anatomy of the brain and spinal cord". Osmium staining was disseminated further through a chapter in the wildly popular Sticker Handbook. A decade later, Ranvier in his seminal 1875-1885 and 1878 books published crisp illustrations of osmium-stained myelinated axons. Ranvier, technological wizard who devoted several chapters to the study of osmic acid, "a reagent of paramount importance for the study of the nervous system" (1878), gave the key why myelin thereafter was not confused with other tissues (1875-1885) Osmic acid is not only used to harden tissues but moreover to color certain elements in more or less intense black, with brown or blue hues. Hence myelin is painted in bluish black and the fat in brownish black." Ranvier mentioned that despite osmic acid fumes were toxic and dissections carried behind a glass wall to avoid conjunctivitis, all histologists used it. Giving beautiful black and white renditions, osmium was far superior to Carmine and had another welcome property to retain the natural anatomy, preventing myelin 'coagulation' in globules. Obviously, with a great future, osmium is used to this day in electron microscopy. Chair of Anatomy at Paris College de France, was interested as much in structure as in function, having trained with homeostasis physiologist Claude Bernard. He loved experimenting on myelin, studying its swelling and disheveling in water, and observed that water stopped nerve conduction. Ranvier was struck by the beauty of raw myelinated nerves he compared to silk having the a wavy lustrous finish white, more or less opaque, shimmering and sparkling as moiré." Ranvier mastered many staining techniques, including classic carmine used by Austrian ophthalmologist Ludwig who first reported the concentric organization of myelin and his eponymous per axonal sheath underneath myelin.

Using Carmine and silver nitrate in mouse thoracic and rabbit sciatic nerves, Ranvier noticed myelinated axons were stained only at regular intervals. He realized myelin had periodic gaps allowing stains to access the axon. Intrigued, Ranvier used a powerful lens to dismiss a possible artifact:" Indeed, under 800 diameter magnification, the nerve tube construction seems formed by a small convex ring confounding itself with the Schwann membrane that I shall designate by the name of constricting ring (annual constrictor), and especially used osmic acid to confirm that myelin was absent at the level of the node. Osmium-blackened myelin glaringly revealed Schwann nuclei nested in the sheath. Surprised, Ranvier determined two important facts: 1) there is only one nucleus per internode, and 2) located at equal distance from each node. Ranvier, a sagacious observer, particularly tracking staining artifacts, realized both sheaths of were not simple envelopes but contained a sheet of cytoplasm running from node to node. The question of whether there was cytoplasm in this sheath was not trivial, because there was no specific stain for cytoplasm at the time. Ranvier hence boldly envisioned the axon enveloped by a muff of cytoplasm with the finite boundary at the nodes. All cellular elements being present: plasmatic membrane, single nucleus and cytoplasm containing myelin, allowed Ranvier to formulate the hypothesis that an internode equated to a sort of elongated adipocyte secreting myelin inside its cytoplasm. In subsequent textbooks, he greatly expanded the analogy with a fat cell pierced by the axon. He speculated further, to the disbelief of his peers, that Schmidt-Letterman incisures were remnant cytoplasmic bridges between the Schwann and the Mouthier sheaths after myelin secretion had completed and filled most the cytoplasm. Ranvier revolutionary hypothesis of a fat cell rolled around the axon paved the way toward the Schwann cell (Boullerne 2016).

2.2. General Biology of Myelin

Myelin is a cholesterol-rich extension of oligodendrocytes and Schwann cells (SCs) the plasma membrane, which renders as a particular insulation sheath for axons in the nervous system. Myelin facilitates axon signal conduction through enabling "saltatory conduction" However, the prominence of myelin in the central nervous system (CNS) is behind its role in rapid signal conduction along axons as its disturbance also cause other severe functional and neurobehavioral disabilities. Myelin is prominence for axon maintenance and function (Nave and Trapp 2008).

Perturbations of myelin structure and function or “demyelination” is associated with an extensive list of CNS pathologies from congenital and autoimmune disorders to metabolic disturbances (Love 2006). Progressive demyelination also results in axonal degeneration due to the disruption of axon-oligodendrocyte signaling. A good cross talk between axons and oligodendrocytes is required to maintain the suitable metabolic role of axons, trophic backing, cytoskeletal arrangement, ion channel organization, and axonal transport (Nave 2010). Axons become ancillary myelinating glia as myelin seems during the development. This notion was explained in PLP/DM20 defective mice where the deficiency of these fundamental myelin proteolipids produced in axonal swellings only in myelinated axons with no evidence of axonal pathology in healthy unmyelinated fibers (Alizadeh Dyck, et al 2015).

The ‘classic’ 18.5-kDa isoform of myelin basic protein (MBP) was primary isolated more than 50 years ago as an encephalitogenic determinant. This protein is an integral part of central nervous system (CNS) myelin, adhering the cytoplasmic leaflets of the oligodendrocyte (OLG) layer to each other to form the major dense line observed in electron micrographs. This essential role is manifestation by the shivered mutant mouse which has an ablation in the MBP gene, and whose CNS myelin is rare and relatively unstructured. The compact myelin phenotype can be “rescued” in these mice transgenically. The 18.5-kDa isoform, which dominates in adult CNS myelin is thus generally considered to be essential for its development and stability. And forms a ‘molecular sieve’ that limits the spread of some layer proteins from paranodal loops into compact myelin. This protein is just an insignificant element of peripheral nervous system (PNS) myelin. The 18.5-kDa MBP has been expression an ‘executive protein’ because of its necessity for CNS myelin formation, unlike other fundamental myelin proteins such as proteolipid protein (PLP) and myelin-associated protein MAG (Harauz and Boggs 2013).

Membrane adhesion – should be a subject of investigation for decades, and researchers advance to analyze molecular features of how this protein is placed inside two membrane bilayers to form the major thick line of myelin. The internal circular of the myelin membrane, where MBP is found, has a complex installation than the outer leaflet, and contains roughly 44% cholesterol, 27% phosphatidylethanolamine, 13%

phosphatidylserine, 11% phosphatidylcholine, 3% sphingomyelin, and 2% phosphatidylinositol, as predestined from biophysical measurements of surface charge. The MBP-lipid interactions rely on equilibrium communications between the fundamental debris of MBP and the acidic head groups of the lipid bilayer, to assemble the proper multilamellar structure seen in myelin sheaths. Of note also is the relatively high proportion of phosphoinositides, particularly phosphatidylinositol-4,5-bis-phosphate (PI(4,5)P2). In vitro and cellule, MBP has been shown to sequester PI (4,5)P2 in membranes. Cholesterol is also important for myelin formation and stabilization and specifically facilitates 18.5-kDa MBP's ability to stack membranes (Harauz and Boggs 2013). Myelin basic protein (MBP) is a necessary protein of myelinated axons. The sequence of MBP predicts the formation of an intrinsically disordered protein carrying a large positive charge, which allows for many protein cover interactions(Harauz, Ladizhansky et al. 2009). This monarchy is exclusive for MBP and is not known in other considerable myelin proteins, such as myelin oligodendrocyte glycoprotein (MOG) or Myelin proteolipid protein (PLP). To preclude MBP from nonspecific binding to molecules, MBP mRNA assembles into ribonucleoprotein complexes termed "granules" which are targeted into oligodendrocyte extensions and translated in-situ. Consistent MBP-membrane communications rely on a precise stoichiometrical equilibrium among the central residues of MBP and the acidic headgroups of the lipid bilayer elements, as well as on copper ions inducing the compaction of the MBP structure. This proposition that reduced myelin stability may relate to changes in levels of diverse myelin components, including designated lipids and their oxidized products, (Ferretti and Bacchetti 2011) copper ions or expression levels of MBP. Upon demyelination, MBP may either rapidly degrade or otherwise aggregate with other myelin components (Frid, Einstein et al. 2015). In the CNS, the major myelin proteins constitute myelin PLP(proteolipid protein) and the associated product DM20, MBP(myelin basic protein), MAG (myelin-associated glycoprotein) and CNP (29,39-cyclic nucleotide 39-phosphodiesterase). PLP and MBP are the divide within the myelin sheath and resemble to be essential to the method of compaction that generates the closely apposed multilayered structure of mature myelin. MAG, on the other hand, is located in the per axonal district and may attend to promote cell–cell interactions between myelin and axonal membranes during the establishment of myelination. CNP is understood less well. Although it exhibits enzymatic activity in vitro, substrates for this action have not been

identified within the myelin sheath, and it has been proposed that this molecule may instead perform a structural role in the myelin membrane (Fulton, Paez et al. 2010). It has been shown in varied in vitro studies that MBP associates immediately with cytoskeletal proteins (actin and tubulin), Ca_2-activated calmodulin (Ca_2-CaM), Src homology 3 (SH3)-domain containing proteins (23,24), and divalent metal cations, and either directly or indirectly with voltage-operated calcium channels. Furthermore, MBP has been conferred to interact concurrently with action and lipid cysts, thus supporting its proposed role in the regulation of the actin cytoskeleton, and in securing it to the inner shiverer mouse (which lacks MBP), actin filaments resembled to be disordered, and the cell methods were less than normal with a larger cell body. Our recent studies with cultured oligodendrocytes have shown that the 18.5-kDa MBP isoform partners with the cytoskeleton through phoebe ester-induced membrane ruffling, and that SH3-ligand modifications of MBP result in attenuated affinity for Fyn kinase and change process differentiation and protein localization. Collectively, these diverse considerations suggest that the communication of MBP with cytoskeletal and signaling proteins is crucial for oligodendrocyte function, and myelin formation and maintenance (Bamm, De Avila et al. 2011).

Myelin degeneration happens after insults to CNS, such as chemical toxicity, traumatic brain injury, and demyelinating disease. Demyelination will induce undesirable inflammation and cell death. Myelin-associated proteins are released after a destruction of the intact myelin sheath. Between them, No go, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein have been classified as inhibitors of neuronal increase by binding to either the No go receptor or to a second receptor, Pire. After the removal of the entire myelin sheath, MBP also disengages from the plasma membrane and acts in a free, membrane-unbound manner in the extracellular pattern. MBP has been well-studied as an antigen to stimulate immune acknowledgment everywhere the nervous system, which induces immune injury, such as in multiple sclerosis (MS). Former studies have also shown that dissolvable MBP changes the compose of platelets, crashes synthetic layer and acidic lipid cysts, excites the generation of astrocytes and Schwann Cells and depolarizes the neuronal layer. However, the effect of MBP on neurons is still largely unclear. (Zhang, Sun et al. 2014).MBP plays important role in protein-protein interactions during membrane and cytoskeletal extension and remodeling in glial cells. In

addition to membrane-association, the 18.5 kDa, and all other classic isoforms can interact with a multitude of proteins, including Ca^{+2} calmodulin, actin, tubulin, and SH3-domain containing proteins, and thus may be signaling linkers during myelin development and remodeling. A highly conserved central domain presents an amphipathic alpha-helix in association with a phospholipid membrane and contains a threnody residue that is phosphorylated by MAP-kinases. In multiple sclerosis, this segment represents a first immunodominant epitope. This helical structure is adjacent to a praline-rich region that presents a classic SH3-ligand, comprises a second MAP-kinase phosphorylation site, and forms a polypro line type II helix. This domain of the protein is thus essential to proper positioning of a protein interaction motif, with the limited amendment and accessibility being modulated by MAP-kinases. Also, the C-terminus of 18.5 kDa MBP has been identified by NMR spectroscopy as a Ca^{+2} -calmodulin-binding site and is of note for having a high density of post-translational modifications. There is an N-terminal binding domain in MBP for Ca^{+2} -calmodulin, in addition to the primary site located in the C-terminus. This MBP Ca^{+2} activity is sufficient for calmodulin induced actin depolymerization. These three domains of MBP represent molecular recognition fragments with multiple roles in both membrane- and protein association. Protease cleavage of MBP with calpain has been extensively studied; its physiological function is poorly understood. The role of MBP in the pathophysiology of various diseases has been implicated, including that of experimental allergic encephalomyelitis (EAE), an animal model of the demyelinating disease multiple sclerosis (MS). In EAE, calpain degrades myelin proteins, including myelin basic protein (MBP), suggesting a role for calpain in the breakdown of myelin in this disease. Subsequent studies Cells concurrent with the loss of axon and myelin proteins. This suggested a crucial role for calpain in demyelinating diseases. There are limited numbers of the methods for isolation and purification MBP for animal immunization for the purpose of generation-specific antibodies against MBP epitopes. All methods use porcine or bovine brain tissue and direct extraction by buffers containing different concentrations of sodium chloride, cation exchange chromatography, buffers containing glycine, and salts .or phospholipase C and calcium chloride solution. myelin is an asymmetric multilamellar membrane wrapped around the axons of the central nervous system (CNS) and consists of alternating extracellular and cytoplasmic leaflets. The bilayer-associated proteins, mainly myelin basic protein (MBP) and proteolipid protein. Any structural changes of the myelin sheath

in the CNS, containing tumor accumulation, destruction of adhesion, expanding of the water gaps, vacuolization, vesiculation, and complete delimitation (demyelination) of the myelin sheath, are indications of several provocative neurological disorders. These types of unrest characterized by a perfect rainbow of neurological manifestations, such as physical and cognitive inabilities, with multiple sclerosis (MS) being one of the substantial common demyelinating diseases. The preponderant size and charge isoform of MBP in natural and overripe myelin has a molecular weight of 18.5 kD and a net positive arraignment of 19.Several studies conducted with model and extracted myelin bilayers confirmed that because of its high content of positively arraignment residues, MBP binds to the negative arraignment lipids of the cytoplasmic circulars of the bilayer via electrostatic interaction in addendum to hydrophobic communications (Lee, Banquy et al. 2014). One primary function of MBP is to bring the defending cytoplasmic surfaces of the myelin membrane closely together. Within the cytoplasmic space, MBP forms a size-selective barrier, which prevents the diffusion of most soluble and membrane proteins into the myelin sheath. Here, we address the underlying principles of MBP assembly (Aggarwal, Snaidero et al. 2013). The blood-brain-barrier (BBB) divides the CNS from the perimeter. It is kept by tight joining proteins inserted endothelial cells, as well as by communications with astrocytic end feet, pericytes, microglia, and some neuronal components. The permeability of the BBB is arbitrated if tight junctions are disrupted. BBB breakdown has been correlated with aggressive demyelination in rats with EAE. In mice, illness severity in the acute phase of EAE was directly related to the degree of BBB permeability. In MS, the disorder of the BBB precedes the formation of demyelinating lesions, and development of clinical signs. A disrupted BBB may be one mechanism by which MBP can leave the brain and enter the periphery to interact with vascular endothelial cell (D'Aversa, Eugenin et al. 2013).

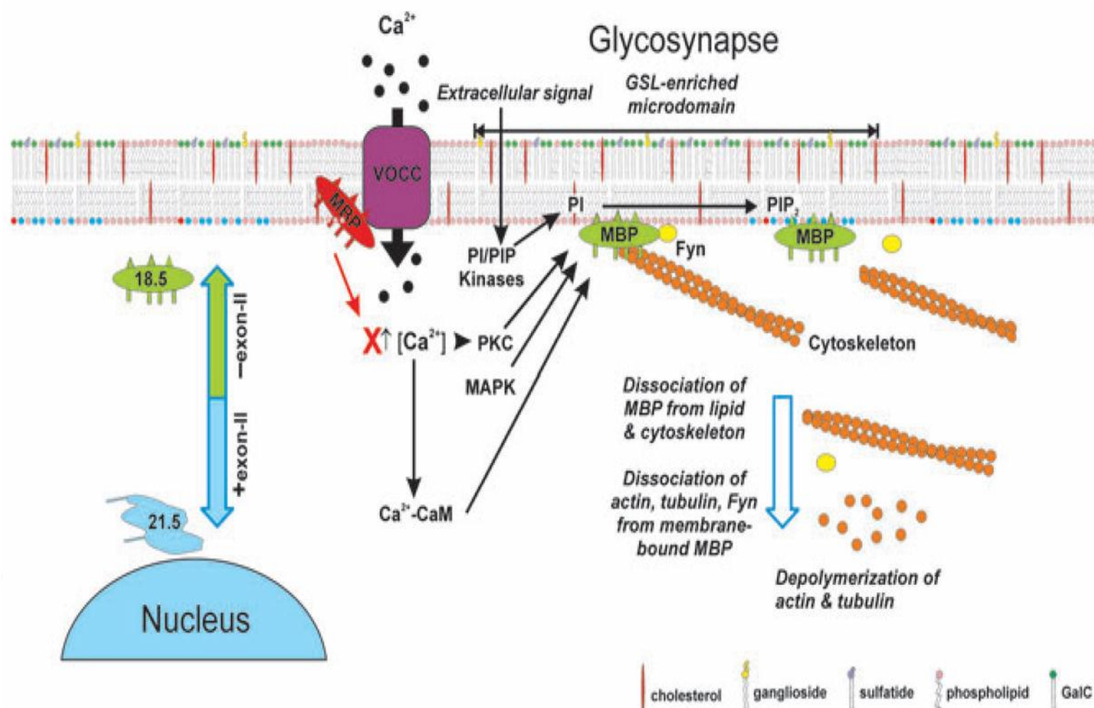


Figure 2.1. Schematic of mechanisms of MBP Interactions

(1) trafficking of the classic 18.5-kDa myelin basic protein (MBP) isoform (green) to the plasma layer and the classic 21.5-kDa MBP isoform (blue) to the nucleus; (2) roles of the conventional 18.5-kDa MBP isoform as a membrane scaffolding protein; (3) regulation of binding of actin filaments, microtubules, and SH3- domain-containing proteins to MBP in oligodendrocytes; (4) coordination of Ca^{+2} entry through voltage-operated calcium channels (VOCC) channels; and (5) of device of extracellular beacons to the cytoskeleton via MBP. The lipids are described as results: PI (4,5) P2 (blue head group), other phospholipids (pink head group), GalC (green head group), sulfatide (purple head group), gangliosides (yellow head group), cholesterol (red rod). Classic MBP that is bound to negatively charged lipids [phosphatidyl-serine and phosphatidylinositol (PI)] on the cytosolic circular also binds actin filaments to the membrane. Extracellular signals that impinge on GSL-enriched lipid rafts or layer signaling domains in the oligodendrocyte (OLG) membrane, such as those imparted by anti- GalC/sulfatide antibodies or by GalC/ sulfatide-containing liposomes, as explained in the text, cause Ca^{+2}-entry (through VOCC or other Ca^{+2}channels) and/or stimulate PI/PIP kinases such as PI4K, and result in depolymerization of the cytoskeleton. The binding of Ca^{+2} to calmodulin causes dissociation of actin from MBP and of MBP from the membrane, as

well as dissociation of microtubules from MBP. The activation of protein kinase C (PKC) by Ca^{+2}auses phosphorylation of MBP, which may reduce communications of MBP with actin filaments, as has been shown for phosphorylation of MBP with MAPK. The kinases PI4K and PI5K cause phosphorylation of PI, and another phosphorylation event appears in increased amounts of PI (4, 5) P2, which are collected by MBP, a ‘PIP module.' The elevated PIP2 increases the negative membrane surface possible inducing dissociation of negatively charged actin filaments from the membrane-bound MBP. The free actin fibers may then depolymerize, also causing depolymerization of microtubules, to which they may be bound, mediated by some cytoskeleton-binding proteins, and possibly also by MBP. The increased negative surface potential also causes a release of SH3-domaincontaining proteins from MBP, thus regulating their activity and regional signaling events. Membrane-targetted MBP (red) can also inhibit Ca^{+2} entry (indicated by a red X) through VOCC channels, possibly by connecting directly or indirectly to them, or by regulation of the cytoskeleton. This figure has been adapted with permission (Harauz and Boggs 2013).

2.3. Myelin Functions

By mid-19th century, the concept of axon had replaced myelin as the essential and active component of the nervous fiber. Myelin origin and function puzzled early investigators by appearing relatively late during development and not investing all tissues in an adult. The following section, by no means exhaustive or comprehensive, presents the most remarkable theories. Nerves were known to transmit electricity, which prompted Virchow to audaciously formulate the first insulation theory “The axis-cylinder [axon] would, therefore, seem to be the real electrical substance of natural philosophers, and we may certainly admit the hypothesis which has been advanced, that the medullary sheath rather serves as an isolating mass, which confines the electricity within the nerve itself, and allows its discharge to take place only at the non-modulated extremities of the fibres.” Ranvier proposed several theories from his significant work on myelin .His elegant comparison with the transatlantic telegraph cables, operational in 1866, drew from Virchow hypothesis «Myelin has perhaps another role; it is probably an insulating envelope. We know that a non-conductive sleeve must isolate electrical connections which are immersed in a conductive medium; construction of submarine cables rests on

this principle. It is conceivable that transmission of the sensitive or motor impulses may have some analogy with the transmission of electricity, so perhaps each nervous tube must be isolated for this transmission to be more efficient.“Ranvier also correctly suggested myelin confers an evolutionary advantage Nerve cells with myelin do not exist in invertebrates. Therefore, they are not essential to the nervous system manifestations; since many animals possess all the nervous functions: sensitivity, motility, Nutrition without having myelin tubes. The nervous cells with myelin seem therefore constitute an improved transmission apparatus particular to the vertebrate nervous system.” By far his most entertaining theory is about the nodes “If liquid myelin were uninterrupted in the entire length of the nervous tube, in example in man sciatic nerve which stands vertically in our usual posture, it would glide to the lowest part by its own weight; there would not remain any more myelin in the superior part of the nerve. But this is not the case; the myelin sheath is interrupted from distance to a distance by transversal partitions which retain it.” Ranvier more sensibly attributed nodes a nutritive role since myelin-insulated the axon so well from dyes “These observations allow us to conclude that the penetration of crystalloid materials or, if you prefer, the diffusible elements necessary for the axis-cylinder nutrition, which, as we know and as I will demonstrate, is the most important part of the nervous tube, would not easily happen if myelin surrounded it in its entire length. At any rate, the penetration is much faster and much easier at the level of the nodes, and we can surmise, without over speculating, that it is through them that nutrition of the axis-cylinder occurs.” Well before saltatory conduction was conceived, joined to a vastly unknown chemistry, the function of myelin unbridled imagination. The sixth edition of an American textbook for medical students is a telling example “May this supposed coating and insulating material, myelin, not be to the nerve what myosin gene is to muscle? The functioning nerve is the seat of increased combustion. This suggests that the nodes themselves. may allow the blood-plasma to filter through them, thus bringing the oxidizing substance in direct contact with the axis-cylinder. Indeed, if the various features enumerated are collectively considered, it will become apparent that the myelin, or white substance of Schwann when in contact with the oxidizing material of the blood-plasma undergoes a reaction in which chemical energy is liberated.” Recent developments have provided an interesting twist to this theory of myelin as energetic fuel. Two independent investigators, Klaus-Armin Nave based in Germany and Jeffrey Rothstein in the USA, have shown that myelin indeed is providing active support to the

axon, which might explain why the axon eventually degenerates upon being stripped of myelin. The fuel currency has been found to be lactate, shuttled by the oligodendrocyte to the axon, in contrast to a self-combustion of the fatty sheath itself as enunciated a century earlier (Boullerne 2016).

2.4. Mechanism of Myelination

The nervous systems of vertebral are regularly classified into gray and white matter based on their manifestation and comparable functional roles. While the gray matter consists mainly of cell organizations and dendrites, white matter contains axons primarily and gets its name from the lipid layer sheets called myelin that is wound tightly throughout those axons. Myelin originates from various classes of glial cells pointed to as oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). The electrical covering presented by the myelin sheets extends axonal function by improving both the energy efficiency and conduction rapidity of action potentials (APs). These two functions may have switched their relative importance during evolution. Myelin first appeared in the Ordovician (485 to 443 mm, or million years already the existing). After the division of lamprey and hagfish parents from the excess of the vertebrate progenitors. With some impressive deviations, myelin or similar structures are found in all vertebrates and is important for the private functioning of their nervous systems. The estimated time of the progression of myelin can be deduced from the known time of divergence between chordates without (Agatha) and with (all other vertebrates) myelin. The myelin wrapping is interrupted by regularly spaced, un-myelinated stretches known as the nodes of Ranvier. Myelin speeds up conduction by restricting transmembrane charge flow through ion channels located within the nodes. Within the so-called internodes, current flows down the axon with little of it passing across the insulated cell membrane. The AP is regenerated at each node where the density of voltage-gated sodium and potassium channels is very high. This process is called “saltatory conduction” since the AP seems to jump from node to node. Disruptions in this rapid-fire the information system can be associated with an arrangement of nervous systems dysfunctions. In numerous respects, axons surface to perform at physical limits.

One compelling example is that the size of axons appears to be restrained by the thermic noise inherent to ion channel proteins; any axon smaller than 0.1µm would be vain for learning transfer due to its excellent noise levels. Intriguingly, 0.1µm is also approximately the smallest axon caliber observed in nervous systems. This and related findings propose that axons and their foundations are finely harmonized biological devices, but that tuning can evidently be disrupted under pathological conditions. Demyelination sets in motion functional changes that are necessary for clinical features but which are not immediately manifested by immunological or radiological changes. Section of a plaque prognosticates what system will be influenced (motor vs. sensory, visual vs. tactile) but not how it will be influenced. This highlights the importance of assessing function (in addition to structure) and how it changes the subsequent demyelination. After including demyelinating diseases, we will explain how the clinical demonstrations of those conditions reflect various pathological differences in axon function. We will argue that understanding those differences and fully serving on that conclusion for diagnostic and therapeutic targets can benefit enormously from computational modeling. In the CNS, two protein classes, namely, myelin basic protein(s) [MBP(s)] and the hydrophobic proteolipid proteins (PLP), account for the significant part of the myelin proteins. Historically, these proteins were first isolated from whole brain or spinal cord, the former from acid extracts of dilapidated brain, the later from brain chloroform-methanol extracts. Considerable indirect evidence suggested that they were indeed myelin components, but confirmation of this hypothesis required the evolution of methods for the separation of myelin and of electrophoretic procedures for visualizing the myelin proteins. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of myelin showed that the PLP and MBP accounted for 60-80% of the protein, but a different collection of minor proteins could also be identified. In general, the laser is found in the "high-molecular- weight" region of the gels (>40,000 Daltons), are insoluble in chloroform-methanol, and are sometimes referred to as the Wolframs fraction. Although initially looked on as contaminants, the high-molecular-weight components are now considered legitimate myelin or myelin-associated components and include the myelin-associated glycoprotein.

2.4.1. Characteristic Myelin Basic Protein

MBP–membrane integrative protein–has been a subject of investigation for decades and investigators continue to dissect molecular details of how this protein is positioned within two membrane bilayers to form the major dense line of myelin. The inner leaflet of the myelin membrane, where MBP is located, has a different composition than the outer leaflet, and comprises roughly 44% cholesterol, 27% phosphatidylethanolamine, 13% phosphatidylserine, 11% phosphatidylcholine, 3% sphingomyelin, and 2% phosphatidylinositol, as estimated from biophysical measurements of surface charge. The MBP-lipid interactions rely on a balance of interactions between the core residues of MBP and the acidic head groups of the lipid bilayer, to assemble the proper multilamellar structure seen in myelin sheaths. Of note also is the relatively high proportion of phosphoinositides, particularly phosphatidylinositol-4, 5-bis-phosphate (PI (4, 5) P2). In vitro and cellular, MBP has been shown to sequester PI (4, 5) P2 membranes. Cholesterol is also important for myelin formation and stabilization (Saher, Brugger et al. 2005). And specifically facilitates 18.5-kDa MBP's ability to stack membranes (Harauz and Boggs 2013). Myelin basic protein (MBP) is an essential protein of myelinated axons. The sequence of MBP predicts the formation of an intrinsically disordered protein carrying a large positive charge, which allows for many protein surface interactions (Harauz, Ladizhansky et al. 2009).

This property is specific for MBP and is not recognized by other many myelin proteins, such as myelin oligodendrocyte glycoprotein (MOG) or Myelin proteolipid protein (PLP). To preclude MBP from nonspecific binding to molecules, MBP mRNA assembles into ribonucleo protein complexes termed "granules" which are targeted into oligodendrocyte extensions and translated in-situ. Consequent MBP-membrane interactions rely on an accurate stoichiometrical balance between the core sediments of MBP and the acidic headgroups of the lipid bilayer elements, as well as on copper ions producing the compaction of the MBP composition. This suggests that reduced myelin stability may relate to changes in levels of diverse myelin components, including designated lipids and their oxidized products (Ferretti and Bacchetti 2011). Copper ions or expression levels of MBP. Upon demyelination, MBP may either rapidly degrade or otherwise aggregate with other myelin components (Frid, Einstein et al. 2015). In the

CNS, the primary myelin proteins insert myelin PLP (proteolipid protein) and the related produce DM20, MBP (myelin basic protein), MAG (myelin-associated glycoprotein) and CNP (29, 39-cyclic nucleotide 39-phosphodiesterase). PLP and MBP are diffused within the myelin sheath and appear to be essential to the method of compaction that generates the closely apposed multilayered structure of mature myelin. MAG, on the other hand, is located in the per axonal sections and may serve to promote cell–cell interactions between myelin and axonal membranes during the establishment of myelination. CNP is understood less well. Though it exhibits enzymatic motion in vitro, substrates for this action have not been identified within the myelin sheath, and it has been proposed that this molecule may preferably perform a structural role in the myelin membrane (Fulton, Paez et al. 2010).

In the mature CNS is initially carried out by MBP (myelin basic protein), which is a remarkably positively-charged, developmentally controlled protein family expressed from the gene in the oligodendrocyte lineage (Golli). Specifically, the 18.5 kDa size isoform has been deemed the 'classic' or 'executive' isoform since it is the most abundant in the adult human brain, and is essential for proper myelin membrane compaction. As previously reviewed, the protein has several other tying companions: cytoskeletal proteins (actin, tubulin), Ca^{+2} -activated calmodulin, and proteins including SH3 (Src homology 3)-domains. The latter include mainly Fyn kinase, but also ZO-1 (zonula occludes 1) and cortactin. This protein's multifunctionality in myelin is derived slightly from its conformational flexibility as an IDP (intrinsically disordered protein) the classic isoforms of MBP are quintessential IDPs exhibiting all of these facets. The interactions of 18.5 kDa MBP with membranes, actin, and Ca^{+2} -calmodulin have been investigated in detail using different spectroscopies, and all involve some degree of political disorder-to-order transition (usually to a α-helix), yet with many segments remaining mobile and dynamic, i.e., 'fuzzy.' We have also sought to characterize the interaction of MBP with SH3-domains using in silico, in vivo, and in vitro experiments. The SH3-domains are a general protein-recognition module, maximum of which recognize and bind proline-rich regions that can adopt a PPII (polyproline type II) agreement and a variety of determination sequences, but often based on a P–x–x–P agreement Specificity in these conversations influx from slight variations in the transaction chains of each SH3-domain, and the hydrophobic and direct interactions of

the residues near the limiting regions of any protein. These communications usually have a KD value in the low-micromolar range and follow a two-state model of binding. Usually, a full characterization of the conversations between these domains and their binding partners is available in vitro by a joining of biochemical and biophysical methods used on the SH3-domain alone, and 7- to 30-residue peptides containing the proline-rich region of the binding protein. The 18.5-kDa MBP isoform contains a proline-rich region (T92–P93–R94–T95–P96–P97–P98, murine numbering) immediately adjacent to a membrane-associated amphipathic α-helix. Both sections are part of a highly conserved central portion and likely include a little change due to the occupancy of MAPK (mitogen-activated protein kinase) sites (T92 and T95) and the strength to support disorder-to-order transitions by adopting α-helical structure. We have shown in previous studies including the full-length protein as well as small MBP peptides (T92–R104, F86–G103, E80–G103 and S72–S107) that the pralines-rich district of 18.5 kDa MBP is involved in SH3- domain binding. We have transferred to the longer S72–S107 peptide earlier as the α2-peptide because it comprises the second of three amphipathic α-helical sections involved in membrane association. In our first comprehensive study of this interaction, we assumed that the proline-rich region containing a single P–x–x–P consensus SH3-ligand formed the typical PPII agreement, and performed in silico rigid-body docking experiments of a model of the (T92–R104) section with the crystallographic composition of Fyn-SH3 (De Avila, Vassall et al. 2014). We have seen that the traditional MBP family comprises a variety of developmentally regulated splice isoforms, which translocate to different cellular compartments (periphery, cell body, nucleus), with multiple biomolecular associations. A ‘dynamic molecular barcode’ of combinatorial post-translational modifications generates further diversity of the family (Harauz, Ishiyama et al. 2004). In exceptional, phosphorylation has arisen several times in the discussion above, and it is worthwhile to discuss this change further at this point Phosphorylation, and dephosphorylation activates or deactivates proteins in cellular signaling performances, by modifying their structure, function, subcellular localization, or interactions. Phosphorylation of MBP is affected by several protein kinase (PK) families and yields several of the MBP charge components isolated from myelin. Phosphorylation of MBP is not a counterfeit event – it occurs immediately before and during myelinogenesis, differences in level during development and with age, and is reduced in multiple sclerosis (Kim, Mastronardi et al. 2003). Phosphorylation of 18.5-

kDa MBP dominates at deposits in areas of higher disorder, as before noted for this protein, and compatible with other IDPs (Harauz and Boggs 2013).

2.4.2. Myelin Proteolipid Proteins

Myelin is a multi-lamellar, tightly compressed membrane that surrounds many axons in the central (CNS) and peripheral nervous systems (PNS). The primary functions of CNS and PNS myelin are identical. Both concentrate Na^+ channels at nodes of Ranvier and thereby promote rapid nerve transmission via saltatory conduction. CNS and PNS myelin also produce trophic support to axons that are essential for long-term axonal survival. Many other features of CNS and PNS myelin, however, differ. Schwann cells form single myelin internodes in the PNS PNS myelin internodes are surrounded by a basal lamina and hold a chain of cytoplasmic channels called Schmidt-Lanterman (S-L) incisures that were intersecting compact myelin and attach the outer and inner margins of the myelin internode. S-L incisures facilitate transport molecules and nutrients to compact PNS myelin. Oligodendrocytes form multiple myelin internodes in the CNS. CNS myelin internodes do not have basal lamina or S-L incisures. There are also variations in the molecular composition of mammalian PNS and CNS myelin. The major structural protein of PNS myelin is P0 protein, a type I transmembrane glycoprotein. Proteolipid protein (PLP), a four-transmembrane-domain protein, is the major structural protein of CNS myelin. Since the extracellular domain of P0 protein is larger than that of PLP, the periodicity, or spacing within compressed myelin lamella, is slightly larger in PNS myelin than in CNS myelin. Finally, myelin–associated glycoprotein (MAG) is present in per axonal membranes of both CNS and PNS myelin internodes but is present in paranodal and S-L incisures membranes of only PNS internodes. The PLP-specific 35 amino acids were apparently produced by the development of an alternative join donor site within intron 3 of the DM20 gene. Both P0 and PLP had moderately high deviation measures until 300 million years gone. It seems that as PLP function was evolving in CNS myelin, it required co-expression of P0. In Reptiles/Aves, the role of PLP became established, allowing the silent drop out of P0 from CNS myelin. From Reptiles/Aves, PLP was the major protein of CNS myelin, though P0 was mainly expressed in PNS myelin. Once this separation was verified, the modification rates of both proteins

descended dramatically, and both proteins are extremely conserved (almost 100%) across all mammalian species analyzed (Yin, Kiryu-Seo et al. 2015).

2.5. Biochemistry of Myelin

It is exceeding the scope of the present review to provide a detailed account of myelin chemical composition discovery. It was known by middl- 19th century that in white matter the axons “consist of protein components very similar to muscular fibrin, the marrow sheath especially of fats from various kinds” Toward the end of the 19th century, the founder of neurochemistry, partially characterized many lipids of myelin, including its most characteristic named galactocerebroside . At the turn of the 20th century, myelin was viewed as a semi-liquid albumin-fatty substance, which chemical composition appeared one of the most complex and included “cholesterin, protagon, lecithin, cerebral and neurokeratin”. At Albert Einstein College of Medicine in New York, one of the most prominent myelin biochemists of the 20th century improved these procedures by devising a sucrose gradient to specifically purify CNS myelin, which culminated in a landmark paper effectively unlocking access to studying myelin chemistry. Norton went on to a seminal work that revealed myelin contains 70–85% lipid and 15–30% protein depending on the source; no lipid is exclusively found in myelin but enriched; large differences in lipid composition exist between CNS and PNS; and myelin is remarkably conserved for both proteins and lipids across vertebrates. Considering we are still mapping the proteins of myelin, the ultimate frontier may be differences in localization -spine versus brain areas- and by oligodendrocyte subtype. Occasional investigations on myelin proteins (lipids are virtually ignored) have uncovered differences between small and large axon myelin, but the majority of studies focus on del Río-Hortega type II oligodendrocyte myelinating small and medium axons (Boullerne 2016).

2.6. Myelin Biogenesis, Structure and Cell Functions

The multilayered myelin layer displays a distinct and complex architecture, i.e., in the process of unwrapping axons, the outer leaflets of the myelin membranes oppose each other, thereby creating the intro period line, while the condensed cytoplasmic surface constitutes the major dense line as immediately reflected by electron microscopy (EM). The myelinated portions of the axons, the so-called internodes, are exchanged with

myelin-devoid sections, named the ‘nodes of Ranvier’, where sodium channels are confined that make a membrane potential that drives the performance potential along the axon in a saltatory manner. Besides axonal enwrapping, myelin compaction takes place, which thus gives rise to areas of compact and non-compact myelin. Recent decisions in rodents suggest that the compaction of the myelin sheath begins from the outer tongue and constantly shifts towards the interior language. Within the internodes, various degrees of compaction can be discerned. While mainly compact within the inter-node, regions at their edges, known as ‘paranodes,' consist of non-compact myelin. Interestingly, the molecular combination of compressed and non-compact myelin changes; i.e., the higher myelin proteins PLP and MBP collectively with the glycosphingolipid GalC remain in compact myelin, whereas other myelin proteins, such as 155-kDa neurofascin (NF155), collectively with the glycosphingolipid sulfatide restrict in the paranodal region of non-compact myelin. The proper ‘compartmentalization’ of the myelin sheath performed in this manner is essential for its role because any modifications in the complex interrelated lipid and protein organization of myelin structure might cause more or less severe demyelinating, dysmyelinating, and hypomyelination diseases. For example, in the hypomyelination diseases Pelizaeus-Merzbacher syndrome PMD) and the few severe spastic paraplegia 2 (SPG2) modifications, deletions or duplication of the PLP gene lead to abnormal myelin formation. The qualifications and deletions affect a revision in the protein adaptation, whereby worrying PLP conveyor to the myelin sheath and exciting intracellular cholesterol/lipid transport to the myelin sheath. In the dysmyelinating disorder met achromatic leukodystrophy (MLD). Arylsulfatase A loss leads to accumulation of sulfatide in lysosomes. This induces a disorder of the myelin composition, which is served by demyelination, and a delay in remyelination. In other conditions, counting multiple sclerosis (MS), myelin is initially formed in a correct manner, but severe myelin loss in the absence of demyelination results in persistent demyelination. The particular cause for demyelination in MS is yet unknown, but in contradiction to MLD, a genetic foundation is not likely. In MS, remyelination failure is likely due to a change extracellular signaling microenvironment that amid others influences the formation of OLG membranes, whatever induces deficits in myelin at the molecular level. For myelin biosynthesis to occur, progenitors cells of the myelin-producing OLGs first have to mature to myelin (Ozgen, Baron et al. 2016).

2.7. Saltatory Conduction in Myelinated Nerve

The majority of the axons invertebrate nervous systems are protected with insulating sheets of back-to-back cell membranes called myelin. The devices by which myelin hurries axonal conduction in veracity and by which damage to myelin manages to loss of axonal conduction in disease have attracted much attention. Still, the causes of myelin-related functional deficits remain incompletely understood. Continuing multidisciplinary examination joining morphology, electrophysiology, and analytical modeling may explain important mechanisms and possibly guide the expansion of powerful medications for medical diseases including myelin damage, incorporating traumatic insult to the brain or spinal cord and demyelinating conditions such as multiple sclerosis. Myelin was developing Schwann cells in the peripheral nervous system or oligodendrocytes in the central nervous system coat around the axon multiple times to create laminated layers of an insulating cell membrane, as presented schematically. Intermittent short pauses in the myelin sheath along the axons must specify width, generally 0.3 to 1 micrometer, are the Nodes of Ranvier, where the intensity of trans membrane channels carrying inward sodium current is high and where trans membrane action inherent are initiated. Directly adjoining to the nodes themselves on each side in the axial dimension are the paranodal regions, where myelin is tightly joined to the underlying axonal membrane The use of analytical models to study devices of saltatory conduction is well precedented. Classical and more modern approaches almost universally involve a form of cable equation or cable model, as originally described by Fitz Hugh. This method salutation successive node as a chain of leaky capacitors having voltage-sensitive ion channels, and combined. In similarity by extracellular and intracellular resistances among the nodes. Courses and voltages at each node are computed from a second order partial differential equation that describes the energies at each node as functions of time and space. The goal of the present paper is to determine quantitatively how myelinated nerve conduction velocity depends upon the historical honesty of the nodes and paranodal precincts at the electron microscopic level of observation and how nerve conduction strength be slowed or blocked by the known pathological changes to these structures. We also explore how drug treatments that prevent potassium conductance in the juxtaparanodes region may act to restore conduction in subtly damaged areas. Moving this end, it is insightful to derive from primary principles a Fitz Hugh style cable model of myelinated axon conduction for

the particular purpose of characterizing the parameters related to subtle injury (Babbs and Shi 2013).

2.8. Myelin Pathology

2.8.1. Multiple Sclerosis

Multiple sclerosis (MS), a persistent inflammatory and demyelination disease, was first identified as a distinct neurological disorder by the French neurologist Jean-Martin Charcot and is the most joint inflammatory disorder of the central nervous system (CNS) in young adults. It was widely accepted that MS lesions are autoimmune in origin, with myelin sheaths and oligodendrocyte being subjected to an inflammatory attack. Consequently, animal models with T cell-driven inflammation and, to some degree, demyelination were commonly applied to study MS pathogenesis and new therapeutic options. The relevance of these animal models, however, the autoimmune nature of the disease has recently come under question. Initial degeneration of oligodendrocytes has been proposed as the primary insult, followed by immune cell infiltration being a secondary phenomenon. Moreover, there is an increasing appreciation of the importance of axonal and gray matter pathology in the genesis and evolution of the lesions, their clinical diagnostic characteristics, and their response to treatments. Thus, it is evident that MS pathology is more complex and heterogeneous than previously assumed. Cortical demyelination can be widespread and is most prominent in patients with either primary, or secondary progressive MS. Cortical lesions are classified according to their neuroanatomical location. Lesions that extend across both white and gray matter are termed leukocortical (type 1) lesions, whereas lesions within the cerebral cortex (Cx) that do not extend to the surface of the brain or the subcortical white matter are classified as type 2. The most common cortical lesion type is the so-called ‘‘subpial’’ type 3 lesion. Type 4 lesions extend throughout the full width of the cerebral Cortex but do not penetrate into the subcortical white matter. The histopathological presentation of gray matter lesions differs from white matter lesions in several aspects. For example, there is less pronounced lymphocyte infiltration, macrophage activity, complement composition, and blood-brain-barrier (BBB) alterations. This diversity in appearance could be due to variations in the structure and role of the BBB or other components of the

cytoarchitecture of the cerebral cortex; that makes it more resistant to inflammation. While it is well reported that degenerated myelin triggers inflammatory responses in MS and EAE, the different myelin content of white and gray matter areas has not been regularly addressed as an underlying mechanism of reduced demyelination-associated inflammation in MS. The attenuated inflammatory response in cortical lesions, especially microglia/macrophage activation, could be simply due to the lower cortical myelin content and in consequence less myelin debris during a demyelinating event (Clarner, Diederichs et al. 2012).

2.8.2. Remyelination

Remyelination, the return of new myelin sheaths to demyelinated axons, is not performed by pre-existing mature oligodendrocytes but involves in most cases the generation of new mature oligodendrocytes from the adult, quiet OPC pool distributed throughout the CNS. The method of remyelination gets the place in several different steps. First, local adult OPCs must switch from a substantially quiescent state to a regenerative phenotype. This transformation seems to be triggered by factors determined from stimulated microglial cells and astrocytes, and not by the demyelination per and leads to OPC proliferation and recruitment to demyelinated areas. Then, the differentiation of OPCs remyelinating oligodendrocytes starts. All following steps—the interactions with unmyelinated axons, the expression of myelin genes, the elaboration, wrapping and compacting of myelin membrane to form myelin sheaths are similar in myelinating OPCs during development, and in remyelinating OPCs during the regenerative process. However, some differences between myelination and remyelination exist; first adult OPCs have a longer cell cycle time and a slower rate of migration. Second the requirements for transcription factor usage seem to be different. Studies in genetically modified mice clearly revealed that the lack of the oligodendrocyte lineage specific transcription agent olig1 is inappropriate with myelination of the brain. Nevertheless, when this lack is reparation by the over expression of the oligodendrocyte-lineage appropriate transcription agent olig2 (as was probably the case in earlier studies, due to the usage of an individual gene targeting cassette, the mice were able to myelinate during development but were unable to repair demyelinated lesions by remyelination. Third groove, the regulator of oligodendrocyte differentiation in development is dispensable during remyelination. Fourth the

association between axon broadness and myelin sheath density and length seen through developmental myelination is less apparent in remyelination, resulting in thinner and shorter sheath segments. The mechanisms underlying this observation remain unclear, but could involve signals obtained from dynamically growing and changing axons with a need for myelination along their entire length during development, or from mature axons focally lacking myelin sheaths during remyelination. Thus, the pathological hallmark of remyelination in the CNS is the presence of axons with unusually thin myelin sheaths about their caliber. Explicit identification of remyelination in conditions of diffuse demyelination is possible at early stages, but tough in old established lesions. In regions of focal demyelination, such as those occurring in multiple sclerosis, demyelination is reflected by shadow plaques. By the basic thoughts expressed above, the recruitment of OPCs and beginning Remyelination is wildish in very beginning stages of demyelination, in injuries which are still infiltrated by macrophages and lymphocytes, and in decorations formed at early stages of the disease. In these new lesions, remyelination might be facilitated by inflammation and infiltrating macrophages which provide the tissue with growth factors. Remyelination largely fails at the later (following) stage of the disease. This failure of remyelination may be additionally ascribed to age, to age-associated changes in the growth factor responsiveness of adult OPCs, and to fewer efficient removing of myelin trash from the lesions which has been shown to restrain remyelination in experimental models (Bradl and Lassmann 2010).

2.8.3. Demyelinating

Demyelination is the pathological replacement of myelin sheaths that circle axons and improve axonal function. Spontaneous remyelination by oligodendrocytes that develop from oligodendrocyte precursor cells may occur the following demyelination, probably allowing a partial, if not perfect, recovery from incompetence. The scales between demyelination and remyelination during demyelinating pathologies is negotiated by the immune system (both the adaptive and the innate) and locate the outcome of the disease (Mayo, Quintana et al. 2012). In toxic-based patterns of demyelination, as dissenting to beginning autoimmune encephalomyelitis (EAE) or virus-induced demyelination, full remyelination gets place spontaneously, which allows for a thorough study of the mechanisms involved in demyelination/remyelination processes. Demyelination is

undoubtedly part of MS pathology; however, in recent years, neuronal loss and axonal loss have been proven to be a consequence of chronic demyelination and the main driving force for neurodegeneration. In demyelinating disorder (Adamo 2014). Additional, more rare forms of demyelinating diseases comprise (i) neuromyelitis visual (NMO), in which there is an adaptive immune response directed against the water channel aquaporin 4 (AQP4), (ii) severe distributed encephalomyelitis (ADEM), which is characterized by a monophasic immune-mediated attack against the CNS and which has been suggested to be a variant of MS, and (iii) paraneo plastic syndromes in which the adaptive immune reply targets cancer antigens shared by epitopes in the nervous system, containing myelin. In demyelinating diseases, the approach of cells from the periphery to the CNS (both governors of the immune response or cells immediately included in the demyelinating process) is critical in disease pathologies. (Mayo, Quintana et al. 2012). Expression of Classic-MBP increases during cortical development. And immunohistochemical labeling of Classic-MBP is cor- related with traditional myelin staining. In contrast, Golli-MBP isoforms (33–35 kDa) are found in new developing oligoden-monocytes, neurons, and immune cells, with roles that extend beyond the myelin sheath and include regulating oligodendria- cute proliferation and migration. Golli-MBP is remarkably expressed prenatally in neurons and oligodendrocytes, even earlier the process of myelination begins and has been called a “molecular link” between the nervous and immune systems (Siu, Balsor et al. 2015). The attenuated inflammatory response in cortical lesions, especially microglia/macrophage activation, could be simply due to the lower cortical myelin content and in consequence less myelin debris during a demyelinating event. Though the pathology of CNP-null mice is similar to PLP1 disrupted mice, the mechanism of degeneration must be different since double knockout mice develop increased axonal degeneration compared to either null mouse alone. Thus, the disruption of oligodendro glial proteins with various cellular functions such as PLP1 and CNP can lead to a characteristic phenotype of severe disruption of axon function and integrity. Recent studies have also investigated the impact of the acute death of oligodendroglial on neuron function and survival. Though some of these mice exhibited abnormalities in myelin composition, overall myelination was not affected, suggesting that axonal injury is not due to demyelination. Additionally, axon damage is not dependent on the secondary immune reaction since it persisted after crossing to an immunodeficient line of mice. These studies demonstrate that oligodendroglia

themselves, and not just components of myelin, are critical for maintaining axons (Morrison, Lee et al. 2013).

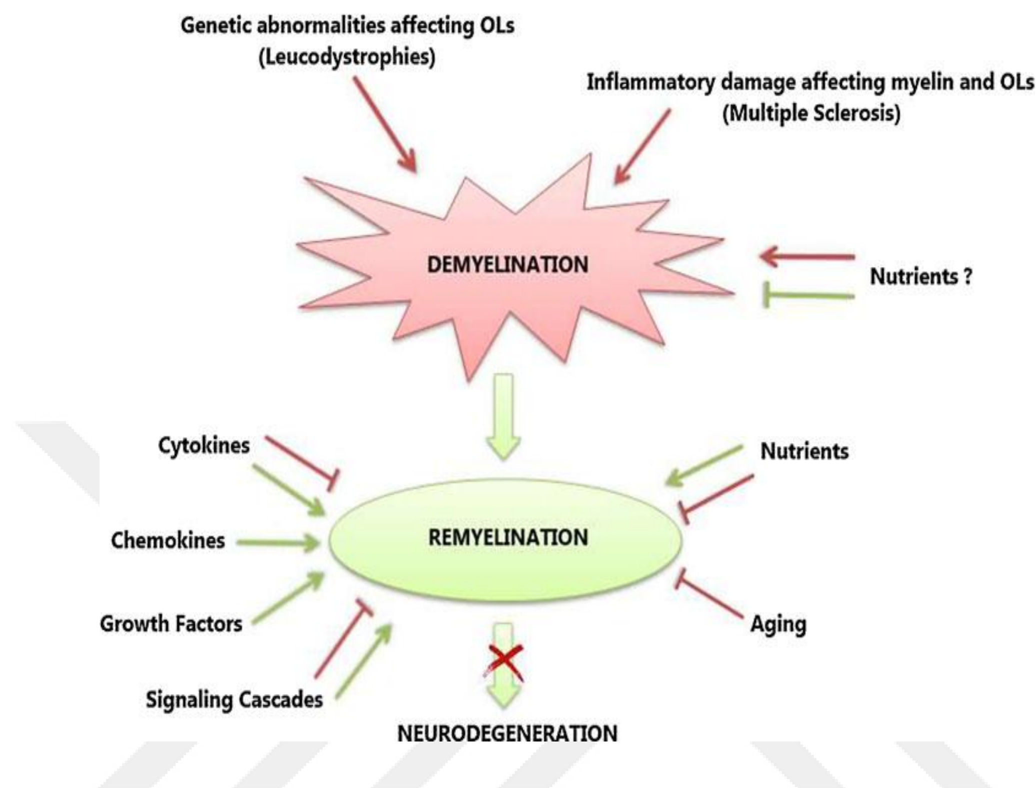


Figure 2.2. Primary demyelination may be caused either by genetic abnormalities affecting OLs or by inflammatory damage affecting myelin and OLs, as is the case in MS

While some nutrients may play a protective role against demyelination (green line), others may play a negative role (red arrow). Remyelination is the physiological response to demyelization. During remyelination, some molecules act positively (green arrows) on the process, such as chemokines and growth factors, while others may play a dual role (green arrows for positive, red lines for negative), such as certain signaling cascades and cytokines. Among environmental factors, nutrients may also play a dual role, and finally, aging has an unequivocally negative impact on the process. The interplay of these factors determines the fate of the remyelination process, whose failure leads to neuro degeneration (Adamo 2014).

2.9. Oligodendrocytes Development

Oligodendrocytes are the myelin-forming cells in the CNS. These cells originate mainly from two types of precursors: the OPCs nestled in the SVZ in the brain and a group of Sox10/Olig1-positive cells in the ventral spinal cord Oligodendrocyte parents develop through a series of stages that have been characterized by their distinct cell morphology and the expression of cell surface proteins: the migratory OPC is typically a PSA-NCAM-positive cell that also expresses a ganglioside recognized by the A2B5 antibody These precursor cells are extremely dependent on the presence of PDGF for proliferation and can be identified because they express high levels of the PDGF receptor while surrounding neurons secrete PDGF. The significance of this neurotrophin in oligodendrocyte construction has been supported by the proceeds from PDGF knockout mice, which show a severe decrease in the numbers of OPCs. Ultimately, the OPCs reach their target regions and develop into myelinating cells and begin expressing model myelin proteins, proteolipid protein (PLP), MBP and MAG. For many years, the origin of the spinal cord oligodendrocyte continued a mystery because of the absence of initial oligodendrocyte-specific markers. Two major actions came from three labs: first, Bill Richardson showed early start of the 1990s that a small group of PDGF positive cells gave rise to OPC. Then David Anderson at Caltech and David Rowitch at Harvard simultaneously reported that a small group of cells in the presumptive motor neuron area, are positive for the bHLH factor olig2. This group of cells divides and migrates radially to cover the spinal cord later in development. That oligogenic are critical for oligodendrocyte significant was additionally supported by the absence of spinal cord oligodendrocytes in the olig1/olig2 double knockout mice. Probably the best-known OPC population is the one initially known as the oligodendrocyteastrocytetype-2 (O-2A) progenitor cells, primarily isolated from the postnatal rat optic nerve and subsequently from the postnatal cerebellum, cortex, brain stem and spinal cord. These OPC cells were observed to have a failure pathway of differentiation into oligodendrocytes, and growth factors could modulate this distinction. For many years, it was reflected that they could also give rise to astrocytes type-2 glial cells. Nevertheless, because upon transplantation these OPC cells distinguish only into myelinating oligodendrocytes, most investigators had concluded that the type-2 astrocyte created in such studies was quite likely an in vitro artifact effected on the precursor by the trophic factors present in the media What is more

important regarding these precursors is that the cells formerly known as O-2A will not differentiate into neurons under any culture conditions tested; in other words, OPC is a right glial precursor. The modern composition is that cortical oligodendrocytes in rodents are born from the cortical sub ventricular zone (SVZ) back birth; nevertheless, recent data from Richardson group submit that many forebrain oligodendrocyte progenitor cells (OPCs) are specified much earlier (between E9.5 and E13.5 in the mouse) in the ventricular zone of the ventral forebrain under the control of sonic hedgehog (Shh) and then migrate into the cortex and that even caudal OPC can compensate loss of cortical oligodendrocytes. As stated for SCs, oligodendrocytes are more than just a myelinating cell. There are quite some mutations of genes affecting oligodendrocyte development and differentiation that cause severe neurological diseases. Apparently, the best known is Pelizaeus–Merzbacher disease, which is a rare, progressive, degenerative CNS disease in which coordination, motor capabilities, and original function deteriorate. The best model is a mouse strain referred as the“Jimpy” mice that carry a mutation in PLP protein. PLP also functions in a paracrine mode to regulate neuronal survival. Co-culture of neurons with non-glial cell lines that overexpress native PLP but not DM20 shortens survival of neurons. This conclusion is mimicked in vivo, where either moderate over-expression of native PLP or its absence leads to axonal abnormalities and neuronal death (Ndubaku and de Bellard 2008).

2.10. Schwann Cell Development

SCs are the principal glia of the PNS, beginning from the neural crest. Like the oligodendrocyte, the SC is an extremely techno scientifically cell that has developed into a myelinating cell. Opposite the oligodendrocyte, it is also a supervision partner for growing axons during peripheral development. A neural crest is a group of cells that begins in the dorsal part of the neural container, goes through an epithelial to mesenchymal development, and later separates – transferring greatly throughout the egg and modifying into a wide variety of cell types – giving rise to the PNS. The beginning of the SC lineage involves three main developmental transitions in neural crest cells. The first is the formation of SCPs from actively migrating neural crest cells. Secondly, the creation of immature SCs from the precursors. Finally, the differentiation of the embryonic SCs into a myelin and non-myelin-forming SC. Whether an SC has a myelin-

forming or a no myelin-forming phenotype, both varieties of SCs are neural-crest-derived and descend from a community precursor in the dorsal neural tube. This is quite a distinct developmental pathway from the oligodendrocyte, which originates from different populations, depending on the location of the precursor neuroblast.

The regulation of the final cell fate of an SC is determined, to a certain extent, by the axons with which they find themselves in communication with. SC progenitors migrate and reproduce ahead, as well as along preexisting axonal tracts, during embryonic development. What determines if a cell will become a myelinating one or not depends ultimately on axonal signals received during their close contact as they enter peripheral tissues. The importance of SCs for neuron development is underscored by the neuropathies seen in mice or humans with mutations in SC or myelin genes. For example, “trembler” mice have a modification in the peripheral myelin protein 22 (PMP22) and show the sensory neuronal loss. Patients with Charcot–Marie–Tooth neuropathy have also axonal destruction, thus indicating the hypothesis that SCs provide the first line of axonal neuroprotection. This suggestion is valuable because SCs also are known to secrete inflammatory cytokines and neurotrophic factors essential to neuronal survival (Ndubaku and de Bellard 2008).

2.11. Oligodendroglia is Critical for Axon Function

Oligodendroglia is technoscientific cells in the CNS that wrap axons with myelin. Diseases of oligodendroglia invariably produce some degree of demyelination (Glossary), which was thought to underlie their clinical signs and symptoms. Over the last ten years, animal education has demonstrated a significant role for oligodendroglia in the support and long-term survival of axons and neurons and may produce clues to the involvement of oligodendrocytes in neurodegenerative conditions. Multiple transgenic models of oligodendrocyte damage have been examined, including several with disturbances of proteolipid protein (PLP; Glossary), 2’,3’-cyclic-nucleotide 3’-phosphodiesterase (CNP; Glossary) knockout mice, and Diphtheria toxin conditional transgenic mice. These animal models produce varying degrees of demyelination and progress over different time frames, but all of the models consistently demonstrate axonal pathology. These genes and models of axonal pathology will be discussed below. PLP is

the most abundant protein already in myelin. Although its exact function remains unclear, it appears to be essential for shuttling some myelin proteins, such as septins and sirtuin 2, from the soma into the myelin sheaths. Several different PLP animal models have been investigated, including naturally occurring point mutations PLP1 over expression rats and mice, and PLP1 knock-out mice It is external the scope of this review to consider these in any detail, but many of these animal models demonstrate axon degeneration joined with (e.g., PLP1 point mutations and overexpressor rodents) demyelination; and in some cases axon degeneration has been immediately linked to lowered axonal transport. The exact mechanism for axon degeneration in these mice is unknown, although it may include abnormal intracellular trafficking of mRNA or protein, for example, metabolic transporters necessary for axonal honesty since the number of myelin proteins are decreased or absent in these animal models. Another oligodendroglia gene that can lead to the axonal lesion is CNP. CNP is an RNA binding protein that may operate to promote intracellular RNA transport by binding RNA to tubulin. Interestingly, these mice do not have demyelination at ages when the axon degeneration is leading. The etiology of axon degeneration is unclear but presented the disrupted paranodal architecture; it likely involves failure of oligodendrocytes to interact with axons. Though the pathology of CNP-null mice is comparable to PLP1 disrupted mice, the mechanism of degeneration must be separate since double knockout mice develop increased axonal degeneration compared to either null mouse alone. In these models, the diphtheria toxin led to rapid and selective oligodendroglial death via the blockade of protein synthesis. Additionally, axon injury is not dependent on the secondary immune resistance since it persisted after dividing to an immunodeficient line of mice. The exact mechanism by which this transpires is not known, but another recurring theme from the animal models is that oligodendroglia intracellular trafficking often seems to be disrupted. Perhaps oligodendroglia support of axonal transport is dependent on one or more molecules in the myelin sheath and failed traffic to the myelin sheath leads to axonal pathology. Though several possible myelin proteins may play a role in supporting axons, recent data suggests that transporters for monocarboxylates are critical for maintaining axon integrity. Failure of these transporters to be expressed in oligodendroglial would reduce the availability of local metabolic energy to the axon, potentially impact energy-dependent processes in the axon such as axonal transport (Morrison, Lee et al. 2013).

3. MATERALS AND METHODS

3.1. Methods of Isolation Rat Brain and Cleaning White Matter

The isolation procedures were performed at +4C^0 the buffer contain the cock tail of the inhibitors proteases benzamidine, leupeptine and phenylmethylsulfonyl fluoride (PMSF). Remove all the meninges and blood vessels carefully to harden the brain, which makes it easier to extract white matter. Collect the white matters, weigh, and homogenize it in Solution A (1:2 ml/g). Add 1m pure propanol and store it at –4C overnight for precipitation. Discard the upper layer. Wash the bottom layer with 2X 90% and then 2X 70% ethanol Dissolve the sample in water (1/1 v/v) and centrifuge at 14,000g for 1h min to remove in dissolved matter. The supernatant Collect the product until you see the solidified protein and the EDTA tube is at roughly room temperature. Dissolve the homogenate in Solution B (1/1 w/ v), with using a pipet to mix thoroughly. Collect the pellet after centrifugation at 14,000g for 30 min. Resuspend again in Solution C (1/1 w/v). Collect the supernatant after centrifugation at 14,000g for 30 min. While isolation of myelin all operations was carried out at 0-4 C^0. Myelin was isolated from freezing rat brains, as described previously, except that the first and second cycle myelin were suspended in 20 volumes of water and centrifuged at 14,000 rpm for 60 min. The resulting pellet was suspended in 20 volumes of water, homogenized, and centrifuged at 14000 rpm for 30 min. The washed myelin pellet was suspended in water. The yield of dry pellet mix with Tris HCl buffer 0.1M pH 2.0 Successive extraction removed Acid Extraction of Basic Proteins from Myelin-Lipids: ethanol (3.2, v/v) and their t 4C^0 as described previously. The resulting pellet was suspended in ice-cold 0.1 N HCl (2 ml of 0.1 N HCl/mg of dry myelin), and centrifuged at 14,000 rpm for one h at 4C^0. The clear supernatant was collected, and the residue was extracted with 0.1 N HC1 two more times as described above. Dialyzed at 4C^0 against 20 volumes of 0.1 M acetic buffer pH 2.0.

3.2. The method of Centrifugation in Difference Organic Solvents for Dilapidation Myelin Proteins

The pellets were resuspended in 500µl of extraction buffer (2.5ml SDS; 50 ml Tris, pH 8.5). The microcentrifuge tubes containing the cell suspension were agitated gently for 1h at 4°C, followed by removal of the cells by centrifugation at 14.000 x g for 1h at 4°C.The extraction was incubated at 100°C for 5 min and then cooled on ice. Subsequently, 24µl of assay buffer (50 ml Tris pH 8.5) were added, and the extraction incubated again for 15 min on ice. The reaction was stopped by the addition of four volumes and precipitation of proteins was left to occur for 20 min on ice. The pellet was removed by centrifugation at 14.000 Rpm for 10 min at 4°C. The MBP were resolubilized in 500µl of sample preparation solution (Malafaia, Guerra et al. 2015).

3.3. Solubilization Proteins with Acid Buffer

Precipitated proteins were suspended in 2.5 mL Tris-HCl and stirred overnight at 4°C to remove all soluble molecules. The samples were centrifuged, the supernatant was removed, and the pellet was suspended in solution, Samples were incubated overnight at 4°C with magnetic stirring and centrifuged (1 h, 4°C, 14,000 Rpm) to remove insoluble proteins. Supernatants were collected for analysis.

3.4. Method of Solubilization Proteins with Acid Buffer. Solubilization with Ionic Detergent Contained the Buffer

Appropriate aliquots of tissue extracts were lyophilized and taken up in volumes of different buffers of pH 4.0 to 10.0 so as to yield a concentration of 10 mg dry weight per ml each. Buffers for the pH units 4.0, 5.0, and 6.0 were those for pH 6.8 and 8.8 from Tris-HC1, Protein samples were equilibrated for 10 min at 4^0C and subsequently centrifuged at 18,000 mg for 20 min at 4^0C to separate soluble and insoluble proteins. The pellets of insoluble proteins were washed once with the appropriate buffer solution.

3.5. The Method of the Proteins Purification with PAAG Electrophoresis. Preparation of Protein Samples for PAAG Electrophoresis

Sample preparation was performed at cold temperatures to avoid denaturation and degradation of the protein. Lysis buffer should be used to enable lysis of cells and to solubilize proteins. Lysis buffer contains 50 mM Tris pH 6,8, 5% glycerol, 3% SDS, 2 mM dithiothreitol, 0,01% bromphenol blue. Lysis buffers used in sample preparation for Western blotting should enable efficient protein extraction and maintain anti-MBP recognition of the protein (MacPhee, 2010). Importantly, quantification and comparison with other samples in western blot analysis are dependent on the protein lysates prepared for polyacrylamide gel electrophoresis.

3.6. Isolation MBP Protein According to Molecular Mass from PAAG Should make with Stained Molecular Weight Markers

PAAG Electrophoresis. PAA gel was prepared manual with concentration acrylamide 15%. The samples are loaded to the PAA gel. One lane was loaded with Molecular Weight marker. These markers was used to determine the molecular weight of the target protein. Another road should include an internal control, ideally with a known concentration and molecular weight to determine.

3.7. Method the Immunization Rabbits with Purified MBP Antigen Should Pass the Requests of Ethical Comments Accordingly

Immunization of the rabbits was carried out white rabbits were immunized Intracutaneously injected. There were two rabbits used for injection MBP antigen in a dose 50 µg - 25 µg for animal at once. For the primary immunization we used 50 µg for animal. After first immunization rabbits were caged three weeks without any treatment. The second immunization was performed with 25 µg for animal at once.

3.8. The Method of Animals Bleeding from Ear Vein Should Pass the Requests of Ethical Committee Accordingly

The collection of antiserum from immunized animals was performed by bleeding from the ear vein. Blood was collected 1 time after the final immunization and anti-MBP were collected and separated from blood cloth. Serum from immunized rabbits centrifuged 15 min 3000 rpm. Separated from blood cells serum conserve with sodium azid 0.01% and save in +4 C^0.

3.9. Isolation and Purification Specific Antibodies Against MBP Realize With 30% Sulfate Ammonium Precipitation

Myelin proteins for SDS/polyacrylamide gel electrophoresis were prepared and separated from one another by the analytical SDS/polyacrylamide gel system previously described Acrylamide was polymerized in a glass column with an internal diameter of 22mm and a total length of 344mm. Briefly, 18ml of 10% (w/v) Acrylamide was added to 20ml of 50mM sodium phosphate buffer, pH7.2, containing 50µl SDS. This was followed by the addition of 10 µl of NNN'N'-tetramethylethylenediamine and 50µl of a freshly prepared solution of ammonium persulphate (8mg/ml). The solution was mixed, 37ml was pipetted into the glass column, and propanol was immediately layered over the gel solution. After polymerization, the surface of the gel was washed with the gel buffer (diluted 1:1 with water). The total length of the polymerized gel was 110mm. Myelin proteins (from one mouse brain) from which lipids had been removed were dissolved in a sample buffer (5mM-sodium phosphate, pH7.2) of an aqueous solution of Bromo- phenol Blue (0.05 %, w/v). The protein was layered on the gel and overlaid with gel buffer (pH 7.2) diluted 1:1 (v/v) with water. The diluted gel buffer was used to fill the lower (anode) and upper (cathode) compartments of the electrophoresis apparatus. The current was maintained increased to 20mA. Electrophoresis was stopped 2h after the Bromophenol Blue had migrated out of the gel. The gel was then removed from the glas plates and left at 4°C for 15h. After this period, the white bands of flocculated SDS-protein complex of all myelin proteins could be readily seen within the gel. These groups correspond exactly to the respective proteins stained with Coomassie Brilliant Blue. Appropriate sections of the gel

containing the proteins were sliced and crushed, and each protein was eluted with 0.1(w/v) SDS containing 0.1 % (w/v) dithiothreitol (20ml/gel section) by incubation overnight at 25°C. After centrifugation to sediment the gel particles, the clear solution was filtered through glass wool. A small portion was freeze-dried and used to determine the homogeneity of eluted myelin proteins by analytical SDS/polyacrylamide gel electrophoresis (Allison et al., 1974). The purity of each myelin protein having been ascertained, the remainder of the extract was concentrated by freeze-drying. Co-chromatographing purified proteins also tested the bands with the mixed myelin.

3.10. Testing of Antibody Specificity with Immunoblot

Polyacrylamide gels are formed from the polymerization of two compounds, acrylamide, and N, N-methylene bis acrylamide (Bis, for short). Bis is a cross-linking agent for the gels. The polymerization is initiated by the addition of ammonium persulfate along with either DMAP or TEMED. The gels are neutral, hydrophilic, three-dimensional networks of long hydrocarbons crosslinked by methylene groups. The separation of molecules within a gel is determined by the relative size of the pores formed within the gel. The pore size of a gel is determined by two factors: the total amount of acrylamide present (designated as %T) and the amount of cross-linker (%C). As the full amount of acrylamide increases, the pore size decreases. With cross- linking, 5%C gives the smallest pore size. Any increase or decrease in %C increases the pore size. Gels are designated as percent solutions and will have two necessary parameters. The total acrylamide is given as a percentage (w/v) of the acrylamide plus the bis-acrylamide. A standard migration buffer (also called running buffer) for PAGE is 1X Tris-glycine: 25 mM Tris base, 190 mM glycine, 0.1% SDS, pH 8.5. Transfer of Proteins. Once electrophoresis is complete, the separated proteins should be moved from within the gel onto a membrane (a western blot) made of nitrocellulose, polyvinylidene difluoride). Transferring proteins from a gel to a membrane should realize 90 – 120 min with constant current 150 – 200 mA. Its main advantages are speed and completeness of transfer. This process uses an electric current to pull proteins from the gel onto the membrane. The effectiveness of protein transfer is dependent on the type of gel used, the molecular mass of the protein, and the type of membrane. Visualization of protein sorption onto membranes: To check for success of transfer, wash the membrane in TBST

(for a TBST recipe, see below). Staining with Ponceau Red: The stock is made of 2% Ponceau S in 30% trichloroacetic acid and 30% sulfosalicylic acid. Incubate on an agitator for 5 min. Wash extensively with water until the water is clear and the protein bands are well-defined. The membrane may be destined thoroughly by repeated washing in TBST or water. When using a PVDF membrane, reactivate the membrane with methanol then wash again in TBST. To block nonspecific binding, the membrane is placed in a dilute solution of protein such as bovine serum albumin and nonfat dry milk. Lock helps mask any potential nonspecific binding sites on the membrane. After blocking, the most popular method is to incubate the membrane with primary antibody, wash, reblock, and then incubate with secondary antibody and wash again. It is important to determine the optimal concentration of antibodies before running all the samples as optimization is a prime determinant of the sensitivity of the assay. The antibody concentration should be optimized to provide the best signal to noise ratio. Both monoclonal and polyclonal antibodies can be used for western analyses, with advantages and disadvantages in using either type (MacPhee, 2010). Detection. The probes that are labeled and bound to the protein of interest need to be detected on the western blot. The primary antibody binds to the protein of interest and the secondary antibody linked to horseradish peroxidase, is used to cleave a chemiluminescent agent. The reaction product produces luminescence, which is related to the amount of protein. Once exposures have been captured, blots can be washed in a buffer and then ‘‘stripped,’’ which involves removing bound antisera to enable reuse of the blog. Blots can then be stored for future reprobing several more times. However, subsequent reprobing can interfere with protein antigens, resulting in a decreased signal (Algeria-Schaffer et al., 2009). Relative quantification and analysis. The blot results can be analyzed by densitometry to measure the relative amount of a particular protein on the blot by comparing it with a control or specific time point. This quantification is necessary to analyze samples. There are commercial software programs for image analysis of bands on film or membrane (Jensen et al., 2007).

4. RESULTS

4.1. Isolation Myelin Proteins from Rat Brain

For MBP isolation in this study was used separation water insoluble proteins with extraction water soluble proteins by tris-buffer pH 8.0. The pellet contained non soluble proteins resuspended in methanol-aceton organic for dilapidation. Later 45 min incubation in +4C^0 and centrifugation pellet contained MBP was separated. Obtained pellet was used for extraction MBP with acid buffer pH 2.0. The extraction was performed 60 min at +4 C^0 with permanent mixing. The results of these steps of isolation and purification presented in (Figure 4.1.).

4.2. Immunization with MBP in Rabbit used Two Light Color

Immunization rabbit by intracutaneous injected 50 µg for each rabbit in the first immunization. Next three weeks rabbits were not treated with antigen MBP. During this while the immune system of rabbits was activated with antigen and protected Anti-MBP antibodies. Second immunization with MBP was performed for rabbit later three weeks. Immunoglobulin to myelin antigen represents genuine antibody activity.

4.3. Testing Affinity and Specify of Anti –MBP by Western Blot

Rat brain 1g with 10ml Tris buffer homogenized with 1ml with 44ml acetone /methanol 1:1. Mixing and cooling in (-20ºC) for (20) mint. Next centrifuge in 14000rpm in (60mint). the result S_1 is the MBP P_1 the pallet saved and dry in (-20ºC). When dried the pellet mix with Tris 0.1 HCL buffer PH 2.0 mixing by centrifuge 14000 Rpm for 30 min. S_1 is the myelin proteins from rat brain, S_1 with MH_4SO_4 saturation 30% centrifuged from 5000 rpm for 30 min. The pallet is p_2 sole in Tries buffer and 0.1 SDS this pellet on this S_2 MBP regimented with S_1. MBP purified for immunization need to antigenic and immunogenic protein characteristics.

Especially MBP polypeptides the polypeptide MBP 18 kDa after immunizations blood MBP isolated by gel electrophoresis for detected Anti MBP polypeptide the MBP its 18 KDa.

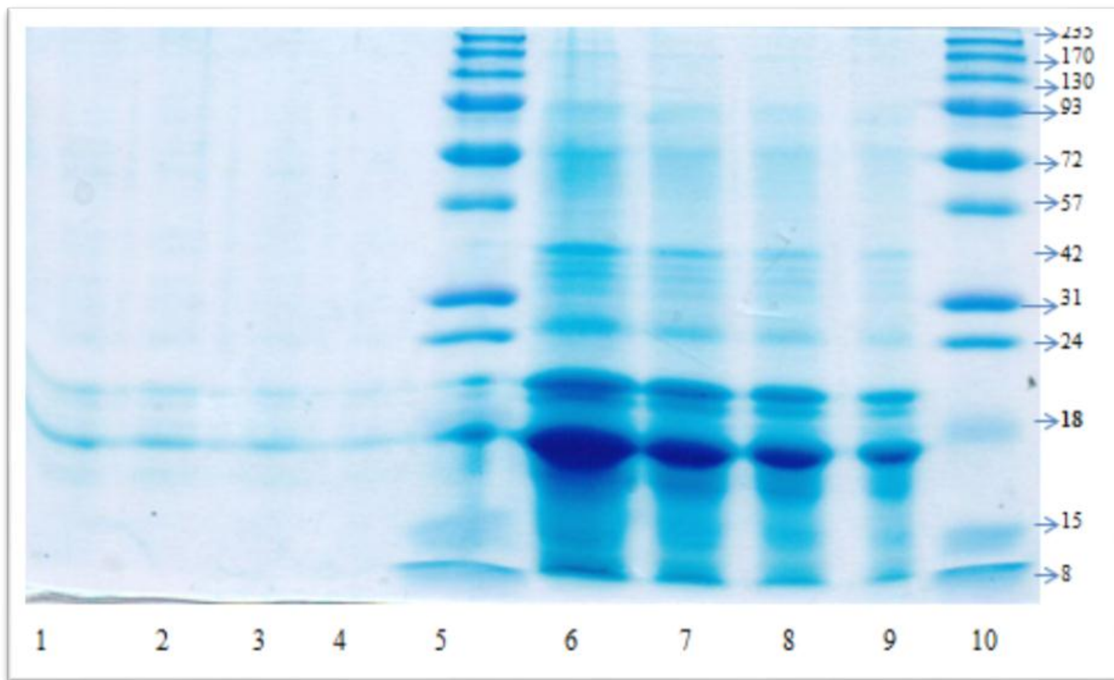


Figure 4.1. Result of electrophoresis in 15% PAAG

1. MBP solution precipitated with ammonium sulfate, 40 µg.
2. MBP solution precipitated with ammonium sulfate, 20 µg.
3. MBP solution precipitated with ammonium sulfate, 10 µg.
4. MBP solution precipitated with ammonium sulfate, 5 µg.
5. Markers of MW.
6. MBP extracted with acid buffer pH 2.0, 40 µg.
7. MBP extracted with acid buffer pH 2.0, 20 µg.
8. MBP extracted with acid buffer pH 2.0, 10 µg.
9. MBP extracted with acid buffer pH 2.0, 5 µg.
10. Markers of MW.

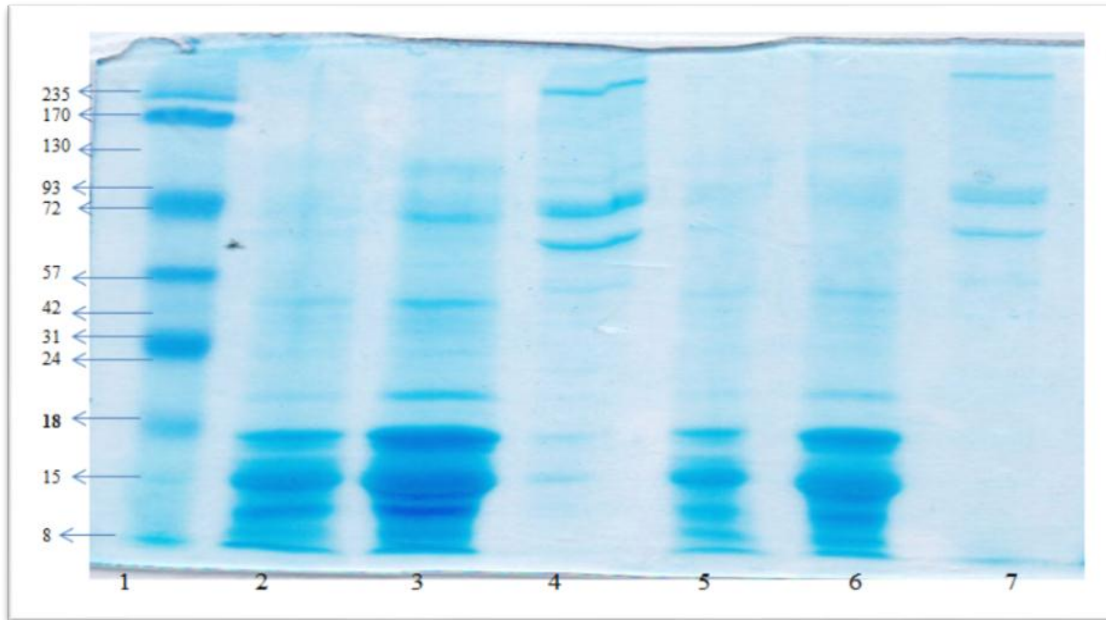
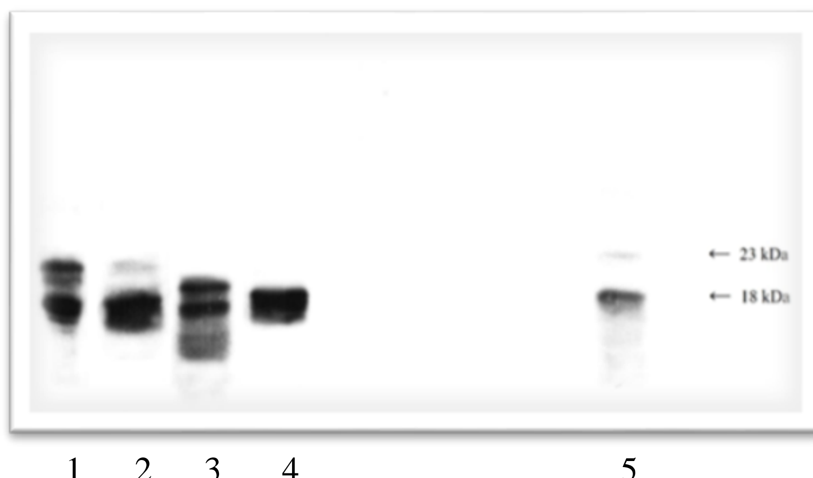


Figure 4.2. SDS/PAAG Isolated membranes were purified MBP (Coomassie- Blue-stained) Experimental section. After removal of radioactivity that had not reacted, the membrane was treated with diethyl ether and the total protein fractions were electrophoresed on SDS/ 15 %-polyacrylamide gels. Lanes of Coomassie Blue staining (a) and radioautography (b) are shown

1. Markers of MW.
2. MBP extracted with acid buffer pH 2.0, 40 µg.
3. Proteins fraction precipitated with acetone/methanol, 40 µg.
4. Supernatant after precipitated with acetone/methanol, 10 µg.
5. Proteins fraction extracted with acid buffer pH 2.0, 20 µg.
6. Proteins fraction precipitated with acetone/methanol, 20 µg.
7. Supernatant after precipitated with acetone/methanol, 5 µg.

For isolation and purification MBP rat brain (marker 20µl, MBPS2 30µl, BSA 30µl, MBP rat precipitated 30µl, soluble rat brain 30µl).after this isolation separated MBP in the gel by buffer



1 2 3 4 5

Figure 4.3. Result of western blot with antibodies against MBP. Dilution of antibodies – 1: 2500 (1-4) and 1:5500 (5)

1. Rat brain extract 0, 5% SDS, 80 µg.
2. MBP solution precipitated with ammonium sulfate, 10 µg.
3. MBP extracted with acid buffer pH 2.0, 10 µg.
4. Concentrated extract of MBP from PAAG, 10 µg.
5. Kidney soluble protein extract, 30 µg.
6. Kidney insoluble protein extract, 30 µg.
7. Liver soluble protein extract, 30 µg.
8. Liver insoluble protein extract, 30 µg.
9. Solution for immunization, 1,0 µg.

In (Figure 4.3.) After immunizing the rabbit by MBP rabbit have antibody for this protein its18 KDa for Anti-MBP, when rabbit after two time injected, in the first time not have anti MBP, in second time immunization of rabbit lead to produce secondary immune response and IgG class anti-MBP antbody. The titer of antibodies in western blot was determinate as maximum dilution for visible results without decreasing intensity of main MBP polypeptides staining on a membrane. This maximal dilution for anti-MBP antibodies was 1:5500. Purification of IgG fraction resulted to obtain specific antibodies against pure MBP from rat brain containing mainly the 18.5 KDa isoform. Obtained specific antibodies against MBP may be successfully used for study demyelinated diseases and olygodendrocyte pathology.

5. DISCUSSION

In this study, we obtained specific antibodies against pure MBP from rat brain that recognize mainly the 18.5 kDa isoform. Different isoforms of MBP are presented in a fraction that were obtained while isolation and purification procedure. The results presented in Figure 4.3. give an evident that antibodies generated after immunization with MBP antigen are high specific to MBP. Especially, the polypeptide 18 kDa is recognized in every sample from rat brain. The pathology of neural tissue is closed associate with olygodendrocytes functions and its potential for myelin metabolism. Obtained antibodies are effective molecular equipment for study and control demyelination and decline of olygodendrocyte functions.

In the recent study, we have identified a novel role of purified MBP as an A_ peptide insulting enzyme in vitro. First, we only read that highly purified MBP from normal human white matter or purified recombinant 6_-hisMBP exhibit serine proteinase autolytic activity. Subsequent experiments revealed that purified MBP degraded both soluble A_40 and A_42 peptides in vitro and Cos-1 cells expression MBP. Further, we manifestation by some averages that purified MBP could diminish fibrillar as well. Mass spectrometry investigation recognized multiple MBP separating sections on solvable and fibrillar A_. Lastly, we showed that cleared human MBP could degrade parenchymal and fibrillar amyloidal deposits in brain tissue sections of APP transgenic mice (Liao, Ahmed et al. 2009). The recent study shows some surprising similarities between antibodies from MS patients and healthy individuals. Firstly, sera from the two groups contained equal quantities of IgM and IgG reactive with MBP.

Accordingly, an addition of MBP to sera from both groups mediated a dose-dependent deposition of IgM, IgG and MBP itself on the surface of monocytes, indicating that formation of MBP/anti-MBP complexes had occurred. Supporting this notion, denaturation of MBP actively reduced its deposition and its ability to induce IgM deposition. A role for the complex net in the process was indicated by the deposition of C3 fragments along with IgM and MBP and by the observation that chelation of calcium strongly inhibited MBP/IgM/C3 fragment deposition. Secondly, disease- associated autoantibodies did not facilitate the production of TNF- and IL-10 by standard MNCs to a greater extent than Naas contained in normal sera. The responses did seem to be antibody-dependent, however, as denaturation of MBP abrogated them. While the propensity of MS-derived MNCs to produce IFN-cand IL-5, in addition to TNF-a and IL-10, appears to be independent of the serum source driving antigen uptake and presentation, and, as such, to be programmed at the cellular level, the enhanced synthesis of these cytokines in the presence of the patient’s own serum does suggest that disease-associated antibodies can amplify the inflammatory cytokine response. More investigation is required to characterize these antibodies further and establish their proinflammatory (Hedegaard, Chen et al. 2009). Purification of IgG fraction resulted to obtain specific antibodies against pure MBP from rat brain containing mainly the 18.5 KDa isoform. Obtained specific antibodies against MBP may be successfully used for study demyelinated diseases and olygodendrocyte pathology.

CONCLUSION

1. The results of testing antibodies show high affinity and specific immune response against MBP antigen.

2. High affinity and specific immune response against MBP antigen was provided by the immunization MBP separated in PAAG and isolated from acrylamide gel by extraction with tris-buffer contained 0,1% SDS.

REFERENCES

Adamo AM, (2014). Nutritional factors and aging in demyelinating diseases. Genes & Nutrition 9(1): 360

Aggarwal S, Snaidero N, Pähler G, Frey S, Sánchez P, Zweckstetter M, Janshoff A, Schneider A, Weil MT, Schaap IA, Görlich D, Simons M, (2013). Myelin membrane assembly is driven by a phase transition of myelin basic proteins into a cohesive protein meshwork. PLoS Biol 11(6): e1001577

Alizadeh A, Dyck SM, Karimi-Abdolrezaee S, (2015). Myelin damage and repair in pathologic CNS, challenges and prospects. Front Mol Neurosci 8

Babbs CF, RSh, (2013). Subtle paranodal injury slows impulse conduction in a mathematical model of myelinated axons. PLoS One 8(7): e67767

Bamm, Vladimir V, Miguel, De Avila, Graham S.T, Smith, Mumdooh AM, Ahmed, George Harauz, (2011). Structured Functional Domains of Myelin Basic Protein: Cross Talk between Actin Polymerization and Ca(2+)-Dependent Calmodulin Interaction. Biophysical Journal 101(5): 1248-1256

Boggs JM, Homchaudhuri L, Ranagaraj G, Liu Y, Smith GS, Harauz G, (2014) Jun, Interaction of myelin basic protein with cytoskeletal and signaling proteins in cultured primary oligodendrocytes and N19 oligodendroglial cells. BMC Res Notes. 24: 7: 387

Boullerne AI, (2016). The history of myelin. Exp Neurol 283(Pt B): 431-445

Bradl MH, Lassmann, (2010). Oligodendrocytes: biology and pathology. Acta Neuropathol 119(1): 37-53

D'Aversa TG, Eugenin EA, Lopez L, Berman JW, (2013). Myelin basic protein induces inflammatory mediators from primary human endothelial cells and blood-brain barrier disruption: implications for the pathogenesis of multiple sclerosis. Neuropathol Appl Neurobiol 39(3): 270-283

De Avila M, Kenrick A, Vassall, Graham ST, Smith, Vladimir V, Bamm, George Harauz , (2014). The proline-rich region of 18.5 kDa myelin basic protein binds to the SH3-domain of Fyn tyrosine kinase with the aid of an upstream segment to form a dynamic complex in vitro. Biosci Rep 34(6): e00157

Ferretti G, T, Bacchetti (2011). Peroxidation of lipoproteins in multiple sclerosis. J Neurol Sci 311(1-2): 92-97

Frid K, Ofira Einstein, Yael Friedman-Levi, Orli Binyamin, Tamir Ben-Hur,and Ruth Gabizon, (2015). Aggregation of MBP in chronic demyelination, Annals of Clinical and Translational Neurology 2(7): 711-721

Fulton D, Pablo M, Paez1, Anthony T, Campagnoni2, (2010). The multiple roles of myelin protein genes during the development of the oligodendrocyte. ASN Neuro 2(1): e00027

Harauz G, DS, Libich (2009). The classic basic protein of myelin--conserved structural motifs and the dynamic molecular barcode involved in membrane adhesion and protein-protein interactions. Curr Protein Pept Sci 10(3): 196-215

Harauz G, JM Boggs, (2013). Myelin management by the 18.5-kDa and 21.5-kDa classic myelin basic protein isoforms. J Neurochem 125(3): 334-361

Harauz G, Ishiyama N, Hill CM, Bates IR, Libich DS, Farès C, (2004). Myelin basic protein-diverse conformational states of an intrinsically unstructured protein and its roles in myelin assembly and multiple sclerosis. Micron 35(7): 503-542

Harauz G, Ladizhansky V, Boggs JM, (2009). Structural polymorphism and multifunctionality of myelin basic protein. Biochemistry 48(34): 8094-8104

Hedegaard CJ, Ning Chen, Finn Sellebjerg, Per Soelberg Sørensen, R Graham, Q Leslie, Klaus Bendtzen, Claus H Nielsen, (2009). Autoantibodies to myelin basic protein (MBP) in healthy individuals and in patients with multiple sclerosis: a role in regulating cytokine responses to MBP. Immunology 128(1 Pt 2): e451-461

Kim JK, Mastronardi FG, Wood DD, Lubman DM, ZR, Moscarello MA, (2003). Multiple sclerosis: an important role for post-translational modifications of myelin basic protein in pathogenesis. Mol Cell Proteomics 2(7): 453-462

Lee DW, Xavier Banquy, Kai Kristiansen, Yair Kaufman, Joan M. Boggs, Jacob N, Israelachvili, (2014). Lipid domains control myelin basic protein adsorption and membrane interactions between model myelin lipid bilayers. Proc Natl Acad Sci U S A 111(8): E768-775

Liao MC, Ahmed M, Smith SO, Van Nostrand WE, (2009). Degradation of amyloid beta protein by purified myelin basic protein. J Biol Chem 284(42): 28917-28925

Love S, (2006). Demyelinating diseases. J Clin Pathol 59(11): 1151-1159

Mayo L, Quintana FJ, Weiner HL, (2012). The innate immune system in demyelinating disease. Immunol Rev 248(1): 170-187

Morrison BM, (2013). Oligodendroglia metabolically support axons and maintain structural integrity. Trends in cell biology 23(12): 10.1016/j.tcb.2013.1007.1007

Nave KA, (2010). Myelination and the trophic support of long axons. Nat Rev Neurosci 11(4): 275-283

Nave KA, BD Trapp (2008). Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci 31: 535-561

Ndubaku U, and ME de Bellard, (2008). Glial cells: Old cells with new twists. Acta histochemica 110(3): 182-195

Saher G, Brügger B, Lappe-Siefke C, Möbius W, Tozawa R, Wehr MC, Wieland F, Ishibashi S, Nave KA, (2005). High cholesterol level is essential for myelin membrane growth. Nat Neurosci 8(4): 468-475

Schaecher KE, Shields DC, Banik NL, (2001). Mechanism of myelin breakdown in experimental demyelination: a putative role for calpain. Neurochem Res 26(6): 731-737

Siu CR, Balsor JL, Jones DG, Murphy KM , (2015). Classic and Golli Myelin Basic Protein have distinct developmental trajectories in human visual cortex. Front Neurosci 9: 138

Yin X, Kiryu-Seo S, Kidd GJ, Feltri ML, Wrabetz L, Trapp BD, (2015). Proteolipid protein cannot replace P0 protein as the major structural protein of peripheral nervous system myelin. Glia 63(1): 66-77

Zhang J, Sun X, Zheng S, Liu X, Jin J, Ren Y, Luo J. (2014). Myelin basic protein induces neuron-specific toxicity by directly damaging the neuronal plasma membrane. PLoS One 9(9): e108646

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