Transcriptional regulation of brain derived neurotrophic factor (BDNF) by methyl CpG binding protein 2 (MeCP2): Implication in re-myelination and/or myelin repair in an animal model of multiple sclerosis (MS)

by

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A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

College of Pharmacy, Faculty of Health Sciences

University of Manitoba

Winnipeg

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisor Dr. Namaka for the continuous support of my Master's program and giving me an amazing opportunity to be a part of this project. I am grateful for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. Besides my advisor, I would like to thank the rest of my committee members: Dr. Klonisch and Dr. Lakowski for their insightful comments and encouragement and also for their illuminating suggestions.

I thank my fellow lab mate Ms. Crystal Acosta for the stimulating discussions, all the patience in training me and for all the fun we have had in the last two years.

My sincere gratitude also goes to Dr. Khaled AlTaweel for his words of wisdom, training, and unending technical support. My grateful thanks are expressed to our summer student: Harpreet Bhullar. Thank you for the tireless effort and assistance in the lab and all the fun times.

I am really indebted to Ms. Claudia Cortes Perez. To her I express my deepest appreciation and gratitude. Without her training, persistent help, and guidance the completion of my project could not have been possible.

Last but not the least, I would like to thank my family: my parents and to my brother for supporting me spiritually throughout writing this thesis and my life in general.

Finally, I would like to acknowledge the college of Pharmacy at the University of Manitoba and the Manitoba Multiple Sclerosis Research Network Organization (MMSRNO) for their on-going

support, as well as, our funding/ supporting agencies: The Manitoba Medical Service Foundation Grant (MMSF), Canadian Paraplegic Association (CPA), University of Manitoba Research Grants Program (URGP) and Bayshore Health Care Systems. Furthermore, I would like to acknowledge endMS research and training network for their continued support of research, training and education to advance our knowledge in the specialized field of MS.

For those living with multiple sclerosis (MS)

Who never give up Hope

And for all people trying to

End MS

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ABBREVIATIONS

AC: Active control

ANOVA: Analysis of variance

BBB: Blood brain barrier

BDNF: Brain derived neurotrophic factor

CNS: Central nervous system

CSF: Cerebrospinal fluid

CX3CL1: Fractalkine

CX3CR1: Fractalkine receptor

DRG: Dorsal root ganglia

DMT: Disease modifying therapies

DPI: Days post induction

EAE: Experimental autoimmune encephalomyelitis

ELISA: Enzyme linked immunosorbent assay

GA: Glatiramer acetate

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

IFN-γ: Interferon –γ

IL: Interleukin

MAG: Myelin associated glycoprotein

MBP: Myelin basic protein

MeCP2: Methyl CpG binding protein 2

MHC: Major histocompatibility complex

MOG: Myelin oligodendrocyte glycoprotein

MS: Multiple sclerosis

NC: Naïve control

NDS: Neurological disability scores

NGF: Nerve growth factor

NK: Natural killer

NPP: Neuropathic pain

OGs: Oligodendrocytes

OPCs: Oligodendrocyte progenitor cells

PNS: Peripheral nervous system

RRMS: Relapsing remitting multiple sclerosis

SC: Spinal cord

SCI: Spinal cord injury

SPMS: Secondary progressive multiple sclerosis

Th: Helper T cells

TNF α: Tumor necrosis factor alpha

Trk: Tropomysin kinase receptor

WB: Western blot

qRT-PCR: Quantitative real time polymerase chain reaction

All FIGURES AND ILLUSTRATIONS PRESENTED IN THIS THESIS REPRESENT ORIGINAL WORK THAT I HAVE PRODUCED IN CONSULTATION WITH OUR NEUROIMMUNOLOGY RESEARCH TEAM

CHAPTER 1: GENERAL INTRODUCTION

1.1. BACKGROUND AND RATIONALE FOR THESIS

Multiple sclerosis (MS) is a chronic progressive, neurological disease characterized by the targeted immune system mediated destruction of central nervous system (CNS) myelin [1]. MS affects approximately 2.1 million people worldwide [2]. This white matter disease characteristically presents with axonal degeneration that follows demyelination. As such leaves the patients with permanent disabilities, from which they never fully recover [3]. Current available treatments for MS such as immunomodulatory and/or disease modifying drug therapies at best only reduce the severity of the disease and slow the progression. At present, there is no definite cure for MS [4]. However, current research is exploring treatments that promote re-myelination and myelin repair of the regionalized areas of MS-induced myelin damage. Re-myelination strategies target the generation of new myelin in areas that have been damaged along the nerve axons of the CNS. Myelin is an insulating material responsible for the normal propagation of electrical impulses that are essential for normal physiological functioning. In addition, myelin also has a critical role in protecting neurons from degeneration which is integral in the neurological recovery of patients following an MS attack [5]. Henceforth, next generation MS treatment strategies are being investigated for their combined ability to suppress the inflammatory immune system response that drives the induction and maintenance of the disease plus their ability to promote re-myelination and/or myelin repair [6-8].

Neurotrophins such as brain derived neurotrophic factor (BDNF) or nerve growth factor (NGF) belong to a family of closely related, small polypeptide molecules that are thought to have a key role in the structure, function and production of CNS myelin as well as neuronal survival [7, 9-

13]. Henceforth, current treatment strategies are being investigated to enhance the role of these beneficial neurotrophins. As such, these treatments offer new promise for neurodegenerative disorders such as Alzheimer's , Parkinson's , Huntington's , Amyotrophic lateral sclerosis (ALS), and MS [8, 14]. Recent investigation into the MS medication glatiramer acetate (GA) also more commonly known as copaxone® [15] has re-directed the focus of its mechanism of action toward BDNF production. Specifically GA is thought to exert its effects by enhancing BDNF production from GA reactive Th2 lymphocytes. GA exhibits random, high-affinity binding to the major histocompatibility complex molecules on antigen-presenting cells, thus mimicking myelin and myelin-like antigen presentation to T cells. Although GA-specific helper T cell (Th)1, Th2 and Th0 cells are involved in BDNF production, *in vitro* studies have suggested that Th2 cells play a predominant role in GA modulation of relapsing remitting MS (RRMS) *via* enhanced BDNF production [15-20]. Pre-clinical studies involving experimental autoimmune encephalomyelitis (EAE) rodents support this mechanism of GA-induced BDNF production. For example EAE animals treated with GA show elevated BDNF levels, compared with a treatment naïve group [21]. This increase in BNDF levels is sustained over the disease course, and correlate with indicators of myelin repair [21, 22]. Similarly, rodent studies involving BDNF gene delivery into the CNS using transformed bone marrow stromal cells as a vehicle, exhibit delayed onset of MS disease, enhanced clinical recovery, reduced apoptosis, and reduced demyelination following EAE [23, 24]. Other studies have also provided evidence that BDNF contributes to the re-myelination of MS-induced lesions [23, 25].

Furthermore, according to a study involving EAE induced BDNF KO mice; the absence of BDNF resulted in a significant increase in myelin structural damage compared to control animals. This increase in myelin damage was attributed to the abnormal distribution patterns of

the myelin proteins [26]. Similarly, other studies have also implicated BDNF in regulating the distribution patterns of myelin structural proteins [27-29]. In addition to governing the structural integrity of myelin, BDNF also induces oligodendrocyte progenitor cells (OPCs) proliferation [30], migration and differentiation at the site(s) of injury [31], which are essential steps in myelin formation [28, 32]. Our recent publication using an EAE model of MS confirms that BDNF is up-regulated and delivered to the spinal cord (SC) from the dorsal root ganglia (DRG). We also identified the small to medium sized sensory neurons housed within the DRG as the cellular source of the BDNF [33]. Based on our previous publication, DRG-derived BDNF is recognized as an integral source essential for CNS myelin repair and neurological recovery following an immune system-mediated attack.

Human studies also support the beneficial role of BDNF in myelin repair but there is no consensus on exact mechanism. Higher serum levels of BDNF were detected in patients during an MS attack in contrast to those seen during the stable phase of the disease [25, 34]. These results suggest that during an MS attack, BDNF levels are up-regulated in order to facilitate myelin repair and subsequent neurological recovery during the remission phase of the disease. However, it has also been shown that peripheral blood mononuclear cells of relapsing remitting multiple sclerosis (RRMS) patients secrete lower levels of BDNF, both in the remission and relapsing phase of the disease, as compared with healthy individuals [35]. These results suggest that RRMS patients consistently have overall lower BDNF levels compared with healthy controls. As such, it may predispose them to myelin damage and limits the ability to achieve complete myelin repair even during periods of remission. Other researchers have reported that during an acute MS attack, serum BDNF levels are significantly elevated above those seen during the stable, remission phase of the disease [36]. These results suggest that, although BDNF

levels rise during an MS attack, they do not reach sufficient levels to efficiently counter the immune system-mediated damage to the myelin. Furthermore, other studies have confirmed that BDNF is expressed in and around human MS lesions [37-39]. Therefore, BDNF has a key role in the localized repair of a lesion, possibly through induction of OG migration and/or precursor proliferation/differentiation at the site of myelin injury. Numerous other studies also support the role of BDNF in myelin repair in response to MS [35, 36, 40-45].

Interestingly, additional studies involving repair of myelin damage caused by traumatic spinal cord injury (SCI) also support the beneficial role of BDNF. Increased BDNF expression in the SC after complete SC axotomy has been shown to facilitate myelin repair [33, 46, 47]. Additional supportive evidence comes from studies that have examined the expression of BDNF in the SC after incomplete crush injuries to the SC [47-50]. Specifically, these studies have shown that the significant increase in BDNF levels in the damaged SC was responsible for the promotion of OPCs proliferation and re-myelination of damaged axons [47]. Therefore, transcriptional regulation of BDNF could be critical in designing treatments in order to enhance re-myelination and/or myelin repair.

Interestingly, a key biological target called methyl CpG binding protein 2 (MeCP2) is currently being investigated for its pathological effects in a childhood white matter disorder called Rett Syndrome. MeCP2 is thought to be responsible in the pathological development of the white matter damage known to occur in Rett syndrome due to its ability to repress the transcriptional expression of BDNF [51-53]. Identified in 1989, MeCP2belongs to the family of methyl binding proteins [54]. Research has confirmed that MeCP2 is known to be involved in the transcriptional regulation of BDNF [55, 56]. Specifically, it exerts its transcriptional repressor effect [55, 57] by binding its methyl-CpG binding domain (MBD) to the methylated CpG binding site of the BDNF

target gene. Following this its transcriptional repressor domain (TRD) forms a co-repressor complex with histone deacetylase (HDAC) and Sin3A [58, 59]. Both studies in mice [57] and rats [55] indicate MeCP2 repression of BDNF could be reversed by membrane depolarization, phosphorylation and release of MeCP2 from the BDNF promoter. However, MeCP2 can also indirectly regulate BDNF gene expression by affecting the expression of other BDNF transcriptional repressors like RE1 silencing transcription factor (REST) and CoREST [60]. REST and CoREST are two transcriptional repressors that are involved in transcriptional repression of BDNF gene. As MeCP2 is involved in their transcriptional repression, they are over expressed in MeCP2-deficient mice. Interestingly, studies done in MeCP2 null mice [53, 61, 62] and human tissues [63] show decreased expression of BDNF gene and protein. Therefore, MeCP2's ability to directly and indirectly effect BDNF expression warrants its attention as a key biological target by which novel targeted interventional strategies can be established to promote myelin repair.

MeCP2 has two biologically active isoforms, MeCP2E1and MeCP2E2 [64, 65]. These two isoforms have been shown to have differential biological effects regarding neuronal survival [66] and embryonic development [67]. Based on our previous research [9], we believe these two isoforms have distinctly different affects in regard to the re-myelination and/or the myelin repair process due to their differential effects on BDNF expression. Our current research suggested that the MeCP2E1 isoform is involved in the transcriptional repression of BDNF while the MeCPE2 isoform may be involved in the activation of the BDNF gene. In addition to its direct effect on BDNF at the level of the transcript, MeCP2 has also been shown to be involved in governing the structural integrity of myelin by affecting the regulation of expression of the specific myelin genes that comprise the structure of myelin [52, 68]. For example, in a recent

study using small interference RNA to knock down MeCP2 in cultured oligodendrocytes, increased expression of myelin proteins was reported [52] .

In order to establish novel treatment approaches for re-myelination and/or myelin repair we designed specific experiments to determine the mechanism by which MeCP2 alters the transcriptional expression of BDNF. We used a well-known and established animal model of MS known as experimental autoimmune encephalomyelitis (EAE). Specifically, we used a myelin oligodendrocyte (MOG) mouse model to induce EAE. The research findings suggest the importance of designing novel epigenetic interventional treatment strategies to enhance BDNF expression by reducing the expression of the pathological MeCP2E1 isoform.

1.2. Project Summary:

Based on the research that I have conducted during the completion of my Master's degree, we evaluated the temporal gene and protein expression of MeCP2E1, MeCP2E2 and BDNF in a MOG-induced EAE animal model of MS. In addition, we correlated these temporal changes in gene and protein expression to corresponding changes in neurological disability scoring (NDS). Our research supports the involvement of the immune system mediated pathological induction of the MeCP2E1 isoform in the repression of BDNF with corresponding neurological disability during acute and chronic phase of the disease. The results presented in this study also confirm the importance of the homeostatic balance between MeCP2E1 and MeCP2E2 isoforms in regulating re-myelination and/or the myelin repair process. Therefore, MeCP2 represents a key biological target that requires further investigation in order to unveil novel treatment approaches aimed at promoting re-myelination and/or myelin repair following an MS attack.

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PROLOGUE TO CHAPTER 2: RATIONALE FOR THE MeCP2 REVIEW MANUSCRIPT

This manuscript was written because it represented the opening of another dimension to Dr. Namaka's previous published research platform involving the molecular signaling triad between cytokines, neurotrophins and chemokines (TNF-α, NGF, BDNF) that govern re-myelination and myelin repair. Based on the scientific evidence that I have obtained during the development of this published review article on MeCP2, it became evident that I would pursue my research project on the upstream transcriptional effects of MeCP2 on BDNF expression. The concept of MeCP2 regulation of BDNF expression and its implications in re-myelination and/or myelin repair following an MS-induced attack formed the foundational basis of my original research work presented in Chapter 3 of my research. In addition to providing a comprehensive review on MeCP2, this published manuscript provides a comprehensive review of emerging role of epigenetic therapeutic interventions in future of MS treatment. Furthermore, this manuscript emphasizes the importance of anatomical connection between peripheral nervous system ganglia called the dorsal root ganglia (DRG) and the CNS in regard to re-myelination and myelin repair. In addition, my involvement in the preparation of this publication set the foundation for my advanced understanding of the importance of BDNF in regard to its beneficial effects on re-myelination and/or myelin repair.

CHAPTER 2: Published Manuscript Review Article: Transcriptional regulation of brain-derived neurotrophic factor (BDNF) by methyl CpG binding protein 2 (MeCP2): A novel mechanism for re-myelination and/or myelin repair involved in the treatment of multiple sclerosis (MS)

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This manuscript was accepted for publication in the peer-reviewed journal, Molecular Neurobiology in December 29, 2014 and published January 13, 2015. Molecular Neurobiology: Doi 10.1007/s12035-014-9074-1. PMID: PMID: 25579386.

STATEMENT OF CONTRIBUTION

I am listed as the first author for this manuscript published January 2015. I was responsible for the inception and design of the manuscript, as well as, writing the initial draft. In collaboration

with my co-authors, I performed the majority of literature searches and was involved in the preparation and revision of the final manuscript and copy proofing.

2.1. ABSTRACT

Multiple sclerosis (MS) is a chronic progressive, neurological disease characterized by the targeted immune system mediated destruction of central nervous system (CNS) myelin. Auto-reactive $CD4^+$ T-helper cells have a key role in orchestrating MS-induced myelin damage. Once activated circulating Th1-cells secrete a variety of inflammatory cytokines that foster the breakdown of blood brain barrier (BBB) eventually infiltrating into the CNS. Inside the CNS, they become re-activated upon exposure to the myelin structural proteins and continue to produce inflammatory cytokines such as tumor necrosis factor α (TNFα) that leads to direct activation of antibodies and macrophages that are involved in the phagocytosis of myelin. Proliferating oligodendrocyte precursors (OPs) migrating to the lesion sites are capable of acute re-myelination but unable to completely repair or restore the immune system-mediated myelin damage. This results in various permanent clinical neurological disabilities such as cognitive dysfunction, fatigue, bowel/bladder abnormalities and neuropathic pain. At present, there is no cure for MS.

Recent re-myelination and/or myelin repair strategies have focused on the role of the neurotrophin brain-derived neurotrophic factor (BDNF) and its upstream transcriptional repressor methyl CpG binding protein (MeCP2). Research in the field of epigenetic therapeutics involving histone deacetylase (HDAC) inhibitors and lysine acetyl transferase (KAT) inhibitors are being explored to repress the detrimental effects of MeCP2. This study will address the role

of MeCP2 and BDNF in re-myelination and/or myelin repair and the potential of HDAC and KAT inhibitors as novel therapeutic interventions for MS.

Key words:

Multiple Sclerosis (MS), epigenetics, histone deacetylase (HDAC) inhibitors, experimental autoimmune encephalomyelitis (EAE), methyl CpG binding protein 2 (MeCP2), brain-derived neurotrophic factor (BDNF), autoimmune disease.

2.2. INTRODUCTION

Multiple sclerosis (MS) is a chronic progressive, neurological disease characterized by the targeted immune-mediated destruction of central nervous system (CNS) myelin [1]. While the exact cause of MS is unknown, it is widely considered to be an autoimmune disease [2]. Specifically, CD4+ T-helper 1 (Th1) cells have been implicated in mediating the inflammatory processes and myelin damage associated with MS [3]. However, several studies also demonstrate that other CD4+ cells (Th2, Th17), CD8+ cells, and B cells are also important in the pathology of MS [4, 5]. According to the autoimmune theory, CD4+ Th1 cells become auto-reactive towards CNS myelin in response to an antigen resembling myelin proteins. However, the exact antigen remains unknown. Upon activation, circulating Th1 cells secrete inflammatory cytokines, such as TNFα, interferon γ (IFN-γ), and interleukins (IL)-1,-6, and -12 [6-12], that facilitate the fenestration of the blood-brain barrier (BBB) to allow the infiltration of Th1 cells into the CNS. Subsequently, these Th1 cells become re-activated in the presence of CNS myelin proteins and continue to produce inflammatory cytokines, such as TNFα, that leads to the direct activation of phagocytic cells that subsequently damage myelin. This destruction of myelin disrupts electrical conduction in neurons, resulting in various neurological deficits [3].

Proliferating oligodendrocyte precursors (OPs) migrate to the sites of lesions where they differentiate resulting in acute re-myelination but are unable to completely repair damaged myelin or restore myelin ensheathment. As the disease progresses, this inability to repair and/or restore myelin ultimately leads to permanent neurological disabilities such as ataxia [13], neuropathic pain [10, 14], spasticity [15] and cognitive deficits [16].

Current available treatments for MS are limited to the immunomodulatory and disease-modifying drug therapies that attempt to improve neurological function by reducing the severity and slowing progression of the disease [17-19]. None of the available treatments for relapsing remitting MS (RRMS) are able to reverse the immune system mediated damage to CNS myelin that are characteristically referred to as MS lesions or plaques [20]. As such, the inability to re-myelinate or repair damaged myelin results in the permanent neurological deficits that result in the late progressive phase of the disease. The concept of re-myelinating lesions as a treatment for MS has gained wide acceptance in recent years and research efforts have continued to direct their focus to re-myelinating strategies [21]. Re-myelination can occur spontaneously but this process is incomplete and not sustainable against the ongoing immune system mediated attack on CNS myelin. Therefore, understanding pathological molecular mechanisms that interfere with re-myelination and/or myelin repair represents the focal point of research [21, 22].

2.3. ROLE OF EPIGENETICS IN MULTIPLE SCLEROSIS

Epigenetics involves heritable changes in gene expression not derived from DNA sequence including DNA methylation, microRNA and a variety of post-translational modification to histones [23]. Epigenetic modifications are known to be affected by genetics [24], diet [25], disease [26] and a variety of environmental factors [27, 28]. For example, it is widely accepted

that genetic factors alone do not dictate development of MS and that infectious agents, such as the Epstein - Barr virus (EBV), and environmental factors, including smoking and vitamin D, play a pivotal role in individual susceptibility [29]. These factors can also exert their effects through epigenetic changes [30]. Although the exact pathophysiological mechanisms underlying this susceptibility are unknown, it is proposed that EBV can up-regulate DNA methyl-transferase (DNMTs) [31], while Vitamin D is thought to alter histone post translational modifications [32]. Clinical and pre-clinical animal studies have confirmed epigenetic dysregulation in DNA methylation and histone acetylation patterns lead to inflammation and demyelination [33, 34]. Therefore, the inability for endogenous mechanisms to completely re-myelinate damaged axons may result from some underlying epigenetic mechanism that, for example suppresses the expression of genes critical for re-myelination such as BDNF.

2.4. METHYL CPG BINDING PROTEIN 2 (MeCP2):

2.4.1. MeCP2 Protein Structure and Isoforms

Discovered in 1989, methyl CpG binding protein (MeCP) is a nuclear protein that non-specifically distinguishes between methylated and non-methylated DNA [35]. MeCP2 is able to bind a single methyl-CpG pair [36]. In mice and humans, alternative splicing of the X-linked Mecp2/MECP2 gene gives rise to two predominant protein isoforms: MeCP2E1 (also called MeCP2B) and MeCP2E2 (also called MeCP2A) [37, 38]. Both isoforms have a transcriptional repressor domain (TRD) [39] and methyl CpG binding domains (MBD). MeCP2E1 is the predominant isoform in the adult brain and shows more uniform expression levels [40]. In contrast, MeCP2E2 is more extensively expressed in placenta, liver, and skeletal muscle [37, 41-43]. MeCP2E1 and E2 isoforms exhibit some functional overlap [44]. For example, both

isoforms are involved in neurite formation [45, 46], but have different effects on neuronal survival [47] and embryonic development and demonstrate differential sensitivity to DNA methyltransferase inhibitors, such as decitabine [48]. It was initially postulated that MeCP2E1 was a mutant isoform of MeCP2 associated with Rett syndrome (RTT), but it is now known that both isoforms are present in this condition [49, 50]. Therefore, identifying isoforms specific of MeCP2 that regulate re-myelination and /or myelin repair mechanisms could be of clinical relevance to establish a pattern of gene expression that mediates myelination.

2.4.2. MeCP2 a Transcriptional Regulator of BDNF

Methyl CpG binding protein 2 (MeCP2) is a transcriptional regulator [51] and is a key biological candidate that is influenced by epigenetic modifications [52-54]. MeCP2 binds to methylated CpG islands and recruits a transcriptional co-repressor complex containing Sin3A and histone deacetylase 1 (HDAC1) and 2 (HDAC2) [55-57]. The subsequent de-acetylation by the MeCP2/Sin3A/HDAC1/2 complex of acetylated histones associated with a target gene results in the repression of gene expression [54]. In this way MeCP2 directed transcriptional repression is indirectly mediated by changing histone lysine acetylation. However, it is important to mention that histone de-acetylase-independent mechanisms also have been postulated for MeCP2 repression mechanism [58]. Although the entire spectrum of MeCP2 target genes remains to be elucidated [59], one important target with respect to MS is BDNF [60, 61]. This suggests a role of MeCP2-dependent repression of BDNF gene expression resulting in the inhibition of downstream BDNF-induced endogenous myelin repair mechanisms that govern the degree of re-myelination and/or myelin repair. It has been postulated that MeCP2 mediated repression of BDNF expression disrupts a critical signaling triad between cytokines such as TNFα and neurotrophins such as BDNF and nerve growth factor (NGF) [62]. This cytokine-neurotrophin

signaling pathway has been identified as a critical signaling network involved in the regulation of re-myelination and/or myelin repair [62]. As such, aberrant activity of the MeCP2/Sin3A/HDAC1/2 repressor complex can disrupt the delicate homeostatic balance between cytokines and neurotrophins via its ability to repress BDNF expression. Therefore, MeCP2 has moved to the forefront of lead biological candidates warranting further investigation in understanding the underlying pathogenesis of MS. In addition, MeCP2 is also being investigated in several other white matter diseases such as RTT [63], mental retardation [64] and autism spectrum disorders [65], including classical autism [66]. Moreover, the over expression of MeCP2 is correlated with autoimmune diseases, such as rheumatoid arthritis [67].

2.4.3. MeCP2 is an Epigenetic Factor That Regulates Transcriptional Activation and Repression

Epigenetic mechanisms control gene expression independently from the underlying DNA sequences [68-71]. Epigenetic information is embedded in chromatin, a structure consisting of DNA and its associated histones [68]. Epigenetic information is transmitted in three important ways, DNA methylation, post-translational modifications to histones, and microRNA (miRNA). Two common DNA marks are 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) located on CpG dinucleotides [70, 72, 73]. While 5mC is usually associated with inactive genes [68, 70], 5hmC is found within active genes [74]. The crosstalk between DNA (5mC or 5hmC) and histone modifications determines expression of individual genes. MeCP2 was originally discovered as a repressor that binds to 5mC to inhibit transcription via multiple mechanisms [75], however, it is now known that MeCP2 also binds to 5hmC [74]. Therefore, depending on the DNA marks and the target gene, MeCP2 can act either as a repressor or an activator [76]. Interestingly, a recent study shows that MeCP2 can bind to non-CpG methylated sequences

called CpH methylated (H = A/C/T nucleotides) regions. Binding of MeCP2 to CpH sequences could also lead to transcriptional repression [77]. CpH methylation occurs in adult human brain and is especially prominent in regions with low level of CpG methylation [77].

Epigenetic studies involving modifications to DNA methylation and/or histone acetylation have linked MeCP2 to the pathogenesis of MS [78]. For example, pre-clinical studies involving the experimental autoimmune encephalomyelitis (EAE) animal model of MS support the pathological role of MeCP2 in MS [79]. These pre-clinical studies are also supported by research in humans. For example, samples from peripheral blood mononuclear cells (PBMCs) and brain tissues of MS patients showed altered promoter methylation, such as hyper- [80] and hypo-methylation [81] that can led to the pathological expression of inflammatory proteins [82] and de-myelination [81]. Furthermore, histone acetylation is another key epigenetic mechanism affecting neuronal death and immune system regulation that links MeCP2 to pathology of MS [69].

2.4.4. *MeCP2 Implication in MS*

Histone acetylation is the most prominent epigenetic modification altered in MS patients [83]. The pattern of acetylation is decreased in oligodendrocytes (OGs) in early MS lesions but, chronic MS lesions show increased acetylation [78]. In addition, many EAE studies show an increase [34] or decrease in histone acetylation of key genes involved in Th17 differentiation [79]. These studies found that the expression of MeCP2 target genes, including BDNF, may be regulated through CpG island methylations and histone acetylation. Therefore, epigenetic modifications to the MeCP2 target gene may represent both the key mechanism behind the diverse pathology and explain the lack of a specific genetic link responsible for causing MS.

Accordingly, the development of epigenetic therapeutic agents that target the transcriptional regulatory mechanisms by which MeCP2 alters the expression of BDNF represents a novel approach to re-myelination of MS-induced myelin damage. For example, many studies conducted in various EAE models show the beneficial role of HDAC inhibitors [84] such as Trichostatin A (TSA) [85], Valproic acid [86, 87] and Vorinostat [88], and lysine acetyl transferase (KAT) inhibitors such as Curcumin [89-91] as novel epigenetic treatments for MS. The benefits of these inhibitors is attributed to their role in modulating histone acetylation of MeCP2- regulated target genes involved in ameliorating the disease process such as BDNF [60, 92]. In this regard, further research evaluating epigenetics mechanisms regulating BDNF expression via MeCP2 has the potential to introduce novel therapeutic approaches to promote re-myelination and/or myelin repair.

2.5. THE DORSAL ROOT GANGLIA (DRG)-SPINAL CORD (SC) ANATOMICAL CONNECTION: IMPLICATION IN MS

Recent studies support the involvement of the dorsal root ganglia (DRG) in the pathogenesis of MS [10, 62, 93, 94]. For example, we have recently published a study showing that the gene and protein levels of TNFα are up-regulated in the DRG and SC of animals induced to a state of EAE [94]. Specifically, the EAE-induced elevated levels of TNFα in the DRG have been shown to induce the expression of neurotrophins, such as BDNF [95, 96] and NGF [97, 98]. The elevated level of DRG-derived BDNF is transported from the DRG to the SC in an anterograde fashion [93] where it facilitates re-myelination and/or myelin repair of EAE-induced myelin damage.

According to our MS-induced [62] model of re-myelination and/or myelin repair (Fig.1), activated Th1 cells in the periphery enter the DRG via its highly permeable capillaries [10]. Once

inside the DRG, activated Th1 cells release a variety of inflammatory cytokines such as TNFα, IL-12, and IL-6 which subsequently trigger the release of TNFα [94], BDNF [93], and NGF [62] from the sensory neurons housed within the ganglia. These neurotrophins and cytokines can undergo anterograde transport to the SC from the DRG [93, 94]. Therefore, the DRG acts as the initial reservoir for TNFα [94], BDNF [93], and NGF (cytokine-neurotrophin signaling triad). Due to the transport of the immune-system induced mediators between the DRG and SC, the same immune system induced signaling triad is also developed in the SC [62]. Hence, the physical connection between the DRG and SC is a critical anatomical pathway utilized in the re-myelination and/or myelin repair process of CNS myelin [62]. The confirmed importance of this DRG-SC connection pathway has changed the paradigm of MS research to now include the involvement of the DRG in the CNS myelin repair strategies.

2.6. BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF):

2.6.1. BDNF Signaling:

BDNF is a neurotrophin [99] that is initially secreted as a precursor protein, called proBDNF (34 kDa), which is proteolytically cleaved to produce the mature form (13 kDa) [100-102]. BDNF signaling is regulated via a receptor complex that encompasses two different receptor classes: the tropomyosin-related kinase (Trk) receptor B (TrkB) [103, 104] and the p75 neurotrophin receptor (NTR) (p75NTR). The p75NTR is a member of the TNF receptor superfamily [103, 105]. Mature BDNF only signals via the TrkB receptor [104] and proBDNF interacts with the p75NTR [106] to mediate biological actions distinct from those of TrkB [103, 107, 108]. Studies show that proBDNF promotes myelination via p75NTR- mediated activity independent of TrkB [109]. Specifically, the inhibition of p75NTR receptor activity in Schwann cells was shown to

prevent the expression of the myelin structural protein, myelin associated glycoprotein (MAG) [110]. MAG is essential for long-term axon–myelin stability, the structure of the nodes of Ranvier, and maintenance of the axon cytoskeleton [111]. In addition, after peripheral nerve injury, p75NTR in Schwann cells mediated re-myelination process and motor recovery [112]. Thus, proBDNF appears to be critical for the structural integrity of the intact myelin sheath through TrkB-independent pathways in the peripheral nervous system (PNS).

Consistently, studies have demonstrated that mature OGs, the myelinating cells of the CNS, express p75NTR and TrkB [113, 114], indicating OGs are responsive to pro-BDNF and BDNF activity. For instance, the expression of p75NTR has been up-regulated in OGs during MS plaque formation [115] and in the corpus callosum of cuprizone-induced mice models of demyelination [116]. Although this up-regulation is not necessary for OG cell death [116], it can regulate the process of cell death in conjunction with other death pathways or molecules [115]. Furthermore, studies conducted in chronic model of EAE [117] and spinal cord injury (SCI) [118] shows involvement of p75NTR in apoptotic cell death of OGs. However, more studies need to be conducted in regards to the role of p75NTR in regulation of cell death. Similarly, in response to demyelination, the expression of p75NTR is up-regulated in OPs adjacent to sub-ventricular zone (SVZ) [119]. As a result, the up-regulation of p75NTR in oligodendroglial cells in response to demyelinating diseases may mediate re-myelination and/or myelin repair.

It is well established that, in addition to regulating oligodendroglial lineage cells, BDNF directly promotes myelination through its effects on OGs [120-122] and indirectly modifies the proliferation of OPs through its interaction with the TrkB receptor located on these cell types [122-124]. Following a cuprizone – induced demyelination lesion in BDNF heterozygous KO mice, researchers have shown that BDNF was integral in the regulation and control of myelin

integrity, myelin proteins and the number of OPs via its interaction with the TrkB receptor found on various cell types [125]. This study confirms the diverse array of BDNF responsive cells within the CNS that are involved in the re-myelination and/or myelin repair process following an immune system induced demyelinating insult to myelin. Other recent studies have demonstrated that TrkB is expressed in neurons and astrocytes, but not in inflammatory cells or oligodendroglial cells in lesions with ongoing demyelination [126]. As such, BDNF's role in neuron-glia interactions represents another important aspect involved in the regulation of re-myelination and/or myelin repair process. We have also recently published results identifying several types of BDNF responsive cells in the SC following EAE induction. For example, we have reported an up-regulation of TrkB at the gene (*via* mRNA) and protein levels in SC during peak inflammatory acute phase of EAE. These results indicated that the up-regulation of TrkB denoted the general mobilization of BDNF responsive cells to assist in the re-myelination and/or myelin repair process following an EAE-induced myelin insult. Specifically, we identified various TrkB expressing BDNF responsive cells in the SC such as: CD4+ T –cells, Th17 and neurons. However, our results did not detect TrkB in EAE SC glial cells such as: OGs, OPs or astrocytes) [127]. In addition, other studies have identified TrkB containing T cells in the peripheral blood of MS patients that were shown to modulate T cell survival [128]. Interestingly, other studies have demonstrated that mice lacking both B and T cells, exhibited deficits in re-myelination after the induction of non-immune demyelination [129]. This suggests that BDNF responsive T cells support OG mediated re-myelination and regulates the re-myelination efficiency. TrkB immunoreactivity has also been detected in Schwann cells in early stages of nerve regeneration [130]. Contrary to what occurs in CNS, TrkB-containing neurons inhibit myelination actions of Schwann cells in the PNS [131] (Table 1).

Although microglia/macrophages have been shown to be primarily harmful in process of re-myelination and/or myelin repair [132], many studies indicate their eventual beneficial role in animal models of MS [133-135]. One of the possible mechanisms for the beneficial effects is the production of growth factors including BDNF and NGF [136]. Therefore, exploring molecular mechanism [137] involved in CNS microglial proliferation and deactivation could be important following an immune system insult to CNS myelin.

A recent SCI study suggests that a truncated form of TrkB located on astrocytes may prevent the penetration of DRG-derived BDNF into the CNS at the dorsal horn point of entry. These TrkB positive (TrkB+) astrocytes are thought to sequester the BDNF protein prior to reaching its target destination, thereby restricting its ability to regulate molecular mechanisms essential for CNS myelin repair [138-140]. This study further suggests that blocking the TrkB receptor on astrocytes represents a viable means to promote BDNF delivery to the site of myelin damage. These findings provide a plausible explanation as to why DRG-derived BDNF is unable to completely repair myelin damage following an MS attack despite the elevation in its levels and subsequent transport from the DRG to the SC [141]. Thus, a myriad of BDNF target cells exist in both PNS and CNS that contribute to the OG recruitment and maturation within MS lesions. Given the vast array of BDNF responsive cells involved in the re-myelination and/or myelin repair process, additional studies are required to unveil the complex molecular signaling network by which BDNF exerts its beneficial effects in the re-myelination and/or myelin repair process.

2.6.2. The Beneficial Role of BDNF in Re-myelination and/or Myelin Repair

BDNF plays a critical role during neurodevelopment and is involved in processes, such as neurogenesis, as well as, neuron survival and plasticity [142]. In addition, it is known to regulate

the distribution pattern of myelin structural proteins that govern the structural integrity of myelin [143]. For example, EAE BDNF knock-out (KO) mice have significant myelin structural damage compared to control animals that results from the abnormal distribution of the myelin proteins that are required for the structural integrity of myelin [143]. Similarly, other studies have also implicated BDNF in regulating the distribution patterns of myelin structural proteins [144-146]. Furthermore, BDNF has been shown to induce OP proliferation, migration, and differentiation at the sites of myelin damage [124, 147, 148], which are essential steps in the re-myelination and/or myelin repair process [123, 144]. In addition, human studies also confirm the presence of BDNF around MS lesions [126, 149-151] suggesting its role in the localized repair of myelin at the lesion site [124, 147].

Numerous studies support the role of BDNF in myelin repair in response to MS [152-158]. It has been shown that PBMCs of RRMS patients secrete lower levels of BDNF, both in the remission and relapsing phase of the disease, as compared with healthy individuals [154]. These results suggest that RRMS patients consistently have overall lower BDNF levels compared with healthy controls. This suggests lower levels of BDNF predisposes individuals with MS to myelin damage and limits the ability to achieve complete myelin repair even during periods of remission. In contrast, higher serum levels of BDNF have been detected in patients during an MS attack compared to levels during the stable phase of the disease [153, 159, 160]. A possible explanation of these results is that during an MS attack, BDNF levels are up-regulated in order to facilitate myelin repair and promote neurological recovery. However, BDNF levels do not reach sufficient levels to effectively repair myelin damage or promote complete re-myelination. Other studies have also provided evidence that BDNF contributes to the re-myelination of MS-induced lesions [160, 161].

BDNF has been shown to have a beneficial role in the repair of myelin damage caused by traumatic SCI. For instance, an increase in BDNF expression in the SC after complete SC axotomy was shown to facilitate myelin repair [162, 163]. Additional support comes from studies that have examined the expression of BDNF after incomplete crush injuries to the SC [162, 164-166]. McTigue et al. were one of the first groups to explore the use of BDNF in myelin repair [162]. In their study, a rat model of (SCI), transplanted fibroblasts produced BDNF resulting in higher OP proliferation and promoting the re-myelination of damaged axons [162].

A study utilizing a demyelinating model of MS showed that BDNF is critical for recovery from CNS demyelination [125]. Despite being a well-known survival factor for both OPs and neurons [167], the molecular mechanisms linking BDNF to myelin repair are not yet fully understood. Interestingly, drugs utilized for the treatment of MS have recently provided novel insight into the benefits of BDNF in re-myelination and myelin repair. For example, fingolimod, the first oral drug for the treatment of MS, was shown to increase BDNF levels in cell culture neurons [168]. In addition, investigations into glatiramer acetate (GA), one of the most commonly used medications used to treat MS, [169] has re-directed the focus of its beneficial mechanism of action toward its ability to increase BDNF production. GA is now thought to exert its effects by elevating BDNF levels in the CNS. GA binds with high affinity to the major histocompatibility complex (MHC) on antigen-presenting cells, and mimics myelin and myelin-like antigen presentation to T cells. It has been shown that GA-specific Th1, Th2, and Th0 cells increase BDNF production, but Th2 cells plays the most important role in GA modulation of RRMS through the enhanced production of BDNF [161, 169, 170]. Therefore, MS treatment strategies that increase endogenous BDNF or the exogenous supply of BDNF protein could effectively reduce the severity of disease. Pre-clinical studies involving EAE in rodents support this

mechanism of GA-induced BDNF production. For example, animals induced to EAE and treated with GA show elevated BDNF levels, compared with a naïve treatment group [171]. This increase in BNDF levels is sustained over the disease course, and correlate with indicators of myelin repair [170, 171] which supports the role of BDNF in re-myelination. Although GA is approved for MS treatment, has also been shown to increase BDNF levels in an animal model of RTT with mutant MeCP2 where reduced BDNF levels are associated with disease pathology. This increase was comparable to the levels of BDNF in naïve animals [172]. This shows the potential of GA as a drug treatment in other white matter disorders and confirms MeCP2 control of BDNF expression. Recent findings further suggest that immunomodulatory drugs (GA) and/or ABR-215062 (Laquinimod®)) used to treat (RRMS), exert their beneficial effects by up-regulating BDNF expression [152, 155, 158, 173, 174]. While this suggests that treatment strategies capable of promoting BDNF expression can potentially induce myelin repair mechanisms, it remains unclear why such therapies do not result in complete re-myelination. Thus, more detailed knowledge on the regulation of BDNF gene expression is required to provide insight into processes that potentially suppress re-myelination and/or myelin repair mechanisms.

2.7. MeCP2 REGULATION OF BDNF:

With respect to MS, BDNF is one of the most interesting target genes whose expression is under the control of MeCP2 [60, 61]. Currently, the specific molecular mechanism(s) linking MeCP2 to BDNF's ability to re-myelinate and/or repair damaged myelin are unknown.

The BDNF gene is very complex, four promoters have been previously described in the rodent BDNF gene [60, 92], each giving rise to a different transcript variant. Aid *et. al.* identified ten

exons in the human and rodent BDNF gene [142]. MeCP2 binds to methylated DNA in the BDNF promoter region of exon III in rats [60] and exon IV in mice [92]. Using chromatin immunoprecipitation assays (CHIP) in neuronal cultures from rats, Chen et al showed that when neurons are not depolarized, MeCP2 is bound to the BDNF promoter III and represses transcription [60]. In contrast, membrane depolarization leads to phosphorylation and the subsequent release of MeCP2, resulting in activated gene transcription [60]. This was associated with reduced DNA methylation at promoter III [60]. Research involving MeCP2 and cAMP response element-binding protein (CREB) in mouse cortical neurons demonstrated that when these neurons are not depolarized, MeCP2 binds with high affinity to the promoter in exon IV, however after depolarization MeCP2 dissociates from the promoter and CREB becomes tightly associated with the region to activate gene transcription and the promoter becomes less methylated [92]. These studies indicate that MeCP2-dependent regulation of BDNF gene transcription is context-dependent. Therefore, it is important to characterize the histone and DNA marks at the regulatory elements of any studied MeCP2-target genes in order to confirm the inhibition or activation by MeCP2.

Most of our knowledge about MeCP2 regulation of BDNF gene comes from studies conducted in RTT in which a mutation in MeCP2 gene is believed to play an important part in the disease pathology. In fact, studies in mouse models of RTT [61, 175-177] as well as human studies involving RTT patients [178] indicate BDNF dysfunction as a hallmark of disease pathology. Interestingly, reversal of pathological manifestations including respiratory dysfunctions has been reported to improve in animal models of RTT by enhancement of BDNF-TrkB signaling [175, 179-181]. Therefore, the concept of increasing BDNF-TrkB signaling as a potential therapeutic target in RTT patients further supports the importance of being able to develop therapeutic

intervention strategies that regulate the repression of BDNF by MeCP2. However, MeCP2 is not the only factor that regulates BDNF. For example, if the gene and protein expression of BDNF were solely repressed by MeCP2, one would expect to see and over expression of BDNF in RTT due to the loss of MeCP2. However, studies involving MeCP2 null mice [61, 175, 176] as well as human RTT post-mortem brain samples [178] show decreased levels of BDNF gene and protein expression demonstrating that repressors other than MeCP2 are involved in the regulation of BDNF expression. In fact, RE1 silencing transcription factor (REST) and CoREST are two transcriptional repressors that are involved in transcriptional repression of BDNF gene [182]. MeCP2 is involved in transcriptional repression of REST and CoREST. Interestingly, in MeCP2 null mice, the absence of MeCP2 results in the over expression of REST and CoREST which intern results in decreased expression of BDNF [182]. Therefore REST and CoREST are also recognized potential biological targets involved in re-myelination and/ or myelin repair process.

2.8. HISTONE DEACETYLASE (HDAC) INHIBITORS AND LYSINE ACETYLTRANSFERASE (KAT) INHIBITORS: IMPLICATION IN RE-MYELINATION AND/ OR MYELIN REPAIR

EAE is the most widely employed animal model of human inflammatory demyelinating MS [183]. The complexity and versatility of the EAE model system has therefore been useful in studying the underlying pathogenesis of MS, including processes of inflammation and demyelination. In addition, the EAE animal model is also used to evaluate new targeted epigenetic therapeutic interventional strategies such as HDAC inhibitors, for their therapeutic effects in regard to re-myelination and/or myelin repair of MS induced myelin damage.

Alterations in histone acetylation have been observed throughout the disease course of MS [78]. In particular, it has been shown that patients with chronic MS have elevated levels of acetylated histone H3 in frontal lobe white matter [78]. This in conjunction with other studies suggest that altered acetylation of histones is associated with MS pathology [184, 185]. Therefore, it follows that drugs effecting histone acetylation may prove beneficial in the treatment of MS. Thus, the concept of modulators of histone acetylation such as HDAC and histone acetyltransferase (HAT) inhibitors as potential interventional strategies have gained acceptance in recent years and are being evaluated in EAE models of MS for their therapeutic benefits [84, 89, 91, 186, 187]. Research also suggests that HDAC inhibitors exert their beneficial effects in EAE due to their anti-inflammatory and neuroprotective properties [85] (Table 2). Sodium butyrate was the first HDAC inhibitor to be evaluated in adoptively-induced EAE [188] and it showed improvements in the clinical symptoms of EAE in SJL female mice [188]. Other studies [85] subsequently evaluated the effects of TSA, a natural compound that specifically inhibits HDAC I and II [189]. In this study, TSA treatment of mice induced to a state of EAE using the MOG antigen showed significant improvement in neurological disability. TSA was also shown to improve the EAE-induced neuropathology by reducing SC inflammatory infiltrates, demyelination, and axonal loss [85]. This research also identified the reduction of several pro-apoptotic proteins, such as Bax, Bid, and caspase-2, [85] further confirming the beneficial effects of this HDAC inhibitor. Furthermore, TSA treatment was thought to be responsible for the elevated expression of estrogen receptor-α and insulin-like growth factor-2 (IGF-2) [85]. IGF is a neurotrophic factor that exerts its effect via IGF-2 and could promote re-myelination by oligodendrocyte progenitor cells [190]. TSA has also been demonstrated to block the proliferation of Th1 cells and suppress IFN-γ production [191]. Furthermore, other *in vitro* studies in human lung cancer cells indicated

that TSA can reduce the activity of matrix metalloproteinase 2 (MMP-2) [192]. This effect of TSA on metalloprotease activity is pertinent to a disease such as MS where increased metalloprotease activity is known to be responsible for the weakening of BBB [193]. Interestingly, this study suggests that TSA treatment can regulate the extent of BBB break-down and effectively reduce T cell infiltration into the CNS. Perhaps the most important activity of TSA is that it can reverse MeCP2-mediated transcriptional repression by inhibiting histone deacetylase activity of the MeCP2/Sin3A/HDAC complex [194]. HDAC inhibitors would therefore prevent MeCP2-dependent deacetylation of histones of MeCP2-target genes such as BDNF. As such HDAC inhibitors would prevent the MeCP2 transcriptional repression of BDNF. Therefore, evidence suggests that by removing the transcriptional repression of BDNF, localized SC levels of BDNF would increase to sufficient levels at sites of myelin damage that would be conducive for complete rather than partial myelin repair.

Epigenetic studies also suggest that hyper-acetylation mediated by TSA stimulate or promote neuronal growth. Especially TSA has been shown to induce the expression of HATs that will impact the acetylation status necessary to lead transcription of genes involved in neuronal growth. Interestingly, increase in markers for axonal growth and synaptogenesis like growth-associated protein 43 (GAP-43) has been reported following TSA treatment [195]. As such this beneficial effect could facilitate axonal regeneration in diseases such as MS.

According to a recent study conducted in an animal model of ischemic stroke induced white matter damage, TSA and sodium butyrate were shown to increase oligodendrogenesis mediated by BDNF-TrkB signaling pathway [196]. Furthermore, other studies conducted on OP cells [197, 198] also supported the beneficial effects of HDAC inhibitors in terms of developmental plasticity.

Curcumin, the major polyphenolic compound found in turmeric, has been shown to exert DNA methyl transferase inhibition, as well as, KAT and HDAC inhibition [199]. Several studies performed in various EAE models of MS have shown the beneficial effects of curcumin in improving clinical neurological disabilities [89, 91, 186, 187]. Curcumin has been shown to have anti-inflammatory effects, as demonstrated by its ability to reduce tumor growth factor β (TGF-β), IL-6, IL-7, and IL-12 levels [89, 91]. This suggests curcumin treatment may be beneficial in the treatment of MS because it can modulate immune-mediated inflammation by down-regulating inflammatory cytokines. Furthermore, it has also been shown that curcumin can decrease MeCP2 binding to the promoter region of Neurog1 by acting as a DNA methyltransferase inhibitor [199]. This suggests that altered DNA methylation can reduce MeCP2 binding and subsequent recruitment of Sin3A and HDAC. Without the deacetylation activity of the MeCP2/Sin3A/HDAC complex MeCP2-target genes can retain their histone acetylations and are active. Therefore, curcumin treatment could promote the gene expression of BDNF by effectively reducing MeCP2 binding to DNA via altered DNA methylations.

Charcot – Marie - Tooth (CMT) disease is an inherited peripheral neuropathy caused by mutations in myelin genes that lead to Schwann cells death by apoptosis [200]. Results obtained with an animal model of CMT showed that oral administration of curcumin improved the motor performance, decreasing apoptotic Schwann cells which increased number and size of myelinated axons in sciatic nerves [201]. In addition, curcumin has been reported to improve Schwann cell myelination by decreasing the expression of transcription factors SCIP that is involved in peripheral nervous system hypomyelination [202]. Recently, it has been reported that curcumin could induce myelin basic protein (MBP) expression, one of the major components of myelin sheath in animals treated with the demyelinating neurotoxin arsenic [203]. The scientific

evidence involving treatment with curcumin suggests its potential as a novel therapeutic interventional strategy in the management of peripheral neuropathies and demyelinating conditions. However, additional studies are required to establish the beneficial role of curcumin in demyelinating diseases with similar neuropathological manifestations as displayed by MS.

Valproic acid is a low affinity HDAC inhibitor that has recently been shown to increase the number of re-myelinating axons in EAE lesions of rats [87]. These animals exhibited faster recovery from clinical neurological disabilities [87]. Additionally, valproic acid has been shown to reduce the severity and duration of EAE symptoms in rats [86]. This improvement in clinical outcome and disease course corresponded with reduced gene transcription of inflammatory mediators, TNFα, IL-1β, and IL-17 [86]. Similarly, the anti-cancer HDAC inhibitor, Vorinostat, improves neurological deficits associated with EAE through the inhibition of Th1 and Th17 responses [88]. These findings suggest HDAC inhibitors may work by down-regulating the transcription of genes involved in the inflammatory response mediating the disease process. As a result, HDAC inhibitors could potentially prevent the MeCP2/Sin3A/HDAC complex from deacetylating histones associated with the BDNF gene, allowing BDNF gene transcription to take place Fig 2.

2.9. CONCLUSION

Emerging evidence in the field of MS emphasizes the prominent role of epigenetic factors in regulation of genes such as BDNF involved in re-myelination and myelin repair. As such, epigenetic therapeutics is a growing field of interest being explored for MS. Based on known critical cytokine – neurotrophin signaling triad between NGF, TNF α and BDNF and the promising role of MeCP2 as an upstream transcriptional repressor of BDNF, additional research

is required to unveil complex regulatory effect that MECP2 has on re-myelination and/or myelin repair *via* BDNF expression. Epigenetic mechanisms such as DNA methylation and modifications to histones regulate chromatin structure and gene expression. Immune system induced epigenetic changes in these mechanisms are linked to the pathological consequences associated with MS. As a result, epigenetic research has moved to the forefront of research priorities. The use of epigenetic regulators such as HDAC inhibitors have been shown to modulate autoimmune diseases such as MS by suppressing the pathogenic immune system induction of MeCP2. The resultant increase in BDNF expression promotes re-myelination and/or myelin repair by restoring the homeostatic balance of cytokine and neurotrophin signaling that govern this repair process. Henceforth, epigenetic therapeutics is an expanding field of research for the treatment of MS. In addition, further research into the key anatomical connection between the DRG and SC is required to optimize DRG-derived BDNF delivery to the SC where it can orchestrate its beneficial effects on re-myelination and/or the myelin repair process. The epigenetic therapeutic interventional strategies being utilized in MS have the diverse applicability to other white matter diseases such as epilepsy, Parkinson's, amyotrophic lateral sclerosis (ALS), spinal cord injury and Alzheimer's disease [204-209].

2.10. Acknowledgments:

Special thanks to the Manitoba Multiple Sclerosis Research Network Organization (MMSRNO) for their continued support of advanced research in MS. The authors would also like to acknowledge the support from The Manitoba Medical Service Foundation Grant (MMSF) Canadian Paraplegic Association (CPA), University of Manitoba Research Grants Program (URGP), Bayshore Health Care Systems, College of Pharmacy at the University of Manitoba, and endMS for their continued support of research training and education in the field of MS

2.11. TABLES

Table 1: BDNF biological effects on re-myelination and myelin repair

Differential targets and biological effects of BDNF in regard to re-myelination and/or myelin repair.

Target	Receptor	Biological function
Schwann cells	*via* P75 ^{NTR}	Improve re-myelination (increase MAG) in rat Schwann - DRG neurons co-culture [70]
	via P75 ^{NTR}	Improve re-myelination and motor recovery after peripheral nerve injury [71]
	via P75 ^{NTR}	Increase peripheral myelination [72]
OPs	*via* P75 ^{NTR}	Enhance motility and migration of precursor cells in SVZ region to adjacent plaques in MS demyelinating brain [73]
	via TrkB	Reduced TrkB expression in OPs, increases proliferation *in vitro* [74]
	via TrkB	Increase proliferation and development of basal forebrain OPs through MAPK pathway in OPs cell line derived from basal forebrain of postnatal rats [30]
OG	*via* P75 ^{NTR}	P75 is up-regulated in OG – corpus callosum during early phases of

		cuprizone model [75]
	via P75 ^{NTR}	P75 ^{NTR} is up-regulated in MS plaque [76]
	via P75 ^{NTR}	Induces cell apoptosis in grey matter of chronic EAE model [77]
	via TrkB	TrkB- FL enhance CNS myelination[78]
	via TrkB	Promote myelin thickness and expression of myelin proteins in mice development [74]
	via TrkB	Enhance myelination by MAPK/ ErK pathways in OG cell cultures of postnatal rats [79]
	via TrkB	Mediate basal forebrain OG differentiation and expression of MBP, PLP and MAG in basal forebrain OG cultures of postnatal rat basal forebrain [80]
	via TrkB	Mediate basal forebrain OG differentiation by MAPK pathway in OG cultures of postnatal rat basal forebrain [29]
Neurons	*via* P75 ^{NTR}	Improve axonal signaling that controls peripheral myelination in NGF dependent DRG neurons [81]
	via P75 ^{NTR}	Increase peripheral myelination [72]

	via P75 ^{NTR}	Improve re-myelination (increase MAG) in rat Schwann - DRG neurons co-culture [70]
	via TrkB	Modulation and recovery of inflammatory damage to the myelin during the acute phase of EAE [10, 33]

MAPK: mitogen-activated protein kinase pathway, ErK: extracellular signal-regulated kinase, OPs: Oligodendrocyte precursors, OGs: Oligodendrocytes

Table 2: Epigenetic Drugs evaluated in EAE model

HDACi and KATi evaluated in EAE model and their potential beneficial effects in MS.

iNOS: inducible nitric oxide synthase

Inhibitor	Epigenetic Mechanism	Mechanism	Reference
Sodium Phenylbutyrate	HDACi	Induce microglial expression of iNOS and activation of NF-kappaB	[188]
TSA	HDACi	Decrease spinal cord inflammatory infiltrates, demyelination and axonal loss	[85]
Valproic Acid	HDACi	Increase number of re-myelinating axons at the lesion site	[87]
		Promote faster recovery from clinical neurological disability	[87]
		Reduce gene transcription of TNF-α, interleukin-1β (IL-1β) and IL-17	[86]
Vorinostat	HDACi	Inhibition of Th1 and Th17	[88]
Curcumin	KATi	Reduce IL-17, tumor growth factor β TGF - β , IL-6 and IL-12	[91]

2.12. FIGURES

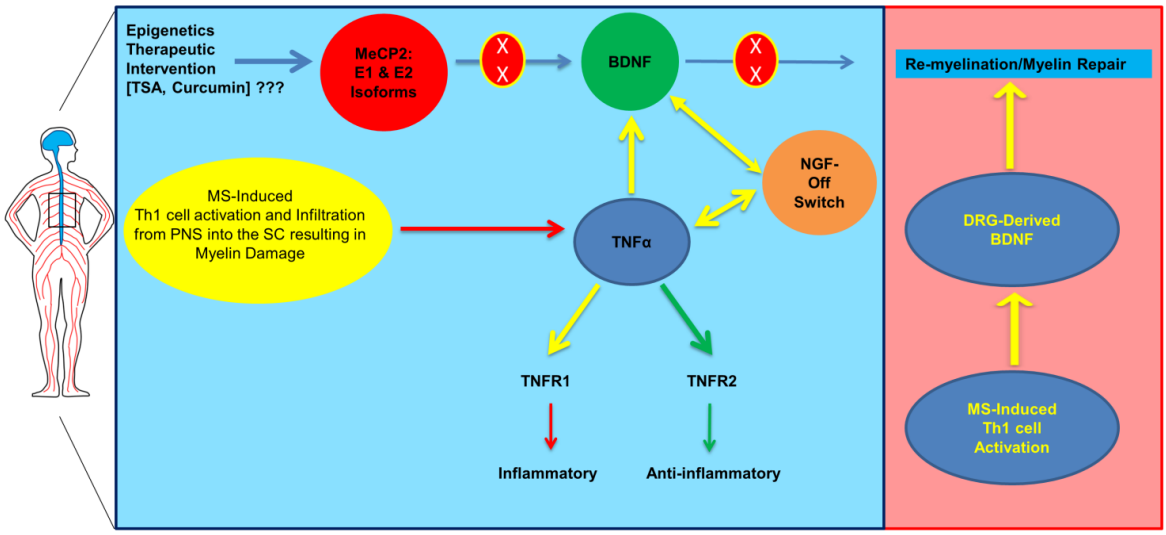

Fig 1: Schematic representation of the nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and tumor necrosis factor alpha (TNFα) signaling triad

T cell activation results in TNFα expression leading to the induction BDNF and NGF expression. BDNF acts as a chemo-attractant cue that draws NGF into regions of elevated BDNF. BDNF also induces NGF expression and TNFα expression. The elevated expression of NGF suppresses TNFα from signaling *via* the tumor necrosis factor receptor 1 (TNFR1) suppressing the inflammatory effects of TNFα/TNFR1 – that causes cell damage and apoptosis. Instead, the elevated expression of NGF, promotes the preferential signaling of TNFα through the tumor necrosis factor receptor 2 (TNFR2). As such, the anti-inflammatory effects of TNFα/TNFR2 signaling promote re-myelination and/or myelin repair. MeCP2 is a transcriptional repressor of BDNF. DRG could act as an initial reservoir for BDNF playing an essential role in re-myelination/ myelin repair. Therapeutic interventional strategies involving the use of TSA and/or

curcumin are thought to suppress MeCP2 activity thereby removing its repressive effects from BDNF. The resultant increase in BDNF restores the homeostatic balance between cytokines and neurotrophins that is essential in the re-myelination and/or myelin repair process.

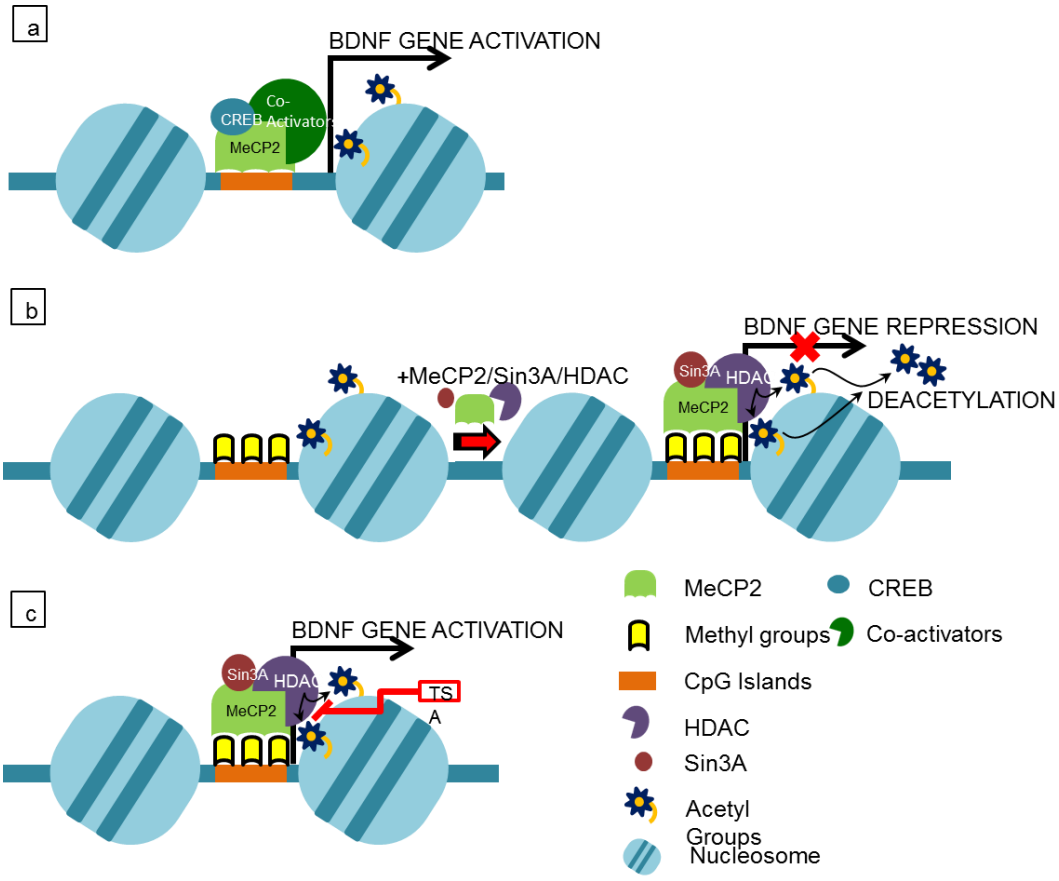

Fig 2: Schematic representation of TSA regulation of BDNF gene expression

(a) In the absence of methylated CpG in the promoter region of BDNF gene, MeCP2 is able to activate BDNF transcription by binding unmethylated promoters and recruiting co-activators and CREB.

(b) An illustration of MeCP2 mediating transcriptional repression: MeCP2 binds to methylated CpG dinucleotide in CpG islands of the promoter region and recruits transcriptional co-repressor complex containing Sin3A and histone deacetylase 1 (HDAC1) and 2 (HDAC2). The subsequent deacetylation by the MeCP2/Sin3A/HDAC1/2 complex of acetylated histones accompanied with chromatin compaction results in the suppression of BDNF gene expression.

(c) An illustration of TSA inhibiting MeCP2 repressive actions: TSA prevents the MeCP2/Sin3A/HDAC complex from deacetylating histones associated with the BDNF gene, leading to BDNF gene activation. As such, BDNF expression will be enhanced following TSA treatment which facilitates the re-myelination and/or myelin repair process.

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PROLOGUE TO CHAPTER 3: RATIONALE FOR MANUSCRIPT

Over the past 5 years, Dr. Namaka's neuro-immunology laboratory has published extensive literature in the field of MS that involve a homeostatic signaling equilibrium network between cytokines, chemokines and neurotrophins which govern the process of re-myelination and myelin repair [1-6]. Based on his previous publications in this area, his laboratory has identified key roles of nerve growth factor (NGF) [1, 2], tumor necrosis factor-alpha (TNF-α) [5], brain derived neurotrophic factor (BDNF) [4, 7] and fractalkine (CX3CL1) [3] in this molecular signaling pathway. In addition, Dr. Namaka's laboratory has also published research indicating the role of tropomyosin-related kinase receptors (TrkB) receptor in BDNF mediated re-myelination and /or myelin repair in SC [7]. Despite these various publications from Dr. Namaka's laboratory, and the publications produced by other laboratories around the world, the transcriptional regulation of BDNF by its two biological active epigenetic regulators; MeCP2E1 and MeCP2E2; has not been investigated in an animal model of MS. As such, the combination of my initial publication on MeCP2 (see Chapter 2) and my scientific review of the published work produced by Dr. Namaka and other international scientists resulted in the establishment of a solid platform from which I began to commence my research interests in this area. Henceforth, the research presented in this chapter of my thesis represents my original research work on MeCP2 as an upstream transcriptional regulator of BDNF.

Upon examining the scientific evidence, I began to understand that BDNF has a key role in re-myelination and myelin repair via its direct effect on oligodendrocytes [8-10] or its indirect effect on proliferation, migration and differentiation of oligodendrocyte progenitor cells [11, 12]. During the development of my review publication on MeCP2, it became apparent that it was known to be a repressor of BDNF expression [13, 14]. In addition, I also found that MeCP2 had

a role in down regulation of the myelin proteins that made up the structural composition of myelin [15]. Based on my extensive background literature search on MeCP2, it became apparent that the distinct role of the MeCP2E1 and MeCP2E2 isoforms in terms of regulating BDNF expression in an experimental autoimmune encephalomyelitis (EAE) model of MS had not been studied. As such, in conjunction with the scientific guidance of my supervisor Dr. Namaka, we decided that exploring the distinct role of the two MeCP2 isoforms in regard to their differential effects on BDNF expression could provide valuable information in terms of developing epigenetic therapeutic agents to promote re-myelination and/or myelin repair.

Based on this rational, we designed our study to determine the temporal gene and protein expression of MeCP2E1, MeCP2E2 and BDNF in a myelin oligodendrocyte glycoprotein (MOG)-induced model of EAE. Furthermore, we aimed to correlate these changes to neurological disability scoring (NDS) of the animals throughout disease progression. The results of our study showed the pathological expression of MeCP2E1 isoform following EAE induction contributes to the repression of BDNF expression with corresponding neurological disability. Our research suggests that the MeCP2E2 isoform may be involved in activation of BDNF gene that could prove to be beneficial in assisting in re-myelination and/ or myelin repair. Furthermore, our study also provides evidence to support the role of DRG-SC pathway in the re-myelination and/or myelin repair process. As such, my research in this area provides the platform to support the design of epigenetic therapeutic interventions that target the restoration of the delicate balance between the MeCP2E1 and MeCP2E2 isoforms.

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CHAPTER 3: Submitted Manuscript: Transcriptional regulation of brain derived neurotrophic factor (BDNF) by methyl CpG binding protein 2 (MeCP2): Implication in re-myelination and/or Myelin repair in an animal model of multiple sclerosis (MS)

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This manuscript was submitted to the *Journal of Neuroinflammation* on December 3th2015. *Impact factor: 5.4*

STATEMENT OF CONTRIBUTION:

For this study, I was involved as the lead investigator. I was responsible for designing and conducting all the animal studies, including EAE induction, animal monitoring, and tissue collection and conducting the RNA and protein extraction for subsequent analysis. Specifically, I established the standard operating procedures (SOP) and conducted the following technical aspects of my research which included: western blot (WB), ELISA, and qRT-PCR. In addition, I was involved in data collection and performing the statistical analysis of the raw data. I was responsible for the preparation of the manuscript, reference selection, and composing the initial draft of this manuscript that we have submitted for publication. In addition, I will also be responsible for copy proofing and revisions of the final manuscript pending submission and acceptance for publication. Overall, I was responsible for establishing all the intellectual decisions in regard to this project following consultation with my primary supervisor, Dr. Namaka.

3.1. Abstract:

Background: Multiple sclerosis (MS) is a chronic neurological disease characterized by the destruction of central nervous system (CNS) myelin. At present, there is no cure for MS due to the inability to repair damaged myelin. Although the neurotrophin, brain derived neurotrophic factor (BDNF) has a beneficial role in re-myelination and/or myelin repair, these effects are hampered by the over-expression of a transcriptional repressor isoform of methyl CpG binding protein 2 (MeCP2) called MeCP2E1. We hypothesize that following experimental autoimmune encephalomyelitis (EAE) -induced myelin damage, the immune system induction of the pathogenic MeCP2E1 isoform hampers the re-myelination and/or myelin repair process by repressing BDNF expression. Methods: Our research identified and compared the temporal gene and protein expression changes of MeCP2E1, MeCP2E2 and BDNF in an EAE mouse model of MS. These temporal expression patterns were correlated with the changes in the neurological disability scores (NDS) over the entire time course of the disease. Results: Our results indicate MeCP2E1 mRNA levels are elevated in EAE animals relative to naïve control (NC) and active control (AC) animals at all EAE time points which is responsible for the repressed BDNF production in the spinal cord that prevents re-myelination and/or myelin repair. However, we observed no significant differences in MeCP2E2 expression among our groups. Baseline protein expression of MeCP2E1 and MeCP2E2 isoforms in the spinal cord (SC) and dorsal root ganglia (DRG) indicate higher MeCPE1/MeCP2E2 ratios in the SC. Conclusion: Our results confirm that the immune system-induction of the pathological MeCP2E1 isoform contributes to the disruption of the normal homeostatic signaling equilibrium network that exists between cytokines, neurotrophins and chemokines that regulate the re-myelination process. As such, the pathological induction of the MeCP2E1 isoform contributes to CNS myelin damage with corresponding

neurological disability. Our research suggests that the elevated ratio of MeCP2E1 expression relative to MeCP2E2 expression correlates to the degree of neurological disability with corresponding myelin damage. Based on MeCP2E1 to MeCP2E2 ratios the DRG appears to be a more conducive environment for BDNF production.

Key words: Multiple sclerosis (MS); Experimental autoimmune encephalomyelitis (EAE); Methyl CpG binding protein 2 (MeCP2); Brain-derived neurotrophic factor (BDNF); Neurological disability scoring (NDS); Myelin repair

3.2. Background:

Multiple sclerosis (MS) is a chronic progressive neurological disease of central nervous system (CNS) characterized by destruction of myelin [1, 2]. It is considered a biphasic autoimmune disease with an acute inflammatory phase followed by a chronic degenerative, demyelinating phase [3]. Myelin is the insulating coating that surrounds nerve axons. It is essential for propagation of nerve impulses to effector target cells. Hence, patients diagnosed with MS have regionalized areas along axons where electrical impulse transmission is compromised due to damaged segments of myelin. As such, MS patients suffer a variety of neurological disabilities that negatively impact their quality of life [4, 1].

We have recently published several publications involving a new model for MS - induced re-myelination and /or myelin repair that revolves around the anatomical connection between the dorsal root ganglia (DRG) and spinal cord (SC) [5-7]. These research efforts have shifted the paradigm of MS research to incorporate the DRG of peripheral nervous system (PNS) into CNS myelin repair strategies. In addition, we have also published several publications that identify a common interactive signaling network between cytokines, chemokines and neurotrophins that regulate re-myelination and/or the myelin repair process [8, 9, 6, 7]. Although our previous research in this area has identified several signaling molecules in this pathway (nerve growth factor (NGF) [5, 10], brain-derived neurotrophic factor (BDNF) [8, 11], tumor necrosis factor-alpha (TNF α) [7] and fractalkine (CX3CL1) [9], our current research is focused on the upstream transcriptional repressor of BDNF called methyl CpG binding protein 2 (MeCP2) [11-13].

BDNF has a well-established role in re-myelination and/or myelin repair [14, 12, 15, 16]. It has been shown to be involved in proliferation, differentiation and migration of oligodendrocyte

progenitor cells (OPCs) [17, 18]. Furthermore, BDNF also has a direct effect on myelination via its effect on oligodendrocytes (OGs) [19-21]. In addition, BDNF has been shown to regulate the distribution pattern of myelin proteins that govern in the structural integrity of myelin [22-24]. Other researchers in this area also confirm the beneficial effects of BDNF in re-myelination and/or myelin repair. For example, several studies have shown that fingolimod [25] the first approved oral drug for MS, glatiramer acetate [26-28] and laquinimod [29, 30] exert their beneficial effect in treating relapsing remitting MS by increasing BDNF levels. In addition to these findings, our neuroimmunology laboratory has also recently published evidence to support the role of BDNF via tropomyosin-related kinase receptors (TrkB) in SC re-myelination and/or myelin repair [11].

Since its identification in 1998 [31], MeCP2 has received a wide range of attention as a genome-wide epigenetic factor [32]. In fact, MeCP2 was the first member of methyl binding protein that was able to bind a single methyl CpG pair [33] by its methyl binding domain (MBD) [34]. BDNF is one of several prominent targets of MeCP2 [35, 36]. MeCP2 was first identified to be a transcriptional repressor of BDNF [35, 37] exerting its effect by directly forming a repressor complex with its transcriptional repressor domain (TRD) and histone deacetylase (HDAC) Sin3A complex [38, 39] (Fig 1a) Specifically, this is the molecular mechanism by which the MeCP2E1 isoform is thought to exert its repressive effects on the BDNF gene. However, it is important to take into account that MeCP2 can also indirectly regulate BDNF gene expression by affecting the expression of other BDNF transcriptional repressors like RE1 silencing transcription factor (REST) and CoREST [40]. Interestingly, studies done in MeCP2 null mice [36, 41, 42] and human tissues [43] show decreased expression of BDNF gene and protein. As such, this research suggests that other factors are involved in the regulation of BDNF expression

besides MeCP2. Irrespective, MeCP2's ability to directly and indirectly effect BDNF expression warrants its investigation as a primary biological target by which targeted interventional strategies can be developed to promote re-myelination and/or myelin repair.

MeCP2 exists in two different biologically active isoforms, MeCP2E1 and MeCP2E2 [44, 45] that have been shown to have differential biological effects on neuronal survival [46] and embryonic development [47]. Based on our current investigative research in this area [13], we believe these two isoforms have distinctly different biological roles in regard to re-myelination and/or the myelin repair process due to their differential effects on BDNF expression [48, 13]. Research suggests that the MeCP2E1 isoform is pathogenically activated by an immune system mediated insult leading to myelin damage. Conversely, the MeCP2E2 isoform is thought to be involved in myelin repair by functioning as an activator of the BDNF gene. Henceforth, the homeostatic balance between the two isoforms may dictate the degree of myelin damage or myelin repair. Interestingly, MeCP2 has also been shown to be involved in regulation of myelin gene expression thereby governing the structural integrity of myelin [48, 49]. A recent study in MeCP2 knock down cultured oligodendrocytes shows transcriptional induction of myelin proteins [49]. Furthermore, several epigenetic studies involving changes in DNA methylation and histone acetylation have linked MeCP2 to MS [50, 51]. Interestingly, other studies have shown a beneficial role of elevated MeCP2 expression in pain suppression via its ability to repress BDNF expression [52, 53]. These studies have shown increased expression of the MeCP2E1 isoform in an inflammatory pain model that utilized complete Freund's adjuvant (CFA) injection as the nociceptive inflammatory pain stimulus [52]. This increase was also correlated with increase HDAC1 and HDAC2 levels both of which are part of MeCP2 repressor complex [52].

To the best of our knowledge, there are no other studies that have evaluated the differential expression of the two biologically active but different isoforms of MeCP2 in experimental autoimmune encephalomyelitis (EAE) animal model of MS. As such, this is the first study to correlate the expression of these isoforms to BDNF expression following EAE-induced myelin damage. Specifically, our current research study examines the temporal gene and protein expression of MeCP2E1, MeCP2E2 and BDNF in SC tissue obtained from a myelin oligodendrocyte glycoprotein (MOG)-induced model of EAE. In addition, we correlated these temporal changes in gene and protein expression with the temporal changes in neurological disability scoring (NDS) during the acute and chronic phases of disease progression.

Our study identifies the importance of the homeostatic balance between MeCP2E1 and MeCP2E2 isoforms in regard to regulating re-myelination and/or the myelin repair process by the differential transcriptional regulation of BDNF by each MeCP2 isoform. Specifically, our research supports the pathogenic involvement of the immune system mediated induction of the MeCP2E1 isoform in the repression of BDNF. As such, this contributes in part to the disruption of the homeostatic signaling balance between cytokines, chemokines and neurotrophins that is essential to re-myelination and/or the myelin repair process. The net result is myelin damage with corresponding sustained neurological disability from which the animals are unable to recover.

3.3. Materials and Methods:

3.3.1. Induction of EAE:

The MOG mouse model of EAE is the preferred model of MS [2, 54, 55]. 10 week old c57Bl/6 mice were randomly assigned to either: naïve control (NC), active control (AC) and MOG-induced EAE. A total of n = 4 mice were used per time point per group. As per our standard in house protocols [11, 9, 6, 7], EAE mice were immunized subcutaneously (SQ) with 200 µg MOGp35-55 in 200 µl of Complete Freund's adjuvant (CFA) at the lower/upper back at day 0 [induction kits from Hooke Laboratories (USA) (Cat, EK-2110)]. Animals received two intraperitoneal (IP) injections of pertussis toxin (PTX: List Biological Laboratories; #179B) (0.2µg in 100µl of PBS at days 0 & 1) to open up the blood brain barrier and facilitate the entry of pathogenic T cells to the CNS. AC mice received all the same treatment as the EAE mice with the exception of the omission of the MOG antigen. NC animals did not receive any treatment (Fig 2). The CFA emulsion contains killed mycobacterium tuberculosis (MT) H37Ra in incomplete Freund's adjuvant (FA) to enhance the immune system response to sensitization. The emulsion was administered SQ at two sites in the upper and lower back area. A 0.1 ml SQ dose of the emulsion was injected at each site (0.2 ml/mouse) at day 0. The DRG and SC tissue were collected during the acute phase at days 12, 15 (first inflammatory phase prior to de-myelination). In addition, DRG and SC tissue were collected during the chronic phase of the disease: days 21 and 27(during 1st remission), day 36 (de-myelinating phase), and day 45 (during the second period of remission: re-myelination phase). The DRG and SC tissue were removed to conduct molecular gene and protein analysis. Female mice were specifically chosen because females are more predisposed to be affected by MS than males [56]. The AC and EAE groups were assessed daily for neurological disability until sacrificed. Neurological disability

scores (NDS) were determined from mean clinical scores measured from a score of 0 (no disability) to 15 (maximal disability) [7]. The total score is the sum of the following individual scores obtained for each of the 6 specified clinical domains, according to the following specifications: tail: 0 = normal, 1 = weakness or partial paralysis, 2 = limp or complete paralysis; right and left hind limbs: 0 = normal, 1 = weakness, 2 = dragging or partial paralysis, 3 = complete paralysis; right and left forelimbs: 0 = normal, 1 = weakness, 2 = unable to support weight or partial paralysis, 3 = complete paralysis; bladder: 0 = normal, 1 = incontinent. The experimental groups were sacrificed at 12, 15, 21, 27, 36 and 45 days post induction (dpi). The disease course could be divided into acute (9-18 dpi) and chronic (18-45 dpi) phase. In order to ensure consistency in regard to the degree of the MOG-induced EAE, only animals that progressed to the NDS of 4 +/- 1 for the acute disease phase and 6 +/- 2 for the chronic phase were included in our study. All other EAE-induced animals that did not meet the criteria were excluded from the data analysis.

All animal experiments and procedures in this study were conducted according to protocols approved by the University of Manitoba Animal Protocol Management and Review Committee, are in full compliance with the Canadian Council on Animal Care, and are in accordance with standards set forth in the 8th Edition of Guide for the Care and Use of Laboratory Animals.

3.3.2. Gene/protein assay:

SC and DRG tissue were harvested at pre-determined time points for gene and protein expression analysis of MeCP2E1, MeCP2E2 and BDNF. Freshly harvested DRG and SC tissue were placed in RNA later stabilization solution (Ambion Cat#AM7020) until being processed. Whole

DNA/RNA and protein was purified using commercially available kits (AllPrep DNA/ RNA/protein, Qiagen) as described in our previous publications [8, 48, 6].

3.3.3. Quantitative real time reverse transcription polymerase chain reaction (qRT-PCR):

RNA purified from SC tissue, converted into cDNA using the iScript™ cDNA Synthesis Kit #170-8891, Bio-Rad and Bio Rad S1000 Thermal cycler instrument. Final concentration of cDNA used in qRT-PCR was 5 ng/µl .The PCR reaction was performed by the CFX96 real-time PCR detection system using SsoFast™ EvaGreen® Supermix (#172-5202) following manufacturers protocols (Bio-Rad, Hercules, CA, USA). MeCP2E1 primers were forward: 5′-GGAGAGAGGGCTGTGGTAAA-3′; reverse: 5′-CTGGAGATCCTGGTCTTCTGA-3′ at annealing temperature of 56 0C. MeCP2E2 primers were forward: 5′-GGAGGAGAGACTGCTCCATAAA-3′; reverse: 5′GGAGATCCTGGTCTTCTGACTT-3′ at annealing temperature of 59ºC. BDNF primers were forward: 5′-AGCTGAGCGTGTGTGACAGTATTAG -3′; reverse: 5′-GGGATTACACTTGGTCTCGTAGAAA 3′ at annealing temperature of 56 0C. The ΔCt method was applied to determine differences in gene expression levels after normalization to the arithmetic mean of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal standard.

3.3.4. Western blot (WB) analysis:

For our NC group we conducted western blot (WB) analysis for MeCP2E1 and MeCP2E2 isoforms. DRG and SC tissues were homogenized and total supernatant protein concentration was determined for each sample using Bicinchoninic Acid (BCA) protein assay kit (Novagen, CN: 71285-3). For each sample, 60 µg total protein was separated by 10% Tris-Glycine gradient

SDS-PAGE (Thermoscientific, CN: 0025269) at 120 V for 1 hour and electrophoretically blotted onto a PVDF membrane (Immobilon, CN: IPFL00010) for 1 hour at 0.35A. The membranes were blocked in skim milk in TBS-T and then treated with primary antibody including: Anti-MeCP2 Isoform B polyclonal anti rabbit IgG (Cat No. ABE 333 Millipore) 1/500, Anti-MeCP2 E2 chicken poly clonal antibody custom-made by Dr. Rastegar (1/100), or rabbit polyclonal IgG against GAPDH (1:1000, Santa Cruz, CN: sc-25778) overnight at 4ºC. GAPDH was utilized as a loading control to ensure equivalent amounts of protein were loaded. The primary anti-MeCP2E1 and MeCP2E2 antibodies were intended to detect the 75 kDa isoform, while the primary anti-GAPDH antibody was intended to detect 37 kDa GAPDH. After incubation with primary antibodies, membranes were washed with TBS-T and incubated with secondary antibodies. The secondary antibodies used were peroxidase-conjugated donkey anti-rabbit IgG (1:10000, Millipore, CN: AP182P) against the anti- MeCP2E1 and GAPDH antibody and peroxidase-conjugated donkey anti-mouse IgY (1:2,000, Thermoscientific, SA1-72004) against the anti-MeCP2 E2 antibody. The antigen-antibody complexes were detected using ECL detection reagent (Pierce, CN: PI32209). Membranes were exposed to chemiluminescence. However, for MeCP2E1 and E2 different exposure time were applied because of difference of efficiency between these two antibodies. Densitometry was performed using a FluorChem 8900 scanner (Alpha Innotech) with Alpha Ease FC software. Densitometry analysis was conducted with ImageJ. The individual MeCP2E1, MeCP2E2 and GAPDH band densities for each sample were normalized to an internal standard. The sample and loading control density ratios obtained relative to the internal standard were subsequently used to calculate the relative density ratio of MeCP2E1 and MeCP2E2 respectively relative to GAPDH.

3.3.5. Quantitative enzyme-linked immunosorbent assay (ELISA) analysis:

Total protein was extracted and protein concentration was determined as described above by BCA assay [10]. Protein concentration was adjusted to 30 µg in 100 µl total sample volume. Sandwich-format ELISA was performed using a BDNF Emax ImmunoAssay System (Promega, Madison, USA cat# G7611) according to manufacturer's instructions. BDNF protein concentration was interpolated from a standard curve with a range of 7.8-500 pg/ml.

3.3.6. Statistical Analysis:

Statistics was performed using GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Data from all samples analyzed from each animal group are reported as mean ± standard error of the mean (SEM). Statistical analysis for ELISA, WB and Real Time-PCR (RT-PCR) was performed using 2-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test.

3.4. Results:

3.4.1. Neurological disability scoring (NDS):

All animals in the EAE groups underwent neurological disability scoring (NDS). All animals in the NC and AC experimental groups displayed a normal NDS of zero. Based on our NDS analysis, we observed an acute disease phase that became apparent during days 9 -18 post-induction. We also observed a chronic disease phase that started between days 18 - 45 post-induction. Our identified phases of disease progression are consistent with other researchers that have used the same MOG model of EAE [2]. We identified that during the acute phase animals begin to develop mild clinical symptoms by day 10 post-induction (tail weakness or paralysis)

[57] (Fig 3). By day 12 to 13 post-induction all animals experience a full range of clinical neurological deficits such as tail and forelimb weakness, loss of bladder control and hind-limb paralysis. As the disease characteristically progresses, the mice enter into a slight remission and regain motor function by day 18 post-induction. The chronic disease phase is followed by a second peak of neurological disability (associated with de-myelination) at 28-30 days post-induction with another slight remission phase identified from days 39-45 post-induction. The control groups (NC and AC) did not show any clinical signs of disability (data not shown).

3.4.2. mRNA Expression of MeCP2E1 and MeCP2E2 isoforms in the SC:

In order to determine which MeCP2 isoform contributes to the regulation of BDNF during EAE disease course, we quantified the changes in MeCP2E1 and MeCP2E2 gene expression in the SC. Quantitative real time RT-PCR (qRT-PCR) analysis was conducted on SC tissue isolated from the three experimental groups (EAE, NC and ACs), at the pre-determined experimental time points (Fig 4a). The MeCP2E1 mRNA expression was assessed in parallel with that of the housekeeping gene (GAPDH). Following ΔCt analysis, EAE animals (red bar) show a significant increase of MeCP2E1 expression in SC over NC (black bar) and AC (blue bars) animal groups at 12, 15 (acute disease phase) and 21 dpi (chronic disease phase). However, NC and AC do not show significant difference at all different time points [**** = $p<0.0001$; *** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$]. We used the same method to evaluate gene expression for MeCP2E2 in SC on our three experimental groups (EAE, NC and ACs), at the pre-determined experimental time points (Fig 4b). However, we observed no significant differences in expression among our groups.

3.4.3. BDNF gene and protein expression in SC:

BDNF is involved in re-myelination and is regulated by MeCP2 [35, 36]. Therefore, we conducted gene and protein quantification of BDNF in SC tissue obtained from NC, AC and EAE animals to determine if regulation of BDNF expression is isoform specific (Fig 5). ELISA was employed to quantify differential protein expression for BDNF. For each sample, results are given as pg BDNF per 30 μg total protein. We identify significant differences between NC, and EAE (**p< 0.01) at day 27 post-induction that corresponds to the second relapse phase of the disease and also at day 45 (*p< 0.05) that corresponds to second partial remission phase of the disease (Fig3). Also, there are significant differences between NC and AC (***p<0.001) at day 45 post-induction (Fig5a). For comparison we measured BDNF gene expression in SC, via qRT-PCR. Significant differences exist between the NC animals (black bars) and EAE (red bars) at day 12 (****p< 0.0001), 15 (****p < 0.0001), 21 (**p< 0.01) and 27 (***p< 0.001) post-induction. There are also significant differences between EAE and AC (blue bars) at day 36 and 45 post-induction (**p< 0.01). Finally, NC and AC showed significant differences (**** p< 0.0001) at all-time points (Fig 5b).

3.4.4. Baseline protein expression of MeCP2E1 and MeCP2E2 isoforms in Spinal Cord (SC) and Dorsal Root Ganglia (DRG) in Naïve Control (NC) Animals:

In order to compare protein expression of MeCP2E1 and MECP2E2 isoforms in DRG and SC, we conducted western blot (WB) analysis. Quantification of MeCP2E1 and MeCP2E2 protein by WB densitometry in the SC and DRG indicates significant differences between MeCP2E1 (***=p<0.001) and MeCP2E2 (*=p<0.05) expression level in DRG and SC (Fig 6).

3.5. Discussion:

At present, the exact pathophysiological molecular mechanisms underlying MS-induced myelin damage is still unknown. However, BDNF has been suggested as one of the lead biological therapeutic target molecules involved in re-myelination and/or the myelin repair processes [19, 20, 58, 21]. BDNF has been shown to regulate OPCs via molecular signaling through TrkB receptors to induce their proliferation, migration and differentiation [18]. In addition, BDNF has also been shown to be involved in distribution and production of myelin sheath proteins that are critical to the structural integrity and function of myelin [12]. The transcriptional control of BDNF by MeCP2 has been extensively researched especially in the field of Rett Syndrome, indicating MeCP2 as an upstream transcriptional repressor of BDNF [35, 37, 59, 60]. This implicates MeCP2 as a pathological factor in BDNF dysregulation and an epigenetic target molecule for therapeutic intervention aimed at promoting increased expression of BDNF to facilitate myelin repair.

In our current study involving a MOG-induced EAE model of MS myelin damage, we hypothesized that transcriptional repression of BDNF by MeCP2E1 isoform disrupts homeostatic signaling network equilibrium between inflammatory cytokines, chemokines and neurotrophins resulting in MS-induced myelin damage with corresponding sustained neurological disability. Therefore, we aimed to study changes in gene and protein expression of MeCP2E1, MeCP2E2 and BDNF in SC during the entire disease course characteristically displayed for this type of EAE model. Our research suggests two potentially opposing and distinctly biologically different functions for the MeCP2E1 and MeCP2E2 isoforms: MeCP2E1 the repressor and MeCP2E2 the activator of the BDNF gene.

As previously indicated in our methods, the animals induced to the state of EAE exhibited two distinct disease phases acute and chronic. The acute phase includes inflammation and the early part of de-myelination and/or myelin damage leading to NDS that peaks by day 18 followed by a chronic demyelinating disease phase that encompasses day 18 to day 45 (Fig 3). The disease pattern displayed in our animal model is consistent with the relapsing remitting models previously described in other studies [61].

Based on our current study, we have shown for the first time the differential expression of the biologically active MeCP2E1 and MeCP2E2 isoforms in a MOG-induced EAE model of MS. In addition, we are also the first to correlate the expression changes of the MeCP2E1 and MeCP2E2 isoforms with that of BDNF expression along with corresponding NDS. Our results demonstrated significant elevations in the expression levels of the MeCP2E1 isoform (relative to NCs and ACs) during the first inflammatory acute phase of disease prior to de-myelination (EAE12-18) (Fig 4a). These results correspond with an identified significant decrease in BDNF expression (Fig 5b) relative to NC's that were also associated with elevated NDS during this same time period (Fig 3). As such, our results suggest that the EAE-induced MeCP2E1 expression, contributed to the repressed BDNF expression resulting in sustained elevated NDS known to occur during an EAE-induced attack on CNS myelin. Conversely, the progressive decreasing levels of MeCP2E1 isoform from EAE 21-27 (1st slight remission phase) promotes the slight but not significant increased expression of BDNF whose sub-optimal levels fail to facilitate re-myelination and/or myelin repair (Fig 3, Fig 4a & Fig 5b). Although, MeCP2E1 isoform expression did appear to continually decrease during the chronic phase of disease progression (EAE 30-36), the levels of MeCP2E1 are still higher than those of NC animals resulting in a continued repression of BDNF leading to the sustained elevations in NDS. As the

disease course progresses, the MeCP2E1 isoform expression continues to further decrease during EAE 39-45 in a failed attempt to facilitate increased BDNF expression that is essential to the re-myelination and/or myelin repair process. However, the persistent elevation of MeCP2E1 expression over that of NC even out to day 45 post-induction continues to repress the production of BDNF, preventing full neurological recovery with sustained NDS scoring that never returns to baseline (Fig 3 & Fig 4a). Overall, MeCP2E1 isoform levels are elevated relative to NCs and ACs at all EAE time points. As such, SC BDNF expression is continually being repressed due to EAE immune system induction which prevents local SC-derived BDNF from having an active role in the re-myelination/myelin repair process.

We believe that the MeCP2E1 induced localized repression of BDNF in the SC, leads to the activation of DRG-derived production of BDNF and its subsequent delivery to the SC [8]. This secondary compensatory mechanism aimed at increasing SC BDNF levels becomes activated in an attempt to assist in re-myelination and/or myelin repair with associated recovery from EAE-induced neurological disability. However, the amount of DRG-derived BDNF produced and transported to the SC is sub-optimal resulting in a failure to provide sufficient amounts of BDNF required to promote re-myelination and/or myelin repair. As such, elevated NDS prevail and the EAE-induced animals never fully recover following an EAE-induced attack on CNS myelin.

Studies have suggested that MeCP2 induced transcriptional repression of BDNF is dependent on several factors including extracellular calcium [35], DNA methylation [37] status and phosphorylation of MeCP2 at serine 421 during neuronal depolarization, all leading to MeCP2 release from the promoter and subsequent transcription of BDNF [35, 60]. The Martinowich research group showed this is associated with reduced methylation at promoter site [37]. These studies show MeCP2 regulation of BDNF gene is context dependent. Recent studies confirm the

complexity of BDNF gene regulation depending on cell types, brain structure and promoter region [59], further confirming the role of MeCP2 as a transcriptional repressor of BDNF. Interestingly, transcriptional activation of BDNF by MeCP2 through cAMP response element-binding protein (CREB) activator complex has been indicated in other studies to activate BDNF expression (Fig 1b) [62, 63]. However, it was not previously determined which isoform is involved in this phenomenon. None of these studies evaluated the role of MeCP2E1 and MeCP2E2 isoforms separately in regulation of BDNF gene. As such, additional research into this area is warranted.

Our research also wanted to determine whether or not the MeCP2E2 isoform may be responsible for activating BDNF expression. We observed that during the 1st inflammatory acute disease phase prior to de-myelination (EAE12-18), the MeCP2E2 isoform expression levels are markedly low relative to NCs (Fig 4b). The marked low expression levels of MeCP2E2 correspond to low BDNF levels during the EAE-induced myelin damage that occurs during the 1st EAE-induced attack (Fig 5). However, the expression levels of the MeCP2E2 isoform continue to increase during EAE21-27 (chronic disease phase) peaking at EAE27 (Fig 4b). We believe that the gradual increase in the MeCP2E2 isoform during this time period represents a failed attempt to activate the BDNF gene. The failure of MeCP2E2 to reach significant levels to counteract the pathological MeCP2E1 expression may result in the sustained elevated NDS throughout the acute and chronic phases of disease. Our results also demonstrate that the reduced MeCP2E2 expression during the chronic disease phase (EAE30-36) (Fig 4b) corresponds directly with the reduction in BDNF expression (Fig 5a) suggesting a gene activation role for the MeCP2E2 isoform in regard to BDNF. As the disease progresses into the farthest part of the chronic disease phase (EAE 39-45), MeCP2E2 isoform levels rise (Fig 4b). This corresponds

with the increase in BDNF expression (Fig 5a). These results, suggest that the MeCP2E2 isoform may be involved in activating BDNF expression.

Overall, our research identified that during the acute disease phase at EAE12, MeCP2E1 expression levels are significantly high (Fig 4a) while MeCP2E2 expression levels are low (Fig 4b) relative to NCs which creates a favorable environment for repressing BDNF expression. However, following the initial immune system activation in the acute phase, MeCP2E2 mRNA expression increases in the chronic disease phase reaching a peak at EAE27 and EAE45. This continual increase in MeCP2E2 expression appears to correspond with the increased expression of BDNF protein in the SC (Fig 5a). As a result, there are two potential explanations for this statistical significant increase in BDNF protein expression at EAE27 and EAE45. Firstly, this may result from the assistance of the MeCP2E2 isoform promoting the transcription of BDNF that ultimately leads to increased BDNF translation. Alternatively, as per our previous publications, the increased BDNF protein at EAE27 and EAE45 may result from the production and anterograde transport of BDNF from the DRG to the SC [8]. Furthermore, it is also plausible that the increased BDNF protein expression at EAE27 may be the result of both effects which contribute to the enhanced expression of BDNF. Irrespective of the elevated BDNF protein levels at EAE27 and EAE45 this effect never reaches statistically significant levels in the SC at any other time points during the acute and chronic phases of the disease. As such, the inability to induce sustained BDNF expression during the acute and chronic phases (except at EAE27 and EAE45) merely represents failed attempts to repair damaged myelin leaving the EAE animals with residual neurological disabilities from which they never fully recover (Fig 3).

Although MeCP2E1 and MeCP2E2 isoforms have over 95% similar sequence homology, other studies support our current research findings which suggest some functional biological

differences between them [47]. For instance, the increased expression profile of MeCP2E1 over MeCP2E2 isoform in the brain is associated with Rett syndrome [64]. In addition, mutations located in exon 1 [65, 66] have also been suggested to implicate MeCP2E1in Rett syndrome. Furthermore, other studies confirm the differential effects on different target gene expression profiles that are regulated by the MeCP2E1 and MeCP2E2 isoforms [67]. For instance, Milacic et al evaluated different gene expression patterns for MeCP2 isoforms in neuronal cells over-expressing predominantly MeCP2E1 or MeCP2E2 [67]. They found that many genes involved in axon genesis, neuronal differentiation and tyrosine kinase receptor signaling are regulated by MeCP2E1 [67]. In addition, they also found that genes involved in tyrosine kinase signaling and immune response were down regulated by MeCP2E1 [67]. However, the MeCP2E2 isoform mostly regulates those genes involved in chromatin organization and transcriptional regulation [67]. These functional biological differences may be related to differences in N-terminal sequence of two isoforms that causes shorter half-life for MeCP2E2 [68] and different DNA binding specificity.

In order to understand how MeCP2E1 and MeCP2E2 affect BDNF gene and protein expression, we evaluated gene and protein expression of BDNF (Fig 5). Interestingly, for BDNF SC protein (Fig 5a), the peak BDNF levels occur at EAE27 and EAE45 without any statistically significant increase in BDNF mRNA levels at these same time points (Fig 5b). This means that there is a disconnection between transcription and translation in SC at these particular time points post-induction. Therefore, the isolated increase in BDNF protein at EAE27 and EAE45 without the corresponding increase at the transcript level could result from a combination of increased levels of MeCP2E2 (Fig 4b) and/or the transport of DRG derived BDNF to the SC [8]. The later effect appears to be the most plausible explanation. However, we believe that the failures of these

compensatory mechanisms are the direct result of the very potent transcriptional repression of BDNF by MeCP2E1. Although we did not evaluate the anterograde transport of BDNF from DRG to SC in the present study, our previous publication demonstrated BDNF transport from DRG to SC by kinesin protein that support this concept [8]. Our research also detected another elevation in BDNF protein at EAE45 in the later chronic disease phase that correlates to second partial remission of the disease. Interestingly, during this later disease phase, the MeCP2E1 mRNA expression had decreased to that depicted by NC's while MeCP2E2 mRNA expression shows a marked increase (Fig 4). Based on our findings, the differential expression of the MeCP2E1 relative to MeCP2E2 isoform appears to dictate the degree of myelin damage as evident by the improvements in NDS during this time period. As such, the expression ratios of the two isoforms may be a useful diagnostic tool since the reduction in MeCP2E1 with the corresponding rise in MeCP2E2 assist in the enhancement of BDNF production. However, despite these efforts, the amount of BDNF increase is still not enough to provide full neurological recovery as displayed by the sustained elevations in NDS during both acute and chronic phases of the disease. Interestingly, during the acute phase of the disease, SC BDNF mRNA levels are repressed in EAE groups compared to NC (Fig 5b). We believe this may be due to elevated SC levels of MeCP2E1 RNA (Fig 4a), suggesting its role as a transcriptional repressor of BDNF. In addition, upon further examination of the BDNF mRNA levels in the chronic disease phase, we see that the BDNF mRNA levels are increased (Fig 5b) which correspond to the decreasing pattern of mRNA expression for MeCP2E1 (Fig 4a) and the marked trend of increased mRNA expression for MeCP2E2 (Fig 4b). Our research supports the concept which suggests the role of MeCP2E1 as BDNF repressor and MeCP2E2 as BDNF activator. Therefore, we believe that EAE animals display residual sustained neurological deficits due to a

disproportionately high production of the pathological MeCP2E1 isoform relative to the MeCP2E2 isoform.

Our research findings report a statistically significant elevation of BDNF protein in EAE animals at day 27 and 45 (Fig 5a). Although BDNF expression appears to have increased in an effort to repair the MS-induced myelin damage, the BDNF levels are sub-optimal as indicated by sustained elevations in NDS (Fig 3). As a result, the ability to block the pathological effects of the MeCP2E1 isoform that is involved in chronic repression of BDNF warrants further investigation. Henceforth, therapeutic interventional strategies aimed at restoring a normalized balance between MeCP2E1 and MeCP2E2 expression represent a novel area for future epigenetic studies. This concept is supported by our data that shows the early immune system mediated induction of MeCP2E1 isoform expression in both the acute and chronic phase of the disease progression (Fig 4a). The trend displayed by MeCP2E1 isoform was not fully counteracted by the MeCP2E2 isoform (Fig 4b). Therefore, our research suggests that an optimal balance between the pathogenicity induced by MeCP2E1 isoform and the BDNF activator isoform (MeCP2E2) is critical to complete re-myelination and/or myelin repair that is required for full neurological recovery following an EAE-induced attack.

Finally we compared the MeCP2E1 and MeCP2E2 protein expression in SC and DRG (Fig 6) in NC animals. Significantly higher protein levels of MeCP2E1 and significantly low protein levels of MeCP2E2 in the SC of NC animals were detected. Specifically, the MeCP2E1 isoform was 9.25 fold higher than the MeCP2E1 isoform in the SC. As such our results suggest that the elevated MeCP2E1 expression at baseline creates a more hostile environment that contributes to blocking the re-myelination and/or the myelin repair process by repressing BDNF. Interestingly, we identified higher baseline protein levels of the MeCP2E2 isoform in the DRG relative to SC

in NC animals. Our findings support the concept that the elevated levels of MeCP2E2 in the DRG relative to the SC produces a more favorable environment in the DRG for BDNF production and subsequent transport to the SC. Furthermore, our results show that the ratio of MeCP2E1 relative to MeCP2E2 is 9.25 fold in the SC compared to the DRG at 1.36 fold. This suggests that the SC is a more hostile environment for producing BDNF. Based on the MeCP2E1 to MeCP2E2 ratios, the DRG appears to be a more conductive environment for BDNF production. This is why the DRG-SC connection represents an important secondary compensatory mechanism that needs to be optimized in order to produce sufficient amounts of BDNF for subsequent transfer to the SC to assist in repairing damaged myelin. This concept involving the anterograde transport of BDNF from PNS to CNS has been confirmed and supported by other studies [69]. As such, the anatomical connection between the DRG-SC represents a novel target that requires further investigation in regard to myelin repair following and EAE-induced insult to CNS myelin.

Research suggests that both mutations and duplication in MeCP2 could lead to neurodevelopmental abnormalities [70, 71]. It is imperative to keep MeCP2 in a narrow range for normal neurological functioning. Our study confirmed for the first time the importance of MeCP2E1 and MeCP2E2 ratio in EAE model of MS in regard to regulating re-myelination and/or myelin repair. We conclude that MS-induced myelin damage may result from an imbalance between MeCP2E1 and MeCP2E2 isoforms. We believe that the pathological over-expression of MeCP2E1 represses BDNF resulting in a disruption of the homeostatic signaling equilibrium between cytokines, chemokines and neurotrophins resulting in incomplete re-myelination and/or myelin repair with corresponding neurological disability (Fig 7) (for detailed description of molecular signaling between cytokines, neurotrophins and chemokines see our

previous publication in this area [13]). On the other hand, the MeCP2E2 isoform may be involved in gene activation thereby promoting BDNF expression in order to facilitate re-myelination and/or myelin repair with corresponding improvement in neurological disability. Hence the ability to upregulate expression of the MeCP2E2 isoform may also prove to be a worthy strategy to facilitate myelin repair following white matter damage. Furthermore, our current research demonstrates the potential importance of the DRG-SC pathway as a critical anatomical connection involved in the molecular signaling of key biological targets (MeCP2E1, MeCP2E2, BDNF) that govern re-myelination and/or myelin repair. Henceforth, the ability to enhance the production and delivery of DRG derived BDNF to the CNS may also prove to be a worthy strategy to facilitate myelin repair following white matter damage.

Epigenetic research represents a new and growing research field that is being investigated in white matter disorders such as MS to establish therapeutic strategies that promote re-myelination and/or myelin repair [72]. Specifically, epigenetic research examines changes to histone acetylation/methylation and/or DNA methylation that are responsible for turning "off" or turning "on" certain target genes involved in re-myelination and/or the myelin repair such as MeCP2E1, MeCP2E2 and BDNF [13]. Histone acetylation alterations are well defined in patients with MS [72]. Studies involving MS patients have shown decrease acetylation in OGs in early MS lesions, while increase acetylation occurs in chronic lesions [50]. Furthermore, animal models of MS show changes in acetylation of key genes involved in Th17 acetylation [51]. As exemplified in (Fig 1a), at the molecular level, MeCP2E1 exerts its repressive effects on the target gene (BDNF) through the binding of HDAC and Sin3A to the transcriptional repressor domain (TRD). The use of epigenetic therapeutic intervention involving selective HDAC inhibitors that reduce the repressive effects of the MeCP2E1 isoform warrants further investigation. Recent research

has suggested the use of indirect epigenetic interventions such as histone deacetylase inhibitors (HDACi) such as trichostatin A [73] and valproic acid [74] and lysine acetyltransferase inhibitors such as curcumin [75] can be beneficial in improving neurological disability in the EAE mice model. Our future studies will evaluate HDACi effects on restoring the normal cytokine, chemokine and neurotrophin homeostatic balance by determining if such interventional strategies can suppress MeCP2E1 repression of BDNF.

In addition to histone modifications, epigenetic studies have also shown that modifications to DNA methylation may be involved in the pathogenesis of MS. This opens a new and growing area of research interest that is being explored for potential therapeutic interventions to prevent or slow MS-induced white matter damage [76, 72]. Specifically, research in this area, identifies two important enzymes involved in the methylation of DNA. DNA methyl transferase is involved in DNA methylation generating 5-methylcytosine (5mC), whereas ten-eleven translocations 1 (TET) enzyme is involved in 5mC oxidation producing 5-hydroxymethylcytosine (5hmC) [77]. In general the literature suggests that 5mC is a repressor while 5hmC is an activator of certain target genes of interest [77]. As such, both 5mC and 5hmC are thought to have different roles in disease pathogenesis or disease prevention depending of the medical condition. However, in regard to MS, epigenetic studies demonstrate that the binding of MeCP2 to 5hmC is associated with transcriptional activation [78]. This finding of 5hmC activation of target genes such as BDNF [79] may have critical implications in advancing our understanding of re-myelination and/or myelin repair of damaged myelin. If the expression of BDNF is regulated by the balance between 5hmC and 5mC at BDNF gene regulatory regions this would identify a critical mechanism for the regulation of BDNF expression and subsequent effects on myelin repair following an MS-induced attack on CNS myelin. Interestingly, MS

studies reveal that 5hmC modifications are decreased in the peripheral blood sample of MS patients [80] thereby suggesting that the low BDNF levels in MS patients are attributed to this reduction in 5hmC. However, to the best of our knowledge there does not appear to be studies that have investigated whether 5hmC is decreased in spinal cord or brain tissues of MS patients. Henceforth, the correlation between the reduction of BDNF levels known to occur in MS patients [81] and the reduction of 5hmC warrants further investigation. As a result of these studies, further investigation involving the use of epigenetic interventional agents such as a TET activator (Vitamin C) to enrich genomic 5hmC may be a novel mechanism to enhance BDNF production in order to facilitate re-myelination and/or myelin repair. Furthermore, research suggests that 5hmC, known as the sixth base, may have a significant role in the pathogenesis of white matter disorders such as Huntington's disease, Alzheimer's disease, and Fragile X syndrome [82]. This novel approach may have the diverse applicability that extends beyond that of MS to include repair of myelin damage associated with other white matter disorders.

3.6. Conclusion

In summary, we have provided evidence to support the pathogenic role of the MeCP2E1 isoform in an EAE model of MS. Our results suggest the potential importance of tracking the expression ratios between MeCP2E1 and MeCP2E2 isoforms. Potential therapeutic implication of this research would be to design a screening test in those patients pre-disposed to the known risk factors for MS and subject them to biological testing of the MeCPE1/MeCP2E2 ratio. For example, patients presenting with a high ratio may be at greater risk for going on to develop MS and therefore, could serve as a useful diagnostic screening test that could be added to the battery of diagnostic tests currently used to diagnose MS patients [83, 84]. MeCP2 represents a key biological target molecule where epigenetic interventional strategies aimed at both DNA

methylation and histone modifications could be optimized to generate a novel therapeutic drug to halt myelin damage associated with MS [85]. Therefore, studying the link between MeCP2 and BDNF and other downstream biological targets such as NGF [10, 5], TNFα [6], and CX3CL1 [9] in MS may lead to new epigenetic therapeutic approaches to promote re-myelination and myelin repair in MS.

3.7. List of abbreviations:

AC: Active control; **ANOVA:** Analysis of variance; **BDNF:** Brain derived neurotrophic factor; **CFA:** Complete Freund's adjuvant; **CNS:** Central nervous system; **CX3CL1:** Fractalkine; **CX3CR1:** Fractalkine receptor; **DRG:** Dorsal root ganglia; **dpi:** Days post induction; **EAE:** Experimental autoimmune encephalomyelitis; **ELISA:** Enzyme linked immunosorbent assay; **GAPDH:** Glyceraldehyde-3-phosphate dehydrogenase**; HDAC:** Histone deacetylase; **MBD:** Methyl binding domain; **MeCP2**: Methyl CpG binding protein 2; **MOG:** Myelin oligodendrocyte glycoprotein; **MS:** Multiple Sclerosis; **NC:** Naïve control; **NDS:** Neurological disability scores; **NGF:** Nerve growth factor; **OGs:** Oligodendrocytes; **OPCs:** Oligodendrocyte progenitor cells; **PNS:** Peripheral nervous system; **PTX:** Pertussis toxin; **qRT-PCR:** Quantitative real time polymerase chain reaction; **SC:** Spinal cord; **SQ:** Subcutaneous; **Th:** Helper T cells; **TNF α**: Tumor necrosis factor alpha; **TRD:** Transcriptional repressor domain; **TrkB:** Tropomyosin kinase receptor; **WB:** Western blot

3.8. Competing interests:

The authors declare that they have no competing interests.

3.9. Authors' contributions:

TKA was the lead researcher performing and analyzing experiments. KA performed and analyzed mRNA analysis. CC performed animal studies and contributed to mRNA and protein analysis. RL performed statistical analysis for mRNA and protein. TML designed experiments and assisted in writing the paper. QGH and JH performed mRNA analysis. XX assisted in writing the paper. XL performed protein analysis. JK assisted in experiment design and writing. LX assisted in protein analysis. MPN designed the study and assisted in writing the paper. All authors reviewed the results and approved the final version of the manuscript.

3.10. Acknowledgment:

Special thanks to the Manitoba Multiple Sclerosis Research Network Organization (MMSRNO) for their continued support of advanced research in MS. The authors would also like to acknowledge the support from The Manitoba Medical Service Foundation Grant (MMSF), Canadian Paraplegic Association (CPA), University of Manitoba Research Grants Program (URGP), Bayshore Health Care Systems, College of Pharmacy at the University of Manitoba, and endMS for their continued support of research, training and education to advance our knowledge in the specialized field of MS. The authors would also like to thank Ms. Crystal Acosta for her kind assistance with the technical aspects related to this manuscript.

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3.12. FIGURES AND FIGURE LEGENDS:

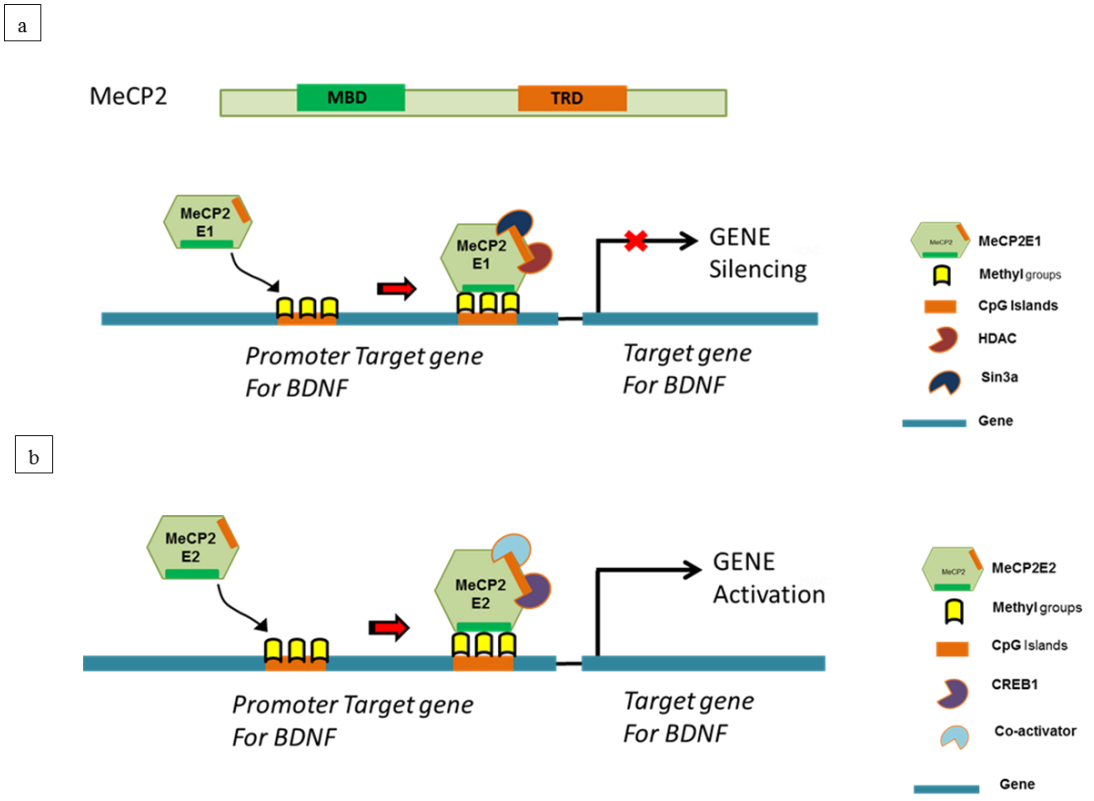

Fig. 1: Suggested Molecular Mechanism of Action of MeCP2E1 and MeCP2E2

BDNF gene is very complex. Aid et al described 10 exons in human and rodent BDNF gene [86]. MeCP2 has been shown to bind to promoter region of exon III in rats [35] and exon IV in mice and humans. a) MeCP2E1 Mechanism of Gene repression: MeCP2E1 binds to methylated CpG dinucleotide in CpG islands of promoter region for BDNF gene with its methyl binding domain (MBD) and recruits a transcriptional co-repressor complex containing mSin3A and histone deacetylase 1 (HDAC1) and 2 (HDAC2) with its transcriptional repressor domain (TRD) to repress the expression of BDNF during an MS attack leading to MS induced myelin damage. b) MeCP2E2 Mechanism of Gene activation: MeCP2E2 binds to methylated CpG dinucleotide in CpG islands of promoter

region for BDNF gene and recruits a transcriptional activator complex containing cAMP response element-binding protein (CREB) and co-activators [62]. This leads to activation of BDNF gene during an MS attack in order to help in re-myelination and/or myelin repair.

Days post – induction (dpi)

0 1 45

Animal Group	Treatment [via]	Days	Clinical Score
EAE	MOG_{35-55} + CFA [SQ]	0 dpi	NDS
	PTX [IP]	0 & 1 dpi	
AC	CFA [SQ]	0 dpi	None NDS
	PTX [IP]	0 & 1 dpi	
NC	No treatment	----	None NDS

Fig. 2: EAE induction by MOG

Our EAE animals were immunized with 200 µg MOG_{35-55} in 200 µl of CFA SQ on day 0. AC animals only received the CFA adjuvant. Both EAE and AC animals received IP injections of pertussis toxin on day 0 and 1. NC did not receive any treatment.

EAE = experimental autoimmune encephalomyelitis, AC = active control, NC = naïve control, MOG = myelin oligodendrocyte glycoprotein, CFA = complete Freund's adjuvant, PTX = pertussis toxin, NDS = neurological disability scoring, dpi =days post induction, SQ = subcutaneous, IP = intraperitoneal.

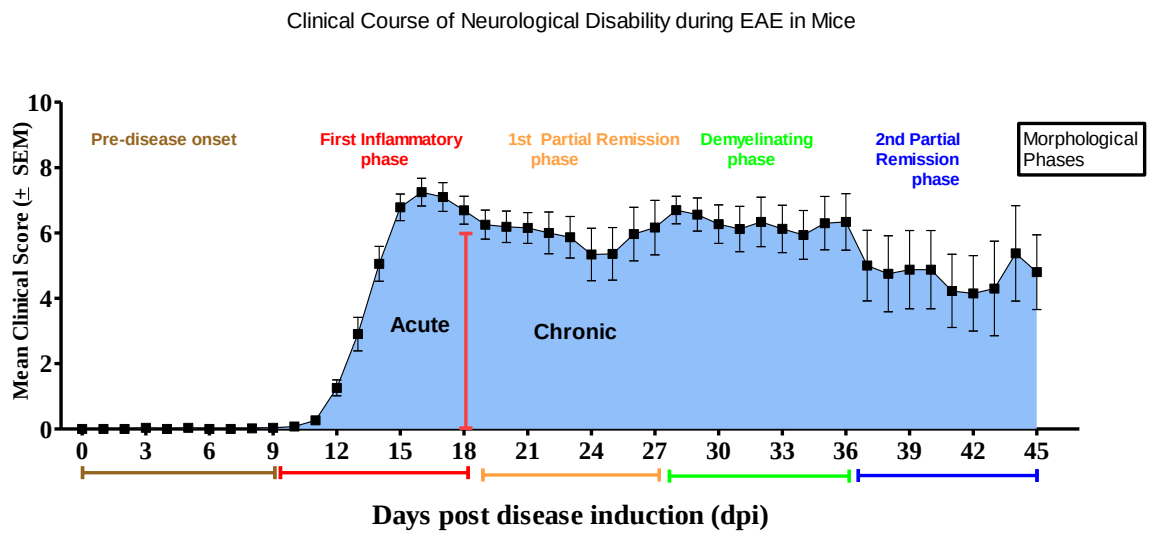

Fig. 3: Clinical course of EAE-induced neurological disability in MOG-induced EAE mice

Preliminary results of mean neurological disability scores recorded in MOG-induced EAE mice at different time points of disease progression. For example, days 3, 6, and 9 (pre-disease onset), days 12, 15, and 18 (first inflammatory phase prior to de-myelination), days 21, 24 and 27 (during 1st partial incomplete remission), days 30, 33, and 36 (demyelinating phase), and days 39, 42 and 45 (during the second period of partial incomplete remission: re-myelination phase). Mean global neurological disability scores were obtained following assessment of all 6 specific clinical domains. Neurological disability scores (NDS) range from a score of 0 (no disability) to 15 (maximal disability). Our preliminary results indicate that MOG-induced EAE mice exhibit clinical signs of the disease activity at ~ 11 days after the initial immunization (disease onset phase) that peaked at ~ day 17 (pre-demyelinating disease phase) and 27 (de-myelinating disease phase). We also observed the remission (re-myelinating phase) at day 21.

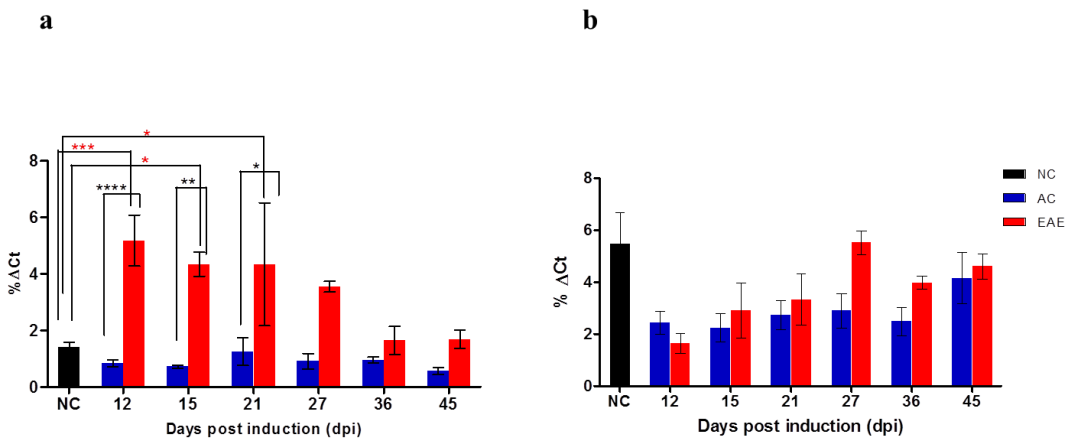

Fig. 4: RNA expression of MeCP2 isoforms in the Spinal Cord (SC)

(a) Quantitative RT-PCR shows MeCP2E1 mRNA gene expression in SC at different times in the disease progression. %ΔCt analysis of real time RT-PCR of MeCP2E1 - EAE animals show a significant increase of MeCP2E1 expression in SC over NC animal groups at 12, 15 and 21 dpi. EAE animals show a significant increase of MeCP2E1 expression in SC over AC at 12, 15 and 21 dpi. In comparison, NC and AC do not show significant difference at all different time points. **(b)** Quantitative RT-PCR shows MeCP2E2 mRNA gene expression in SC at different times in the disease progression. **%**ΔCt analysis of real time RT-PCR of MeCP2E2 expression in SC do not shows significant difference in mRNA expression between the NC control animal and EAE group. [**** = $p<0.0001$;*** = $p<0.001$; ** = $p<0.01$; * = $p<0.05$]. (Two way ANOVA followed by Bonferroni's post hoc test). Values are mean+ SEM.

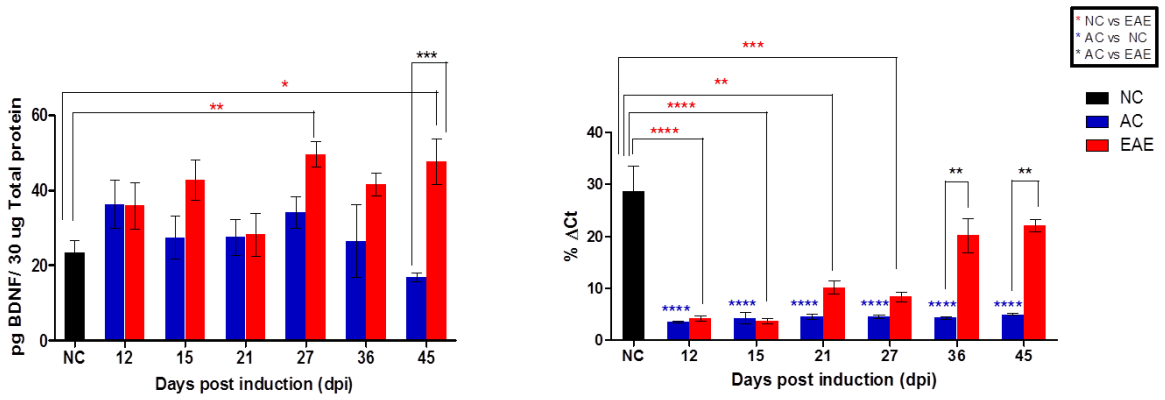

Fig. 5: Transcript and protein expression of BDNF in the Spinal Cord (SC)

(a) ELISA quantification of BDNF expression in the SC. BDNF expression in the SC was quantified using ELISA. There is significant differences between NC and EAE at day 27 (**p < 0.01) and 45 (*p < 0.05) and between AC and EAE (***p < 0.001) at day 45. (Two way ANOVA followed by Bonferroni's post hoc test). Values are mean+ SEM.

(b) RT-PCR quantification of BDNF mRNA expression in the SC. %ΔCt analysis of real time RT-PCR of BDNF expression in SC shows significant differences in mRNA expression between the NC animals (black bars) and EAE (red bars) at day 12 ,15, 21 and 27 dpi. EAE group show significant increase compared to AC animal (blue bars) at day 36 and 45 dpi. There are significant differences between NC and AC at all-time points. [**** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.05]. (Two way ANOVA followed by Bonferroni's post hoc test). Values are mean+ SEM.

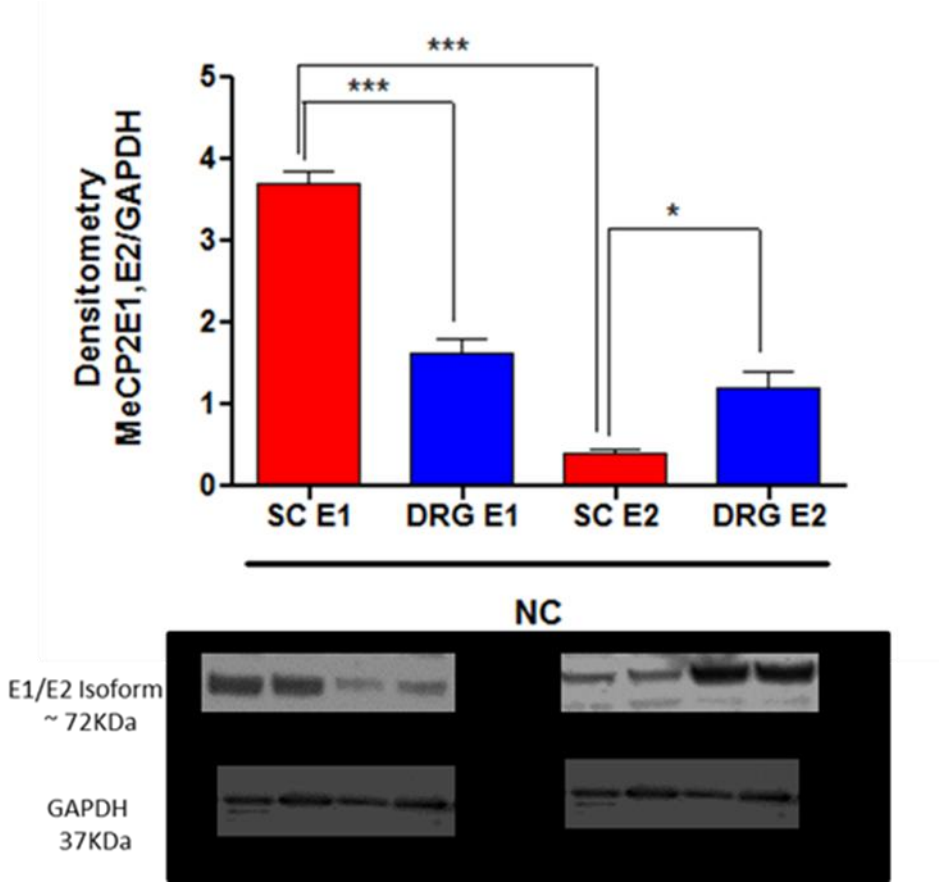

Fig. 6: Baseline MeCP2E1 and MeCP2E2 expression in DRG and SC

Results are shown as the ratio of MeCP2E1/GAPDH and MeCP2E2/GAPDH. There are significant differences between MeCP2E1 (***=$p<0.001$) and MeCP2E2 (*=$p<0.05$) expression level in DRG and SC. Relative expression of MeCP2E1/ MeCP2E2 is 9.25 in SC, whereas it is 1.36 in DRG.

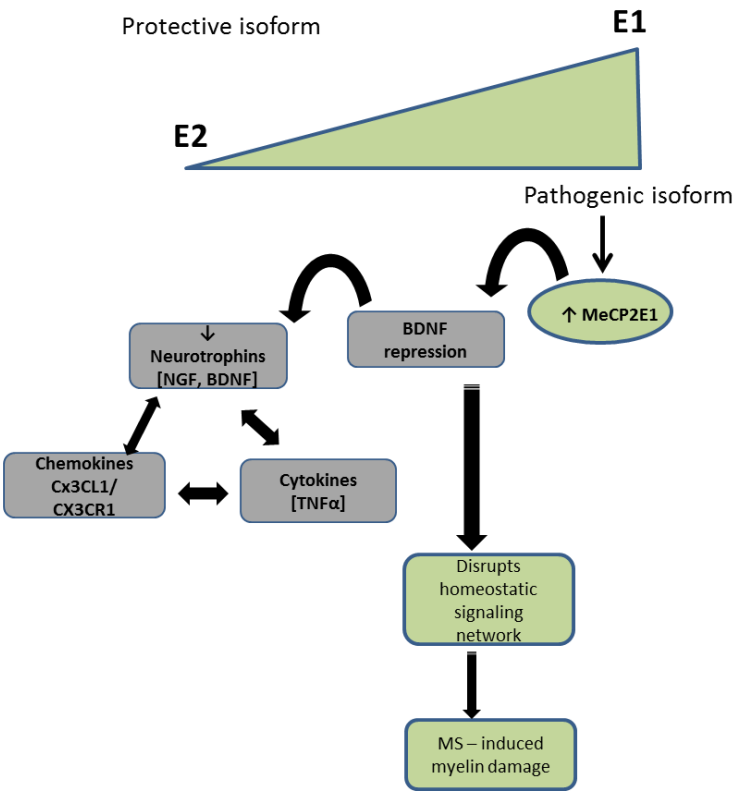

Fig. 7: Pathogenic MeCP2E1 molecular signaling leading to MS-induced myelin damage in CNS

MS results from imbalance between MeCP2 isoforms: increase in pathogenic MeCP2E1 isoform and decrease in protective MeCP2E2 isoform. As MeCP2E1 is involved in repression of BDNF, homeostatic signaling equilibrium between neurotrophins (NGF and BDNF), cytokines (TNF-α) and chemokines (CX3CL1 and CX3CR1) is disrupted leading to MS-induced myelin damage. NGF = nerve growth factor, CX3CL1 = fractalkine, CX3CR1 = fractalkine receptor, TNF-α = tumor necrosis factor-alpha

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CHAPTER 4: GLOBAL DISCUSSION AND CONCLUSIONS

4.1. OVERVIEW

MS is a chronic inflammatory neurological disease characterized by autoimmune response directed against the myelin sheath proteins of the CNS. These myelin proteins are responsible for the structural integrity and normal physiological functioning of myelin that surrounds the nerve axons. Therefore, the resultant myelin damage that occurs following an immune system mediated MS attack interrupts the normal propagation of electrical impulses along nerve axons that required for normal physiological functioning. Patients with white matter disorders such as MS suffer from a variety of disease induced symptoms with associated debilitating neurological disabilities [1]. Inflammation, de-myelination and axonal loss are the hallmark pathological markers of MS [2]. Although current treatments including anti-inflammatory and immunomodulatory therapies reduce the severity and frequency of attacks, to date there is not definite cure for MS [3]. This interventional approach to MS-induced myelin damage is suggested to assist patients in a quicker recovery from the neurological impairments and thereby improve their quality of life.

4.2. Transcriptional regulation of BDNF by MeCP2: A novel mechanism to explore in the development of epigenetic treatment interventional strategies for re-myelination and/or myelin repair in MS

Epigenetic therapeutic intervention strategies involve the use of various therapeutic agents to block or potentiate the expression of the various genes involved in the pathological induction of disease. Therefore, such epigenetic modifications represent a novel area in the expanding field of MS research [4]. Our neuro-immunology laboratory has a specific interest in MS-induced histone modifications that contribute to disease pathology. However, following Dr. Namaka's sabbatical leave, our laboratory is now establishing research collaborations involving MS-induced DNA methylation changes that contribute to disease pathology. Reflecting back on our knowledge of MeCP2, we know that it has two functional domains, MBD and TRD [5]. It binds to methylated DNA by MBD and affects the post-translational modifications of histone by recruiting a transcriptional corepressor complex containing Sin3A and histone deacetylase 1 and 2 (HDAC1, HDAC2) [6-8]. Therefore, our laboratory's future studies of interest will involve the use of epigenetic interventional strategies that attenuate those DNA methylation [9, 10] and histone modifications [11], that contribute to the underlying pathogenesis of MS. As discussed earlier, MeCP2 is the key transcriptional regulator of BDNF [12, 13]. In addition, we have also addressed the well-established role of BDNF in regard to promoting re-myelination and/or myelin repair [14-16]. One of my first future research studies would involve the use of a histone deacetylase inhibitor (HDAC) to block the establishment of the co-repressor complex for MeCP2 via the TRD (described above) that is critical for the MeCP2E1 repressor effects on BDNF. Interestingly, changes in histone acetylation have been recently identified in MS patients [11]. Several EAE studies have confirmed the beneficial effects of HDAC inhibitors in improving neurological disabilities and other pathological features of MS [17-19]. The concept of using epigenetic interventional studies to modulate histone acetylation through the use of HDAC inhibitors has recently gained significant attention [20]. Therefore, evaluating the specific

mechanism of action and benefits of various HDAC inhibitors in MS warrants further investigation. Interestingly, in our EAE animal model of MS, our early preliminary results using TSA (HDAC inhibitor) suggests significant benefit in attenuating NDS associated with disease activity (Figure 1). However, our research laboratory is now just starting to study the histone modifications and DNA methylation changes caused by EAE induction to further advance our knowledge in this area. Our early preliminary results in regard to histone modifications identify that EAE induces both mono and dimethylation of lysine residues. Our preliminary research also suggests that following TSA treatment, TSA significantly reduces the expression of the mono and dimethylated forms of lysine with corresponding reductions in NDS (Figure 2). Interestingly, TSA did not cause significant elevations in acetylation of the lysine residues as we would have expected due to its effects as a HDAC inhibitor. However, our preliminary results in this area, suggest a potentially different beneficial effect of TSA that appears to be linked to its ability to significantly reduce the mono and dimethylation of lysine residues that appear to be driving the EAE disease course. Despite these very early preliminary results much more extensive research in this area is required by our laboratory to definitively confirm that EAE-induced histone changes contribute to disability associated with this disease. Our future research in this area includes evaluating the source of the histones in our tissue to determine their origin. Because we did not expect to see histones from neuronal cells, their cell bodies were not included in the tissue preparations. However, we expected to extract myelin basic protein and histones form glial cells. These intriguing findings represent one area that captivates my interest in pursuing advanced studies in a doctoral level research program.

Overall, epigenetic research strategies aimed at promoting BDNF expression could potentially promote re-myelination and/or myelin repair following an MS-induced attack on CNS myelin

[21]. Current research supports this novel concept. Fingolimod (Gilenya®) the first oral medication used in the management of the relapsing remitting form of MS has also been shown to increase BDNF levels in mice lacking the MeCP2 protein [22]. Although all these studies support the promising role of enhanced BDNF expression in re-myelination and/or myelin repair, more detailed research in this area is required.

BDNF is one of the most well studies genes whose transcription is known to be regulated by MeCP2 [12, 13]. Early studies in rats and mice describe MeCP2 as a transcriptional repressor of BDNF by exerting its effects by binding to methylated DNA and forming a co-repressor complex containing Sin3A and HDAC1 and HDAC2 [6, 8, 23]. However, further studies show that the transcriptional regulation of BDNF by MeCP2 is actually more complex [24] depending on extracellular calcium [12], DNA methylation [25] status and phosphorylation of MeCP2 at serine 421. Conversely, transcriptional activation of BDNF by MeCP2 has been shown to occur through cAMP response element-binding protein (CREB) activator complex [26, 27]. The earliest study in this area shows MeCP2 having a role in transcriptional activation (Nan et al 1997) indicating MeCP2 functions as a transcription activator when binding to unmethylated promoters [7]. Furthermore, using chromatin immunoprecipitation method, it was found that only 6 % of MeCP2 binding promoters are in CpG islands out of which 63% are transcriptionally active including the BDNF promoter [26]. Therefore, these studies confirm that MeCP2 can function as both a transcriptional gene repressor and gene activator of BDNF. However, our current research findings also suggest that the different biological activity of MeCP2 may be a function of the differential biological activity between the MeCP2E1 and the MeCP2E2 isoforms [20]. The ability to design a diagnostic test that could accurately and reliably detect the MeCP2E1/MeCP2E2 ratio may prove to be a useful diagnostic for initial diagnosis as well as

determining the degree of ongoing disease activity. This ratio could potentially serve as a novel key pharmacodynamics marker by which could be tracked as a function of time following treatment to determine therapeutic efficacy of the medication at suppressing disease activity. This would be analogous to diabetic patients following blood glucose levels while being treated with hypoglycemic agents [28].

4.3. Pre-Establishing Criteria for Inclusion of EAE Animals: Implications in Data Analysis

In our current study we used a well-established and highly reproducible MOG-induced mouse model of EAE to mimic the disease course of MS [29, 30]. Based on the literature and our extensive experience with this animal model, the antigenic induction of animals with a MOG peptide produces a relapsing remitting pattern of MS that shares similar presentation to that seen in humans. Our results identify two main disease phases. The first phase that is the acute phase is characterized by inflammation, de-myelination and the onset and peak neurological disability. The second phase is the chronic phase that is characterized by ongoing inflammation, de-myelination with associated immune system flare-ups called relapses followed by only slight periods of remission with sustained neurological disability. Based on our in house expertise with this animal model of MS, all EAE animals exhibited ascending paralysis pattern starting from the tail and progressing to fore limbs. In our model, we included stringent criteria for EAE animals that were harvested for gene and protein analysis. Based on our pre-established inclusion criteria, we only retained the tissue from EAE animals that exhibited NDS of 4 +/- 1 during the acute phase and NDS of 6 +/-2 for the chronic phase. Dr. Namaka's previous extensive experience with this specific animal model was instrumental in establishing this criterion that formed the foundation for which we could establish these parameters that depict a standardized approach to EAE inoculation. Establishing these stringent inclusion criteria was critical in the analysis of the

data collected in order to minimize the degree of experimental error often associated with the use of animal models. When using animal models, researchers accept that each independent animal may respond differently to the same treatment conditions. As such, even under optimal conditions with the exact same EAE inoculation formula, some animals don't develop the same degree of disease severity as others. Conversely, other animals provided with the same EAE inoculation formula go on to develop much more severe disease course than would normally be expected to occur. Hence, we pre-established our EAE animal inclusion criteria to minimize the degree of investigator experimental error associated with the preparation and administration of the EAE inoculation formula as well as to minimize the degree of inter-animal variation in response to the EAE inoculation administration. In this manner, the gene and protein analysis presented in my research represents that obtained from a standardized/normalized EAE reproducible response. The introduction of this type of scientific rigor into our data analysis minimized the skewing effects of data that could have been introduced from those EAE animals that displayed unusually low or high NDS. The results we presented in our gene and protein analysis represent a more true reflection of the EAE-induced effects of disease induction on gene and protein expression.

4.4. SUMMARY

In this regard, our results indicate a statistically significant immune system mediated induction of the MeCP2E1 isoform during acute phase of the disease that is associated with significant decreased BDNF expression and increased neurological disability during the same time period. The MeCP2E1 isoform levels were shown to be higher than NC group at all experimental EAE time points. We believe that this subsequently contributed to the continued repression of BDNF during acute and chronic phase that resulted in sustained NDS during the entire disease course.

Conversely, upon examining the MeCP2E2 isoform that is suggested to be a gene activator of the BDNF gene, we see that its levels are very low compared to NC during the acute phase that also corresponded to decreased BDNF expression during this same time period. However, in the chronic disease phase, MeCP2E2 expression levels increased during the chronic phase and peaked at EAE 27 and EAE 45 that correspond with BDNF increased expression. However, because MeCP2E2 isoform could not counteract the effects of pathological MeCP2E1 isoform, the elevated levels of BDNF were still not sufficient to provide full neurological recovery. Furthermore, our results indicate higher levels of MeCP2E1/MeCP2E2 ratio in the SC compared to DRG (Please see Chapter 3 Figure 6). As such, the SC appears to be a more hostile environment for producing BDNF. However, the MeCP2E1/MeCP2E2 ratios in the DRG are much lower, representing a much more favorable environment within these PNS ganglia for BDNF production. As such, based on our laboratory's previous publications in this area, the anatomical connection between DRG and SC may serve as a secondary compensatory mechanism to transport BDNF from DRG to the SC in order to assist animals recover from neurological disability [31-33]. As such, another area that I am interested in potentially pursuing during my doctoral studies is to further explore the role of the DRG-SC anatomical connection for its role in promoting re-myelination and/or myelin repair.

The research presented in my thesis, demonstrates for the first time the differential expression of the biologically active MeCP2E1 and MeCP2E2 isoforms in a MOG-induced EAE model of MS. In addition, we are also the first to correlate the expression changes of the MeCP2E1 and MeCP2E2 isoforms with that of BDNF along with corresponding NDS. As such, our research supports the concept which suggests the role of MeCP2E1 as BDNF repressor and MeCP2E2 as BDNF activator. Therefore, we believe that EAE animals display residual sustained neurological

deficits due to a disproportionately high production of the pathological MeCP2E1 isoform relative to the MeCP2E2 isoform. As a result, the ability to block the pathological effects of the MeCP2E1 isoform that is involved in chronic repression of BDNF warrants further investigation. Therapeutic interventional strategies aimed at restoring a normalized balance between MeCP2E1 and MeCP2E2 expression represent a novel area for future epigenetic studies.

Currently there is no cure for MS. At present, current available treatments can only improve the symptoms and slow the progression of the disease [34]. Therefore, there is an unmet need for emerging treatments in MS to focus on improving re-myelination and/or myelin repair. Emerging evidence suggests that neurotrophins such as BNDF and NGF [33, 35] have well-established roles in re-myelination and/or myelin repair. Further investigative research in designing new future MS treatments to promote their beneficial effects in this regard is warranted. The recent literature on the new available treatments for MS such as fingolimod, glatiramer acetate and laquinimod also support our independent research findings in regard to the importance of BDNF in this re-myelination and/or myelin repair process [22, 36, 37]. Accumulating evidence also demonstrates the importance of MS-induced epigenetic changes that alter the course of disease and degree of myelin repair. In fact, many studies have already indicated changes in histone acetylation and DNA methylation in patients with MS [11]. Based on laboratory's previous publications, we have also identified the importance of molecular signaling triad including cytokines, neurotrophins and chemokines in the re-myelination and/or myelin repair process. My current research involving MeCP2 has contributed to the research field in this area by identifying it as key upstream biological target that warrant further investigation in regard to its effects on myelin repair. My master's project was focused on transcriptional regulation of BDNF by MeCP2 two biological active isoforms; MeCP2E1 and

MeCP2E2. The research accomplished during my master's program provides insight into correlation between gene and protein expression of BDNF, MeCP2E1 and MeCP2E2 in regards to NDS. The findings unveiled by my research continue to expand and build upon the molecular research platform of our neuro-immunology laboratory. It is our belief that our findings will also contribute to the growing field of epigenetic research that could lead to the discovery of novel treatment advances in this area. Overall, the training and experience that I have acquired during the completion of my Master's degree, has provided me with a key set of scientific skills that I can build upon as I move forward towards my goal of undertaking doctoral studies in the field of MS.

4.4.1. Summary Conclusion:

Our research identifies the differential expression of two biologically distinct biologically active isoforms (MeCP2E1 and MeCP2E2) in a myelin oligodendrocytes glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS). Specifically, we confirm the effects of MeCP2E1 in the pathological repression of brain derived neurotrophic factor (BDNF). This upstream effect of MeCP2E1 on BDNF repression hinders optimal re-myelination and/or myelin repair process resulting in myelin damage with sustained neurological disability. Conversely, our results also suggest that the MeCP2E2 isoform may be involved in the activation of the BDNF gene. However, the pathogenic effects of MeCP2E1 appear to outweigh the benefits of MeCP2E2 resulting in EAE-induced myelin damage. The MeCP2E1/MeCP2E2 isoform ratio is an important indicator of disease activity.

4.5. Figures

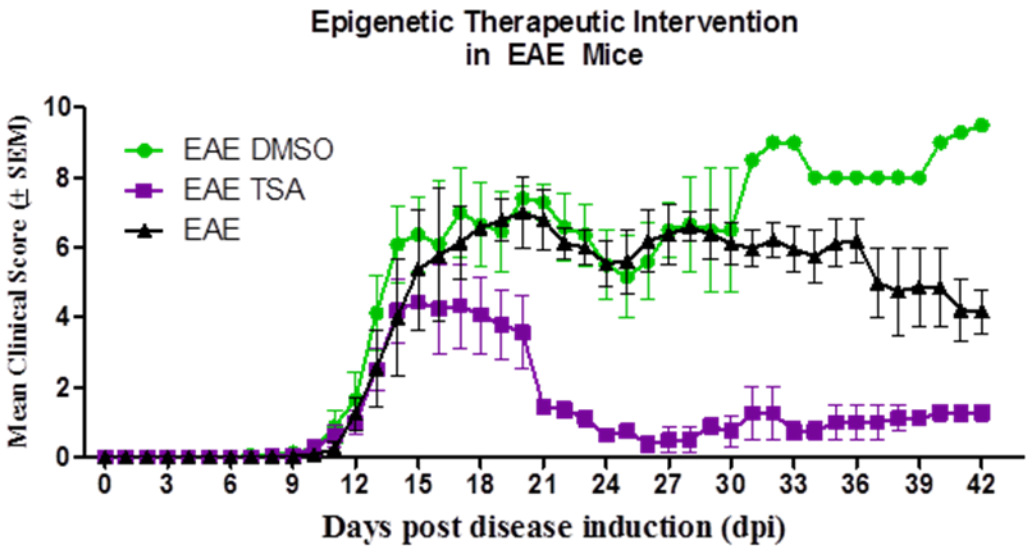

Figure 1: Effect of trichostatin A (TSA) on clinical course of neurological disability in EAE mice: In vivo treatment of mice with TSA (7.5 mg/kg/day i.p.) on days 4–42 post-disease induction (dpi) resulted in a significant improvement in EAE-induced NDS measures during the chronic relapsing phase. EAE + DMSO group are EAE mice treated with dimethyl sulfoxide (DMSO) vehicle. EAE+ TSA are EAE mice treated with TSA.

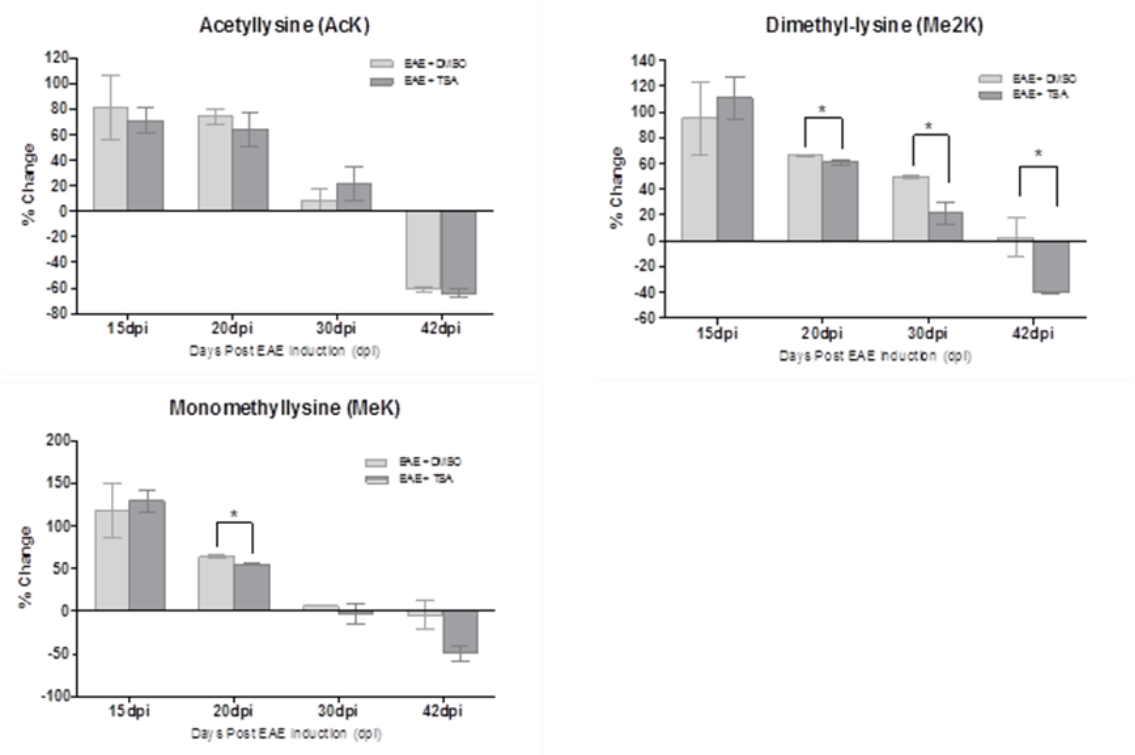

Figure 2: Epigenetic modifications of histones in SC of EAE animals: The epigenetic modifications are derived from LC-MS/MS quantification of total enzymatic hydrolysis of histones. Displayed are the concentrations of the modifications normalized to total SC histone protein as a ratio of EAE disease model normalized to naive control. There is significant decrease in monomethyl lysine following trichostatin A (TSA) treatment at day 20 dpi. Also, significant decrease is observed for dimethyl lysine at 20, 30 and 42 dpi. There is not any significant difference between TSA treated group and vehicle treated animals in lysine acetylation. EAE + DMSO group are EAE mice treated with dimethyl sulfoxide (DMSO) vehicle. EAE+ TSA are EAE mice treated with trichostatin A (TSA).

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