Published in final edited form as:
Trends Mol Med. 2017 June ; 23(6): 546–562. doi:10.1016/j.molmed.2017.04.004.

Emerging Role for Methylation in Multiple Sclerosis: Beyond DNA

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Abstract

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system. The inflammatory and neurodegenerative pathways driving MS are modulated by DNA, lysine and arginine methylation, as evidenced by studies made possible by novel tools for methylation detection or loss-of-function. Here, we present evidence that MS is associated with genetic variants and metabolic changes that impact methylation. Further, we comprehensively review the current understanding of how methylation can impact CNS resilience and neuroregenerative potential, as well as inflammatory vs. regulatory T helper-cell balance. These findings are discussed in the context of therapeutic relevance for MS, with broad implications in other neurologic and immune-mediated diseases.

Keywords

methylation; multiple sclerosis

Altered DNA and Protein Methylation Pathways in MS

MS is a central nervous system (CNS) disease that affects over 2 million young adults worldwide [1]. The pathogenesis of MS involves chronic CNS inflammation and

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demyelination, thought to be driven by myelin-specific immune T lymphocytes of the **T helper (Th) cell subset (See Glossary),** although a primary neurodegenerative cause is also possible (reviewed in [2])(Box 1). Most MS patients develop symptoms between 20–40 years of age, at the prime of life, resulting in substantial socioeconomic loss, and currently, there is no cure.

Box 1

Multiple Sclerosis Pathogenesis

Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system (CNS) in which the myelin sheath, the protective coating that insulates axons, is damaged. Demyelination results in nerve conduction deficiencies that can have varied manifestations, including visual, cognitive, sensory and motor disturbances, as well as pain and fatigue. Approximately 85% of MS patients suffer from **Relapsing-Remitting MS (RRMS)**, where acute neurologic disability relapses are interspersed by remission periods without apparent symptoms. A minority of patients present with **Primary-Progressive MS (PPMS)**, where neurologic disability progresses from the onset without remission, and even RRMS patients eventually develop progressive disease, namely Secondary-Progressive MS (SPMS). The exact cause of MS is not fully known, though the current GWAS studies have pointed mostly toward immune-related genes, suggesting that the immune component of MS is essential to disease pathogenesis. In early stages of the disease, T cells can be found within demyelinated lesions of MS patient white matter and thus, it is suggested that autoreactive T cells promote immune attack against myelin. However, the exact auto-antigen in MS patients is unknown. Although lesions typically occur in the white matter during early stages of disease, and patients can mostly recover from acute relapses of disease activity, there is increased axonal degeneration as the disease progresses, resulting in permanent damage and disability. Current therapies are focused on modulating the immune component of MS, and although they are able to delay relapses in RRMS patients, most therapies are unable to stop progression of the disease and are entirely ineffective in treating progressive disease.

MS is a complex disease precipitated by the combination of genetic and environmental/lifestyle factors. A quarter of MS risk originates from known MS genetic loci, although overall MS heritability is estimated to be larger, between 36–76% [3,4]. The “missing heritability” may originate from yet-undiscovered genes, genetic interactions and **epigenetic mechanisms** that influence gene expression. Environmental factors such as low Vitamin D/sunlight exposure, smoking, obesity and Epstein-Barr virus infection also contribute to risk [5]. Of note, MS incidence and female:male ratio is increasing, and male MS is more severe, lending support to a growing role for environmental factors in MS [1,6]. Remarkably, it has been posited that environmental factors, such as smoking, synergize with MS risk loci via epigenetic mechanisms that amplify pathogenic gene expression patterns in CNS or immune cells [5]. One epigenetic modification, methylation, has received recent attention in the context of MS. Although most studies have focused on DNA methylation in MS, a critical role for protein methylation of **histones** or other proteins is now emerging. Methylation is at the core of gene expression patterns and can regulate gene expression in immune and neurologic pathways driving MS. Recent developments, including **Genome-Wide Association Studies (GWAS)** studies, animal models and improved methylation mark detection tools have translated into novel insights on the impact of methylation in MS.

In this review, we summarize recent work made possible through novel selective methylation inhibitors, as well as new knockout and methylation mark detection strategies. These studies show that methylation changes are common in MS, may stem from genetic changes or dysregulation in methylation pathway genes and metabolites, and thus may provide possible mechanisms that contribute to MS. DNA, lysine and arginine methylation may modulate MS via two arms. Our discussion describes: first, how methylation may impact CNS resilience by regulating myelin stability, antigenicity, oligodendrocyte progenitor cell (OPC) renewal and neuronal respiration. Second, how methylation may modulate inflammatory vs. beneficial Th cell balance by impacting Th1/Th2/Th17 subtypes, as well as regulatory T cell (Treg) differentiation, proliferation and cytokine secretion. These findings have wide-ranging implications for understanding MS pathogenesis and devising effective preventative or therapeutic strategies.

Methylation Reactions in Immune and Neurologic Processes

Methylation is an energy-dependent reaction whereby a methyltransferase enzyme covalently links a methyl group to a DNA or protein substrate [7]. Methylation has long been suspected to play a significant role in immune processes that drive or contribute to MS pathogenesis in mammals. *In vivo*, inhibition of all methylation reactions with methylthioadenosine (MTA) has resulted in reduced inflammation and disease severity in rat models of MS [8,9]. Pan-methylation inhibitors, such as MTA and S-adenosylhomocysteine (SAH), suppress mouse and human $CD4^+$ Th cell proliferation, Th1/Th2 cytokine production [8], and human $CD8^+$ T cell cytotoxicity [10]. Aside from immunomodulation, MTA exerts neuroprotective effects on neurons and astrocytes [11]. For example, MTA was shown to decrease *in vitro* cell death while increasing neuronal sparing following **excytotoxic** insults in brain ischemia, epilepsy and Parkinson's disease rodent models [11]. These effects could be derived from suppression of either DNA, lysine or arginine methylation. The fact that non-selective suppression of methylation suppresses disease in animal models of MS and may also bear a neuroprotective role raises the question as to which types of methylation reactions are responsible for these effects. Based on recent evidence, three reactions, namely DNA methylation, protein lysine methylation and protein arginine methylation, and their respective **writers** (Box 2, Fig. 1) might be behind those effects.

Box 2

DNA and Protein Methylation Reactions

DNA methylation is a reaction catalyzed by DNA methyltransferases (DNMT)1, DNMT3a or DNMT3b. The methyl group is added to cytosine in **CpG sites**, typically near or within a promoter sequence, yielding 5-methylcytosine (5mC) (Fig. 1). The enzymes involved in erasing DNA methylation remain controversial, although Ten Eleven Translocation (TET)1, TET2, and TET3 enzymes have been proposed as erasers [103–

105]. TETs catalyze the oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxycytosine (5caC). 5caC can be excised by base exchange repair, leading to complete demethylation of cytosine. Other proposed enzymes involved in DNA demethylation include Thymine DNA glycosylases and cytosine deaminases [104,106,107].

Protein methylation. Proteins can be methylated on lysine and arginine residues via addition of one or more methyl groups, which can take various conformations. Lysine can be mono-, di- or tri-methylated (Fig. 1) by more than 50 lysine methyltransferase enzymes (PKMTs) classified into a least two families (reviewed in [108]). Over 25 lysine demethylases (PKDMs) are currently known to reverse this process [16]. One large group of PKMTs include all methyltransferases that contain the catalytic SET (SU(var), Enhancer of Zeste and Trithorax) domain. Within the SET domain enzymes, the proteins can be divided into seven families, including SUV3/9, SET1, SET2, SMYD, EZ, SUV4/20, and RIZ. The second large group of PKMTs include all methyltransferases containing the seven β strand (7BS). Both groups can catalyze methylation of histone and non-histone substrates. Arginine can be monomethylated (MM), symmetrically dimethylated (SDM) or asymmetrically dimethylated (ADM) (Fig. 1). Arginine methylation is catalyzed by the protein arginine methyltransferase (PRMT) family. Type I PRMTs (PRMT1-4, 6, 8) catalyze ADM while Type II PRMTs (PRMT5, 9) catalyze SDM, and Type III PRMTs (PRMT7) catalyze MM of arginine [12–14]. Although arginine methylation was considered irreversible, studies now show that arginine methylation is dynamic and regulatory, analogous to phosphorylation [99,109]. However, arginine demethylases have not yet been identified. Some studies have pointed to JMJD6 as an arginine demethylase, though this has been controversial [110]. Peptidyl arginine deiminases (PADIs), which result in the citrullination of arginine residues, may also antagonize the effects of arginine methylation [111].

DNA methylation is catalyzed by three major DNA methyltransferases (DNMTs). DNMT3a/b is responsible for *de novo* methylation reactions, while DNMT1 performs maintenance DNA methylation. Protein Arginine Methyltransferases (PRMTs) 1–9 catalyze arginine methylation as monomethylation (MM), **symmetric dimethylation** (SDM) or **asymmetric dimethylation** (ADM); most cellular SDM and ADM are catalyzed by PRMT5 and PRMT1, respectively [12–14]. Finally, lysine methylation is catalyzed by a complex group of over fifty Protein Lysine Transferases (PKMT) enzymes, including Enhancer of Zeste Homolog 2 (EZH2). The enzymes, responsible for demethylation, or "**erasers**", are less clearly defined, but Ten Eleven Translocation (TET)1–3 appear to catalyze DNA demethylation via oxidation [15]. No arginine-specific demethylases are known. However, lysine demethylases (PKDMs), including lysine demethylase (LSD) 1,2 and Jumonji C (JmJc) domain-containing proteins may catalyze both lysine and arginine demethylation. For more information on these three types of methylation writers, their methylation marks, and methylation erasers, see Fig. 1 and Box 2 [16].

Methylation is a reversible epigenetic process essential for the tight regulation of cell- and tissue-specific gene expression. DNA methylation is the most extensively studied epigenetic modification across species, and is generally transcriptionally repressive, as 5-methylcytosine (5mC) can prevent DNA binding of transcription factors to their binding sequences [17]. Methylation of histone and non-histone proteins plays similar roles [17]. The best-known targets of protein methylation are histones H3, H4, H2A and H2B, proteins, which form an octamer around which DNA wraps to form nucleosomes, the basic unit of **chromatin**. Histone modifications are specifically detected and interpreted by histone modification "**readers**", ultimately modulating chromatin's transcriptional competence or incompetence. Generally, trimethylation of histone 3 lysine 4 (**H3K4Me3**) at transcriptional start sites is associated with increased transcription while **H3K27Me3** and **H3K9Me3** are linked to repression [18,19]. General guidelines for histone arginine methylation are more difficult to achieve, as the effect on expression depends on the locus in question. ADM of arginine residues on histones is typically associated with transcriptional activation, whereas SDM is associated with transcriptional repression, although this is not always the case [20]. This antagonism hints at an intricate regulation, balancing SDM and ADM. In addition to histones, other proteins can be substrates for lysine and arginine methyltransferases [20,21].

Each type of methylation mark has specific biological effects. By contrast, all methylation reactions, whether DNA, lysine or arginine methylation, are regulated similarly. For instance, all methyltransferases require the same methyl-donor group, namely **(S)-adenosylmethionine (SAM)**, which originates from methionine. Similarly, all methylation reactions are tightly regulated through negative feedback inhibition via their own enzymatic by-products **S-adenosylhomocyteine (SAH)** and homocysteine. Alterations in methionine metabolism could therefore have wide-ranging implications to methylation and biology.

Evidence of Deregulated Methionine Metabolism in MS

Methionine can be acquired from dietary sources, particularly meat, fish, eggs and dairy, or produced by the folate or betaine pathways (described in Fig. 2 and Box 3). The folate pathway requires folate, Vitamins B2/B6/B12 and active serine hydroxymethyltransferase (SHMT), methylentetrahydrofolate reductase (MTHFR) and methionine synthase (MS) to generate methionine from homocysteine. Similarly, the betaine pathway requires betaine and betaine hydroxymethyltransferase (BHMT), only found in the liver and CNS [22]. Dietary methionine impacts blood methionine and SAM levels, which in turn promote histone methylation and gene expression changes [23]. Of relevance, alterations in several metabolites of the methionine pathway have been observed in MS patients [22,24], consistent with the idea that methylation dysregulation contributes to MS pathogenesis. For example, plasma methionine was found to be reduced in MS patients while plasma SAM was unchanged in **Relapsing Remitting MS (RRMS),** and increased in progressive MS [25]. This was reported to translate into higher SAM/SAH ratios in **Primary Progressive MS (PPMS)** and Secondary Progressive MS (SPMS), and a trend towards increased ratios in RRMS patients [25]. Higher plasma or serum homocysteine levels in MS patients have also been frequently reported [24,26–29], although other studies have found no differences [30–32]. Homocysteine is a by-product of methylation that may accumulate as a consequence of deficient folate or betaine pathways. Impaired folate pathway enzyme activity or deficiency affecting essential enzyme substrates or cofactors, such as folate or vitamin B12, could result in homocysteine accumulation and deficient methionine/SAM regeneration (Fig. 2).

Since all methylation reactions depend on methyl donor SAM, this is expected to result in deficient methylation reactions. In the CNS, deficient methylation may negatively impact myelin formation or stability and neurologic function, since methylation of myelin has been proposed to increase its stability [33,34]. Indeed, human deficiency in folate/B12 has been linked to transient neurological impairments, such as **peripheral neuropathies** [35]. However, the cause for hyperhomocysteinimia in MS is so far, unclear. Although a couple of studies reported decreases [24,28], most studies have found normal folate and B12 levels in MS patients [24,26,27,30]. Increased homocysteine could stem from reduced activity of enzymes in the folate, betaine or cystathionine pathways. Finally, mutations rendering methyltransferases resistant to inhibition by SAH/homocysteine could in theory, result in unrestrained methylation and homocysteine. Regardless of cause, homocysteine drives T cell proliferation and inflammatory cytokine production [36], raising the possibility that high homocysteine promotes pathogenic inflammatory T cells that drive and perpetuate MS. Therefore, restoring normal homocysteine levels may be beneficial in MS.

Box 3

Folate and Methionine Metabolism Pathways

All methylation reactions require a methyl donor group, namely **(S)-adenosylmethionine (SAM)**. After donating its methyl group to a substrate, SAM is converted into S-Adenosyl Homocysteine (SAH). SAH possesses strong methyltransferase inhibitor activity that negative regulates methylation. SAH hydrolases relieve SAH-mediated inhibition by breaking SAH into homocysteine and adenosine. **Homocysteine** can be degraded via the cystathionine beta synthase pathway or recycled into SAM via the folate or betaine pathways to support methylation reactions (Fig. 2). The strong inhibitory activity of SAH and versatility of pathways to regenerate or degrade the methyl donor SAM highlight the importance of tight control of methylation reactions for appropriate cellular function. Dietary methionine from meats, fish, eggs and dairy provides an alternative source. In the folate pathway, tetrahydrofolate (THF) acts as a methyl carrier in various steps leading to generation of methionine, whereby THF is converted into 5,10-tetrahydrofolate and 5-methyl tetrahydrofolate (5-MTHF) in consecutive steps catalyzed by serine hydroxymethyltransferase (SHMT1)/Vitamin B6 and methyltetrahydrofolate reductase (MTHFR)/Vitamin B2, respectively. Reduced folate carrier 1 (SLC19A1) is the gatekeeper for folate, regulating intracellular folate concentration. 5-MTHF then transfers its methyl group to homocystine in a reaction catalyzed by methionine synthase that requires Vitamin B12, effectively recycling methionine. This pathway is dependent on the appropriate dietary intake of folate, Vitamin B2, B6 and B12. Besides the folate pathway, an alternative pathway catalyzed by betaine homocysteine methyltransferase allows recycling of homocysteine to methionine when betaine is available. An alternative fate for homocysteine is degradation into cystathionine via cystathionine beta-synthase (CBS) activity. Alterations in several metabolites in the methionine pathway have been observed in MS patients (see Fig 2).

In contrast to serum/plasma, homocysteine is not significantly changed in tested MS patients' CNS tissues, including **cerebrospinal fluid (CSF)** or **normal appearing gray**

matter (NAGM) [22,27]. The difference between the blood and CNS compartments could indicate tighter methionine metabolism regulation in the CNS. However, reductions in the universal methyl donor SAM and its precursor betaine have been observed in MS patients' NAGM [22]. As a consequence, reduced histone methylation and reduced mitochondrial respiration were observed, which were thought to be mediated by transcriptional control of mitochondrial respiration [22].

Collectively, alterations in methionine metabolites are commonly observed in MS, although they vary depending on the individual, population, metabolite and tissue compartment tested. These changes, particularly increased peripheral homocysteine levels, may enhance immune activity, while low CNS SAM may reduce resilience to damage. While direct causal relationships remain so far, elusive, the connection between a broad range of methionine/methylation pathway metabolites and MS risk suggests that methylation is an important player in MS.

Genetic Evidence Supporting Altered Methylation Pathways in MS

MS is a complex disease determined by the interplay of genetic, environmental and stochastic factors. Extensive genome-wide association studies (GWAS) have explored the link between small changes found in human nucleotide sequences, namely **Single Nucleotide Polymorphisms (SNPs)**, and MS risk. The first and by far most robust MS contributor revealed by GWAS is the HLA locus that encodes antigen presenting molecules [37], with *HLA-DRB1*1501* its most reproduced allele [38–40].

Outside of the Major Histocompatibility Complex (MHC), more than 200 polymorphisms contributing to human MS have been identified [37,38,40–42]. Among these variants, many involve methionine metabolism or methylation pathway genes (Table 1, Fig. 2). For instance, the A allele for the reduced folate carrier 1 gene (*SLC19A1*, c.80G>A) correlated with delayed MS onset age [43]. This allele results in reduced folate uptake and, presumably, methylation. In contrast, the cystathionine beta-synthase (*CBS)* c. 844_855ins68bp insertion that reportedly enhances its own expression and lowers homocysteine levels, was linked to younger age at MS onset [43]. Although the effects on MS onset are opposite, both changes are expected to reduce folate pathway activity and methylation potential. Further insight on the direct effects of these changes on methylation or immune vs. CNS compartments will be key. The wild-type A allele (c.1298A>C polymorphism) in the human *MTHFR* gene that generates methionine from folate and homocysteine (Box 3, Fig. 2) is associated with protection from MS [44,45]. Protection may stem from normal methylation and improved myelination in the CNS, although the same allele is linked to reduced DNA methylation in healthy human whole blood [46]. This raises the question of whether this allele can have different consequences in two tissue compartments and, if so, how. The low MTHFR activity c.677C>T allele [47] was associated with MS in Iranians [48], but this finding was not reproduced in German and Tunisian populations [44,45]. Recently, a SNP linked to another folate pathway enzyme, *SHMT1*, was identified as a risk factor for MS in a German population GWAS study and validated in a second Sardinian cohort [39]. This allele is in **linkage disequilibrium (LD)** with *HLA-DRB1*1501* and correlated with suppression of *SHMT1* expression by increasing

DNA methylation at the SNP's **CpG sites** [39]. Overall, these data are more consistent with MS being promoted by alterations in the regulation of methylation rather than by global increases or decreases in methylation. Therefore, gaining insight into the impact of these modifications on specific inflammatory and myelination pathways will be essential in appropriately devising targeted therapies.

Finally, other genes associated with methylation readers or erasers have been linked to MS. For example, one of the 48 new human MS variants identified by the International Multiple Sclerosis Genetics Consortium (IMSGC) in 2013 [40] is *TET2*, which encodes for an enzyme involved in DNA demethylation. Ten-Eleven Translocation 2 (TET2) catalyzes the oxidation of 5-methylcytosine (5mC) in DNA **CpG islands** to 5-hydroxymethylcytosine (5hmC) [17]. 5hmC is considered an intermediate in the process of CpG demethylation [49] but may play other roles as an epigenetic mark, particularly in brain tissue that shows the highest level of this DNA modification [50,51]. Human GWAS studies have recently revealed additional MS risk loci linked to methylation genes, namely i) *L3MBTL3*, a methylated lysine reader, ii) *MAZ*, a factor that regulates *MYC* expression and iii) *ERG*, which interacts with the histone methyltransferase ESET [39,42]. However, to what capacity these variants impact MS remains to be determined and requires additional functional studies.

The identification of polymorphisms in various methylation pathway enzymes strengthens the notion that methylation plays an important role in MS. However, it is essential to determine the consequences of each of these polymorphisms on activity and regulation of methionine/methylation pathways and immune and neural pathways driving MS neurodegeneration.

Epigenetic Evidence of Altered DNA methylation in CNS and Peripheral Blood

CNS

Several studies have analyzed the DNA **methylome** in the CNS of MS patients. Initially, immunoblotting showed decreases in global DNA methylation in **normal appearing white matter (NAWM)** of MS patients vs. controls [52]. However, these global differences were not reproduced with the more sensitive **Illumina 450K methylation assay**, which revealed finer differences, both increases and decreases, at specific loci [53]. The nature and function of differentially methylated sites in MS CNS is just starting to be uncovered. Oligodendrocyte-specific, neuroregenerative *MBP, SOX8, BCL2L2* and *NDRG1* gene loci were found to be hypermethylated, negatively correlating with protein expression, in MS patient NAWM [53]. This was accompanied by hypomethylation and increased expression of proteolytic genes *LGMN* and *CTSZ,* which could promote microglial antigen presentation [53]. Further supporting a role for DNA methylation in oligodendrocytes, it was reported that murine oligodendrocyte-specific ablation of *Dnmt1*, but not *Dnmt3a*, caused a hypo-myelinated phenotype due to an impairment in **oligodendrocyte progenitor cell (OPC)** proliferation and differentiation [54].

Peripheral Blood

DNA methylation changes have also been observed in MS peripheral blood. An early immunoblotting study showed a slight increase in 5mC DNA methylation in MS patient peripheral blood mononuclear cells (PBMCs), suggesting increased global methylation [55]. These changes were accompanied by reductions in the 5mc derivative 5hmc, as well as by the TET2 enzyme that catalyzes that conversion. TET1 and TET3 enzymes remained stable, consistent with a non-redundant function of TET2 in the CNS [55]. Remarkably, the recent identification of MS-risk SNPs in the vicinity of *TET2* raises the question of whether the observed changes in TET2 expression and methylation marks are causally linked to this SNP [40]. Recently, more sensitive genome-wide DNA methylation profiling revealed that, while MS $CD8^+$ T cells showed increased global methylation, MS and healthy control $CD4^+$ T cells had similar global methylation [56]. Rather, a mix of differentially hypomethylated and hypermethylated loci in isolated $CD4^+$ T cells of MS patients has been identified [57]. The promoter of protein tyrosine phosphatase *SHP1*, a well-known negative regulator of inflammation, was shown to be hypermethylated in MS patient peripheral blood leukocytes [58], correlating with reduced *SHP1* expression [59]. These locus-dependent methylation changes provide a methylation signature that likely modulates inflammatory activation/ phenotype and may have diagnostic potential. Another current study compared individual methylation sites in PBMCs from patients with RRMS, PPMS, and healthy controls; more hypermethylated sites were detected in PPMS than in RRMS patients or controls, while increased hypomethylated sites were observed in RRMS patients relative to healthy controls [60]. These data imply that methylation plays a role in both RRMS and PPMS pathogenesis, albeit through different mechanisms and gene sets. The increased deregulation of DNA methylation in PPMS suggests that DNA demethylating agents may potentially prove to be useful in this MS subtype that does not respond well to most available therapies.

An interesting recent development is that the locus responsible for most of MS risk, i.e., *HLA-DRB1,* is now linked to methylation; eight tightly clustered sites of hypomethylation in the *HLA-DRB1* locus of MS patients' $CD4^+$ T cells were associated with *HLA-DRB1*1501* [57], raising the possibility that methylation itself is driving MS risk. Although increased HLA loci hypomethylation could be secondary to inflammation and enhanced T cell responses in MS, hypomethylation could also be observed in healthy controls carrying the *DRB1*1501* allele [57]. Strengthening the notion that hypomethylation in HLA loci might not be limited to MS patients, one study identified and validated one long-range and several local **methylation quantitative trait loci (meQTL)** controlling HLA methylation and gene expression in four large cohorts of healthy individual [61]. Remarkably, the minor allele of the long-range meQTL was in linkage disequilibrium with the HLA locus SNPs tagging *DRB1*1501*. The meQTL was associated with higher *HLA-DRB5* and *HLA-DRB1* expression, reduced *HLA-DQB1* expression and reduced white matter **Fractional Anisotropy**, a measure of myelin fiber organization and content [61]. Another study had similarly observed increased *HLA-DRB1* expression in *DRB1*1501* healthy carriers [62]. Interestingly, these observations were made in healthy individuals carrying the MS risk loci, suggesting that increased antigen presentation and reduced myelination may be early events leading to MS [61].

Therapeutic Implications

Studies show that prophylactic and therapeutic treatment of mice with the DNA hypomethylating agent, decitabine (5′-aza-2′-deoxycytidine, DAC) suppressed disease severity in **experimental autoimmune encephalomyelitis (EAE)** [63,64]. DAC reduced CNS inflammatory cytokines and lymphocyte infiltration while increasing anti-inflammatory cytokines. DAC's therapeutic effects are attributed to observable increases in Tregs, mediated by Foxp3 induction, paired with reduced numbers of CNS infiltrating lymphocytes. While these results are promising, DAC has also been shown to increase the expression of antigen-presenting and costimulatory molecules MHC I, CD80, CD86 and CD40 in human chronic lymphocytic leukemia cells and other cell lines [65,66]. Therefore, DAC may have opposing immunoregulatory and immunogenic effects. Indeed, increased myelin-specific **Th cell proliferation** was observed in DAC-treated EAE mice [63], which could perpetuate or enhance CNS immune attack. Additionally, an early study showed that rats treated with DNA methylation inhibitor 5-azacitidine (5-AZA) presented reduced myelin levels and altered action potential formation in the optic nerve [67]. Thus, DNA methyltransferase inhibitors appear to have opposing immune effects and potential neurotoxicity, likely stemming from de-repression of both regulatory and inflammatory gene pathways.

Overall, various studies on DNA methylation in MS patients do not support major global decreases or increases in methylation in MS patients but rather, differences at specific loci. Loci methylation changes appear to promote antigen presentation and T cell autoimmune responses while simultaneously reducing neuroregeneration potential. Consequently, these findings indicate that further mechanistic insight needs to be obtained to better understand the role of DNA methylation in MS, and furthermore, caution should be placed when designing autoimmune disease therapeutic approaches that target DNA methylation.

Protein Lysine Methylation: Potential Role in MS

CNS

Lysine methylation (Box 2) appears to play a dual role in both oligodendrocytes and neurons in the CNS. It is known that OPC differentiation into mature oligodendrocytes is associated with histone deacetylation and chromatin compaction [68,69]. Recently, H3K9me3 and H3K27me3 and their respective writers, *EHMT2* (also known as G9a) and *EZH2,* have been reported to be upregulated during murine and human OPC differentiation, contributing to increased chromatin compaction [69,70]. However, ablation of H3K9 methyltransferases (*Ehmt2* and *Suv39h1*), but not H3K27 methyltransferases (*Ezh1/2*), impaired murine OPC differentiation *in vitro*[71], suggesting that H3K9me3 might be essential for proper OPC function and myelination. Furthermore, reports of reduced H3K4me3 in MS NAGM neurons implicate lysine methylation in neuronal integrity, as evidenced by the correlation of reduced H3K4me3 with reduced betaine and mitochondrial respiration in MS patient brain tissue[22,72]. Therefore, non-lesional MS tissue may be at heightened risk of axonal damage [22] due to the inflammatory oxidative conditions typical of MS lesions. It appears that lysine methylation may be of critical importance in the CNS.

Peripheral Blood

Many studies have explored the role of lysine methylation in modulating the differentiation and function of Th cells, which may drive MS. Global **chromatin-immunoprecipitation (ChIP)-sequencing** of lysine methylation marks has shown reduced H3K4me3 and H3K27me3 enrichment at lineage-specific loci in mouse **Th1, Th2, Th17 and inducible Treg cells** compared to naïve or natural Treg cells [73]. Other lysine marks, H3K9me2 and H3K9me3, have been implicated in the maintenance of Th lineage integrity[74,75]. The lysine methyltransferase Ezh2 modulates mouse Th cell differentiation and plasticity [76–81]. However, initial reports on the consequences of Ezh2 deficiency in model systems have been contradictory; two murine studies observed that *Ezh2* deficiency selectively suppressed Th1 cell cytokine production *in vitro*, which correlated with protection from aplastic anemia or graft vs. host disease *in vivo* [77,78]. However, another study in *Ezh2*-deficient mice reported enhanced Th1 and Th2 populations in a model of ovalbumin-induced allergic asthma [76]. These controversial results might be explained by murine Ezh2 playing multiple opposing roles, including promoting T cell effector survival and function while suppressing effector Th1, Th2 and Th17 cell differentiation [77,78,80,81]. For example, another study found that Ezh2 deficiency increased Th1/Th2/Th17 cell death resulting in inefficient bacterial clearance in *Listeria monocytogenes* infected mice, even though increased Th1, Th2 and Th17 differentiation was noted *in vitro* [81]. Similarly, *Ezh2* was required for effector T cell-mediated control of *Toxoplasma sp. in vivo* infection, in mice [80]. By contrast, Ezh2 may promote Treg differentiation and function. Indeed, Treg-specific *Ezh2* deficiency in mice resulted in Foxp3-dependent transcriptional program instability and Treg loss, leading to spontaneous development of autoimmunity *in vivo* and interfered with resolution of EAE [79]. In agreement, *Ezh2* deficiency in $CD4^+$ T cells impaired the ability of Tregs to suppress autoimmunity in EAE and colitis mouse models [79,80]. *Ezh2* deficiency also debilitated neutrophil and dendritic cell adhesion and migration due to deficient methylation of the cytoskeletal protein talin, which has been implicated in reducing EAE disease progression in mice [82,83]. From another angle, the lysine demethylase Jmjd3, which specifically demethylates H3K27, has also been shown to play an important role in murine Th cell differentiation, although current results are conflicting. For instance, one report indicated that Jmjd3 can specifically promote Th17 differentiation *in vitro* and *in vivo*, and Th cell-specific *Jmjd3*-deficient mice are highly resistant to EAE development [84]. However, another study showed that murine T-cell specific *Jmjd3* ablation promoted murine Th2 and Th17 cell differentiation in the intestine and colon, while suppressing Th1 cell differentiation *in vitro*, as well as in a Th1-induced adoptive transfer colitis mouse model [85].

Overall, lysine methylation is significantly involved in control of differentiation and plasticity of Th phenotypes, some of which are known to drive MS, although a direct demonstration of their involvement in MS is lacking. Variable results among studies highlight the possibility that different lysine methyltransferases on T cell differentiation may depend on chromatin status and context, under diseased vs healthy conditions.

Therapeutic Implications

Therapeutic restoration of lysine methylation may be beneficial to restore oligodendrocyte function and/or neuron integrity in MS. For example, treatment with the antihistamine clemastine, which induced high levels of H3K9me3 in oligodendrocytes, restored myelin thickness and structure in socially isolated mice due to enhanced OPC differentiation [86]. Moreover, betaine treatment during oxidative stress restored H3K4me3 levels and improved mitochondrial respiration in human neurons [22], a response that could potentially prevent susceptibility to axonal degeneration in MS. A potent inhibitor of H3K27 demethylases, GSK-J4, ameliorated EAE disease in mice, which could be attributed to a tolerogenic DC phenotype, but likely affects other cell types [87]. Although the observation that *Jmjd3* deficiency can suppresses Th17 responses and EAE is in agreement [84], other studies suggest however, that *Jmjd3* deficient mice in a colitis model have enhanced Th2 and Th17 responses in the small intestine and colon, and suppressed Th1 responses in the small intestine and spleen [85]. Therefore, the data so far do not conclusively support considering EZH2 or JMJD3 as targets for therapeutic treatment in autoimmunity. Nevertheless, small molecule inhibitors might provide invaluable insight on their therapeutic potential in MS and autoimmunity.

Protein Arginine Methylation in MS

CNS

Links between arginine methylation (Box 2) and MS have long been suspected due to the importance of arginine methylation in both CNS and immunity. Some level of arginine methylation is likely required for normal CNS activity, since both MM and di-methylation of arginine, including SDM on **myelin basic protein** [88] can be observed in normal CNS tissue. The main methyltransferase responsible for SDM is Protein Arginine Methyltransferase 5 (PRMT5), an epigenetic modifier enzyme. PRMT5 promotes stem cell renewal and is essential during ontogeny, including a requirement for PRMT5 in neuronal stem cells for murine brain development [89]. Additionally, severe hypomyelination is observed in CNS-specific *Prmt1*-deficient mice [90], and PRMT5 deficiency in oligodendrocyte progenitor cells (OPCs) leads to upregulated expression of Inhibitors of Differentiation *Id2* and *Id4*, as well as an immature gene expression profile, suggesting PRMT5 is required for proper OPC differentiation into mature oligodendrocytes [91]. Although complete PRMT5 deficiency is incompatible with brain development, its requirements in the adult brain are less well defined and excess SDM is associated with brain pathology. For instance, myelin SDM is increased in MS patients' brains [33], with unclear consequences. Arginine methylation has been proposed to increase the stability of myelin [34], and so, the increase observed in MS [33] could be the result of a neuroprotective process. Conversely, myelin modifications could increase myelin's autoantigenic potential [92]. Therefore, the precise effects of methylation on myelin's stability or autoantigenicity, as well as how those are modulated by various modalities of arginine methylation, need to be further elucidated.

Peripheral Blood

A role for protein arginine methylation in T cell responses has long been surmised [8]. Early studies showed increased dimethylarginine, and arginine dimethylation of an important T cell receptor signaling protein Vav1 after CD28 co-stimulation of human and mouse Th cells [93]. Moreover, PRMT1 has been linked to mouse Th1 and Th2 cell cytokine production and proliferation after non-specific stimulation [94] while PRMT5 has been shown to promote IL-2 production in Jurkat T cells [95]. After several years of low activity in this field, the interest in arginine methylation in immune processes has reemerged due to technological advancements and the development of several small molecule inhibitors including PRMT5 inhibitors BLL1, EPZ015666 and HLCL65 [96–98]. A proteomic study in primary human $CD4^+$ T cells recently demonstrated that arginine methylation was poised to play key regulatory roles in T cell activation and differentiation [99]. Indeed, arginine methylation of Runx1 by Prmt1 has been found to be essential for mouse $CD4^+$ T cell maintenance in the periphery [100]. Additionally, our laboratory recently reported that PRMT5 is up-regulated during mouse and human memory Th cell activation and can drive myelin-specific memory Th1 and Th2 cell proliferation [98], the process that expands autoimmune T cells in MS. *In vitro* treatment with novel PRMT5-selective inhibitors suppressed inflammatory memory Th1 cell proliferation more potently than regulatory Th2 cell proliferation [98]. *In vivo*, pathogenic Th1 and Th17 cell responses were also suppressed in the CNS and peripheral tissues, while Treg responses were maintained [98]. Taken together, the increased resistance of Th2 and Treg cells to PRMT5 inhibitors may provide an opportunity to restore the balance between inflammatory and 'beneficial' T cell populations in MS.

Therapeutic Implications

Therapeutic effects of pan-methyltransferase inhibitor MTA in T cell responses and EAE have been proposed to stem from arginine methylation inhibition [8,9]. Indeed, similar effects on $CD4^+$ Th1/Th2 cell cytokine production or proliferation have been observed with pan-arginine methylation [94,101] or PRMT5 inhibitors [98], respectively. Interestingly, *in vivo* PRMT5 inhibitor treatment effectively stopped murine EAE progression and reduced acquired disability [98]. EAE suppression was accompanied by a reduction in myelin-specific T cell proliferation and pro-inflammatory Th1 and Th17 responses, as evidenced by reduced tritiated thymidine incorporation and frequency of IFN-γ^+, ROR-γ t^+IL-17^+ and ROR-γt^+T-bet^+ Th cells [98]. This raises the possibility that PRMT5 inhibitors might potentially be beneficial in MS, as they suppress Th1 and Th17 populations that drive MS. Although evidence of enhanced PRMT5 activity in MS patients is lacking, MS risk has been linked to SNPs in the PRMT5 driver *MYC* [41,102]. Beyond PRMT5, the role of individual PRMT family members in EAE and MS is vastly unexplored.

Thus, arginine methylation appears to play important roles in inflammatory immune responses that are implicated in MS pathogenesis and/or progression, with PRMT5, possibly playing a prominent role. With the impressive development of genetic models and selective inhibitors that target individual PRMT family members [96], further understanding on how arginine methylation regulates T cell responses as well as its impact on CNS neurodegenerative/reparative pathways should be forthcoming.

Concluding Remarks

The advent of improved methylation detection, new selective arginine and lysine methyltransferase inhibitors and methyltransferase animal knockout models have placed methylation back into the spotlight. GWAS studies have identified novel loci associated with methylation pathway genes or methylation QTL, with the intriguing observation that the strongest MS risk locus determines MHC II promoter methylation status. A combination of hyper and hypomethylated DNA loci can be observed in MS brains and T cells, which may lead to hyperactive T cells, enhanced CNS antigen presentation and reduced regenerative potential. Decreased lysine methylation and neuronal respiration is observed in CNS brains and may also impact the ability of OPCs to repair damage while lysine methylation exerts multiple controls over Th1/Th2/Th17/Treg differentiation. Finally, arginine methylation of myelin is increased in MS brains and PRMT1 and PRMT5 have been implicated in the expansion and development of Th1/Th2/Th17 cell responses. While these findings (Fig. 3) settle the importance of methylation in MS, many critical questions remain (Outstanding Questions Box, Box 4). The key to therapeutically harnessing methylation in MS will be to dissect the pathways by which environmental, genetic or metabolic risk factors modify methylation. A better understanding of how *HLA DRB1*1501* allele-determined methylation changes impact inflammatory T cell and neurodegenerative pathways in MS will also be important. Similarly, identifying key driver methylated loci/protein targets and which cells or tissue compartments they impact should help devise more targeted strategies and reduce side effects. The speed with which novel more potent and selective epigenetic modifier drugs and conditional animal models are being developed promises exciting discoveries ahead.

Box 4

Clinician's Corner

- A surge of new evidence strongly suggests a role for methylation reactions in MS disease development and pathogenesis.
- MS-associated SNPs and alterations of metabolites in the methylation pathway are apparent in MS patients. Further work is required to determine if these alterations have the power to predict MS disease development.
- Early studies have shown that inhibition of all methylation reactions can suppress disease severity in EAE, a mouse model of MS. Recent work has also indicated the EAE therapeutic benefit of selectively targeting methyltransferases.
- Methylation and methylation metabolites likely play various roles and are regulated differently in the peripheral blood and in the central nervous system. Understanding these nuances is essential to therapeutically targeting methyltransferases in MS.

Outstanding Questions Box

- What are the qualitative effects of methylation pathway SNPs or metabolite changes on methylation, gene expression and cell phenotype?
- Do effects of SNPs and metabolites on methylation, gene expression and cell phenotype differ in CNS vs. peripheral immune compartments?
- What is the impact of diet and other environmental factors on CNS/immune cell methylation and function?
- What is the functional impact of the *HLA-DRB1*1501* meQTL on thymic tolerance, Treg development, antigen presentation and effector T cell responses?
- Are there functional impacts of the *HLA-DRB1*1501* meQTL on CNS development or susceptibility to insults?
- What are the specific protein arginine and lysine methylome marks that are altered in MS patient T cells?
- Which methyltransferases are responsible for the EAE therapeutic effects of non-selective methylation inhibitors?
- What are the effects of available selective DNA, lysine or arginine methyltransferase inhibitors on Th cell differentiation and CNS repair?

Acknowledgments

L.M.W. is supported by NIH T32 rGM068412 and M.G-de-A. is supported by NIH NIAID R01AI121405 (M.G-de-A) and NIH NIAID 1R21AI127354 (M.G-de-A). We thank Dr. Stephanie A. Amici for insightful discussion.

Glossary

Asymmetric dimethylation (ADM)
addition of two methyl groups to an arginine residue in an asymmetric manner

Cerebrospinal fluid (CSF)
clear, colorless fluid found in the brain and spine that serves as a cushion for the brain’s cortex

Chromatin
Compact structure in the nucleus of a cell containing DNA wrapped around histone proteins

Chromatin Immunoprecipitation (ChIP)-sequencing
experimental method of investigating protein interactions with DNA

CpG island
a large region of DNA containing a high frequency of CpG sites

CpG sites

regions of DNA containing a CG dinucleotide sequence. Such sequences can generally be methylated

Demyelination

damage to the myelin sheath, the protective covering that surrounds neurons in the brain and spinal cord

Epigenetic eraser

enzyme that can remove a specific epigenetic mark upon recruitment

Epigenetic modifications

potentially heritable changes in gene expression not due to a change in DNA sequence. Instead, these regulate gene expression by altering chromatin structure and DNA accessibility

Epigenetic reader

proteins that are recruited to and recognize specific epigenetic marks, ultimately affecting chromatin competence

Epigenetic writer

enzyme that catalyzes the addition of modifications to histones or DNA

Excitotoxic

causes neuron damage and cell death due to overactivation of receptors for excitatory neurotransmitters, such as glutamate.

Experimental autoimmune encephalomyelitis (EAE)

mouse model of MS driven by inflammatory Th1 and Th17 cells, recapitulating many features of human MS

White matter Fractional Anisotropy (FA)

a measure used in neuroimaging to reflect fiber density, axonal diameter, and myelination of white matter.

Genome-wide association studies (GWAS)

analysis of genetic variants within the entire genome to identify variants associated with a disease

Homocysteine (Hcy)

non-protein coding homolog of the amino acid cysteine, also a degradation product of SAH

Histones

basic proteins around which DNA is wrapped to form chromatin

H3K4me3

trimethylation of histone H3 lysine 4; mark of transcriptional activation

H3K9me3

trimethylation of histone H3 lysine 9; mark of transcriptional repression

H3K27me3

trimethylation of histone H3 lysine 7; mark of transcriptional repression

Illumina 450K methylation assay

array that probes for more than 485,000 methylation sites, covering 96% of CpG islands, at a single nucleotide resolution

Linkage disequilibrium (LD)

non-random association of alleles at different genomic loci, indicative that alleles are likely co-inherited

Methylation quantitative trail loci (meQTL)

polymorphic loci, or SNPs, that can influence the level of DNA methylation at nearby (short-range) or distant (long-range) CpG sites

Methylome

set of methylation modifications in the genome

Myelin basic protein (MBP)

important for the myelination of axons; a typical antigen in EAE

Normal appearing gray matter (NAGM)/Normal appearing white matter (NAWM)

MS lesion-free regions within the brain. The white matter is rich is myelinated axons and demyelinated regions in MS

Oligodendrocyte progenitor cell (OPC)

precursor to myelinating oligodendrocyte, a subtype of glial cell in the CNS that supports axons and forms the myelin sheath

Peripheral neuropathy

a result of damage to peripheral nerves that often results in pain and numbness

Primary progressive MS (PPMS)

rare form of MS, diagnosed in 10% of MS patients, characterized by continuous worsening of disability

Relapsing-remitting MS (RRMS)

form of MS, diagnosed in 85% of MS patients, characterized by acute periods of disease activity followed by periods of remission

S-adenosylmethionine (SAM)

or AdoMet; methyl donor used in methylation reactions

S-adenosylhomocysteine (SAH)

or AdoHcy; product of a methylation reaction in which a methyltransferase transfers a methyl group from SAM to its substrate

Severe Combined Immunodeficiency (SCID)

caused by various genetic defects, in which there is a complete lack of T and B cells

Single nucleotide polymorphism (SNP)

single nucleotide variation at specific genome locations that normally occur in human populations

Symmetric dimethylation (SDM)

addition of two methyl groups in a symmetric manner to an arginine residue

T cell proliferation

process of T cell division and expansion to form a T cell population of shared specificity triggered by recognition of cognate antigen

T helper (Th) cells

subset of T cells, characterized by $CD4^+$ expression, that orchestrate the activation of other immune cells. Subtypes of Th cells include **Th1** (produce IFN-γ; drive inflammatory responses in infection resolution and autoimmunity), **Th2** (produce IL-4, drive anti-helminth and allergic reactions), **Th17** (produce IL-17: drive anti-fungal immunity and autoimmunity), **Treg** (important for negative regulation of immune responses)

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Trends Box

- Novel MS-risk SNPs are linked to methylation pathway genes (e.g. *SHMT1, L3MBTL3, TET2, SLC19A1, MTHFR* and *CBS*)
- The most robust and reproduced MS risk allele, *HLA-DRB1*1501*, is linked to a meQTL that controls DNA methylation and gene expression
- Improved tools allow for sensitive detection of methylation marks, as well as knockout models and selective inhibition of DNA, arginine and lysine methyltransferases
- Specific hypomethylated and hypermethylated loci favor antigen presentation and T cell activation while reducing OPC renewal and myelination
- Reduced lysine methylation in MS CNS has been associated with decreased neuronal mitochondrial respiration and resilience
- Arginine methylation enzymes PRMT1 and PRMT5 modulate Th cell balance and myelination

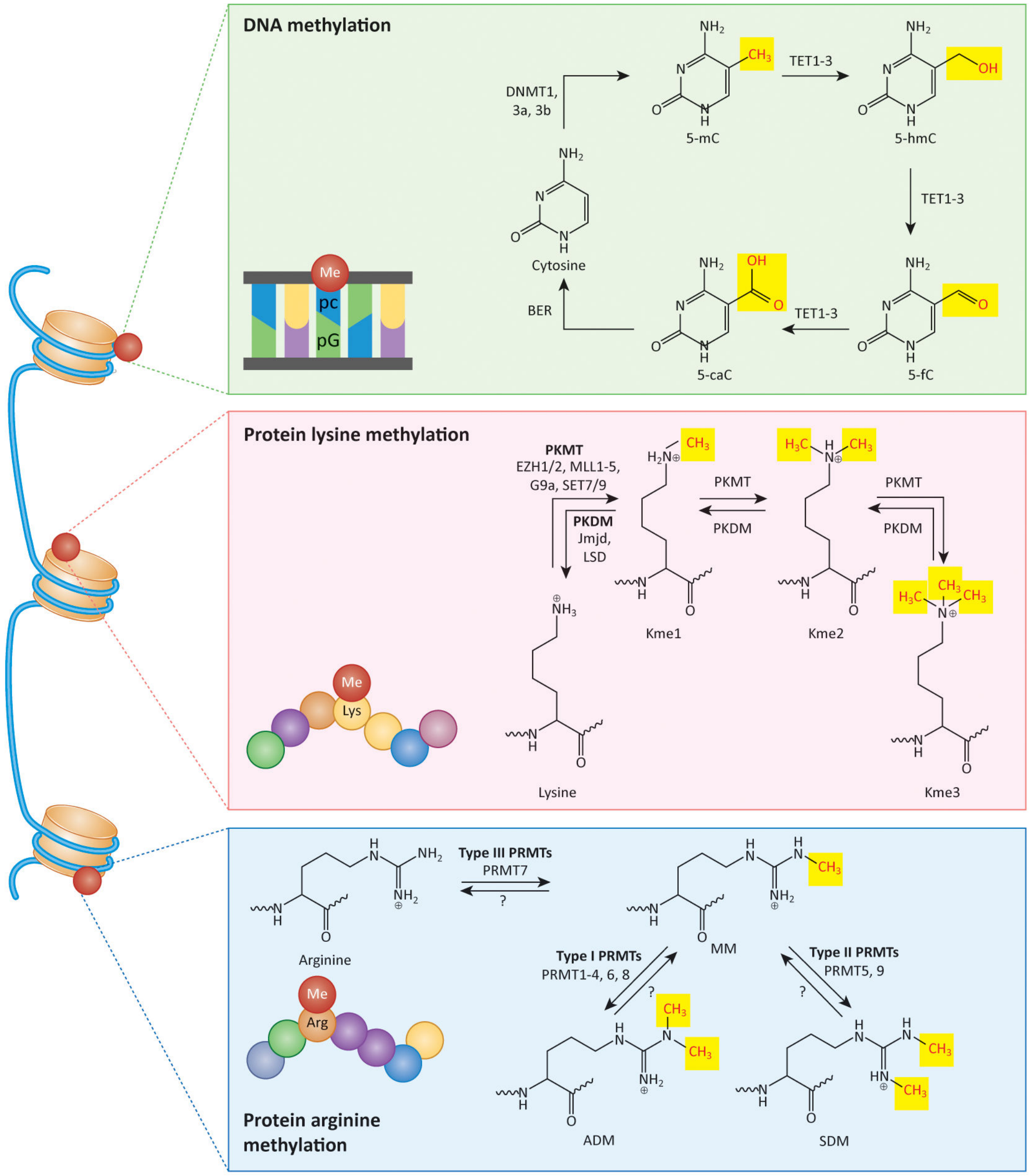

Figure 1. Biological Methylation Reactions

Methylation is the transfer of a methyl group (-CH_3) onto a substrate. The most frequent biological methylation reactions occur on DNA and lysine or arginine residues of proteins. In DNA methylation, cytosine at a CpG site is methylated by a DNA methyltransferase (DNMT) to form 5-methylcytosine (5-mC). DNMT3a/b catalyze *de novo* methylation reactions, whereas DNMT1 is responsible for the maintenance of DNA methylation. Ten-Eleven Translocation (TET) 1–3 are responsible for the removal of this methyl group, catalyzing the oxidation of 5-mC to 5-hydroxymethylcytosine (5-hmC), then to 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC). Finally, the base-excision repair (BER) pathway is able to replace 5-caC with cytosine. In lysine methylation, the ε-nitrogen atom of the amino acid lysine can be mono-, di-, or tri-methylated by a diverse set of lysine methyltransferases (PKMTs), including Enhancer of Zeste Homolog (EZH) 1/2, Mixed Lineage Leukemia (MLL) 1–5, Euchromatic Histone Lysine Methyltransferase (EHMT) 1/2, and many more [107]. Although this is not an inclusive list, there are two families of PKMTs including those with a SET (SU(var), Enhancer of Zeste and Trithorax) domain and those with a seven β strand (7BS) domain. The reversal of lysine methylation can be catalyzed by lysine demethylases (PKDMs), including lysine demethylase (LSD) 1,2 and Jumonji C (JmJc) domain-containing proteins [16]. Arginine methylation is catalyzed by arginine methyltransferases (PRMTs), which transfer a methyl group onto the ω-nitrogen of arginine. All PRMTs catalyze monomethylation of arginine (MM). However, PRMTs then diverge upon dimethylation into Type I PRMTs, which catalyze asymmetric dimethylation (ADM) of arginine, and Type II PRMTs which catalyze symmetric dimethylation of arginine (SDM). Type III PRMTs catalyze MM. The enzymes that catalyze the reversal of arginine methylation are currently unknown, but may include JMJD6 and/or other known lysine demethylases.

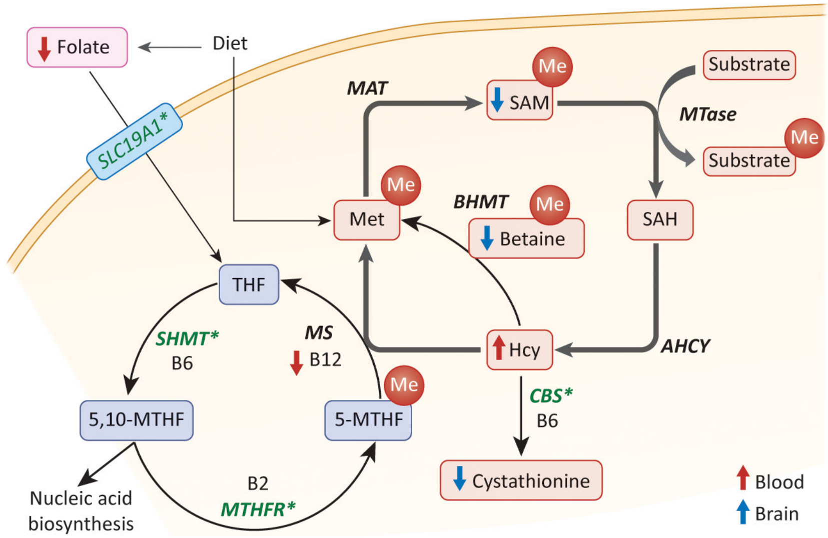

Figure 2. Methionine Metabolisms Pathways and Alterations in Multiple Sclerosis

Folate and methylation pathways are intimately linked. The essential amino acid methionine (Met) is converted to (S)-adenosylmethionine (SAM) by methionine adenosyl transferase (MAT). SAM serves as a methyl donor for the vast majority of methyltransferase reactions. Upon methyl donation, SAM is converted to (S)-adenosylhomocysteine (SAH). SAH is a potent negative regulator of methyltransferases, but is rapidly converted to homocysteine (Hcy) by SAH hydrolase (AHCY). Hcy can be used to form cystathionine, catalyzed by cystathionine beta synthase (CBS), or remethylated to Met. Hcy can be remethylated to Met by one of two pathways: 1) 5-methyltetrahydrofolate (MTHF) transfers its methyl group to cobalamin (B12), which then transfers the methyl group to homocysteine, catalyzed by methionine synthase (MS), or 2) Betaine-Homocysteine Methyltransferase (BHMT) catalyzes the transfer of a methyl group from betaine to homocysteine. 5-MTHF is generated by the folate pathway, which requires entry of dietary folate into the cell via the reduced folate carrier 1 (SLC19A1). Then, serine hydroxy methyltransferase 1 (SHMT1) methylates tetrahydrofolate (THF) to form 5,10-methylene tetrahydrofolate (5,10-MTHF). 5,10-MTHF can be utilized for nucleotide biosynthesis, or reduced to 5-MTHF by methylenetetrahydrofolate reductase (MTHFR) to contribute to remethylation of Hcy to Met. Genes are italicized. Genes with green lettering have SNPs identified as MS risk factors. Asterisks also denote genes associated with MS risk. Red and blue arrows indicate that at least one study reported changes in metabolite levels in MS patient blood or brain, respectively. See "*Evidence of deregulated methionine metabolites*" for more details on conflicting evidence.

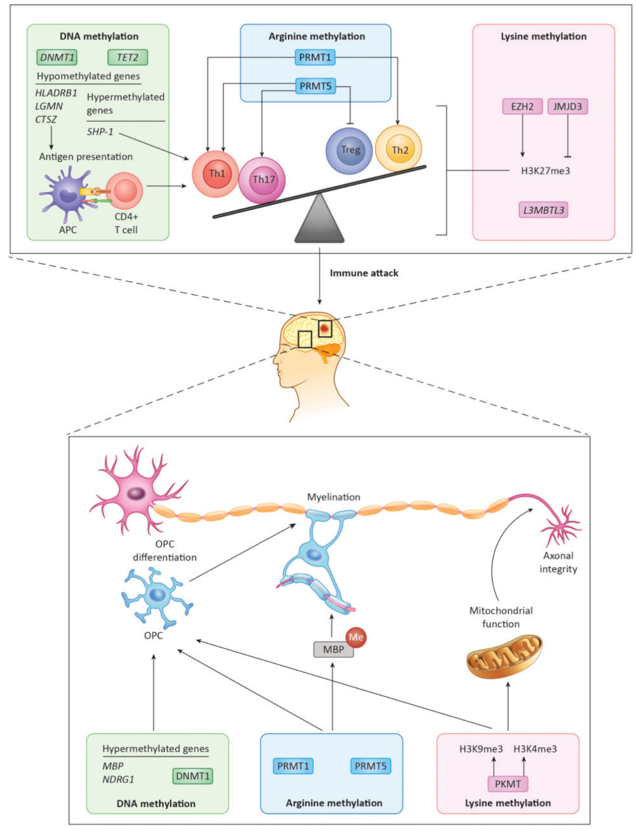

Figure 3, Key Figure. Model of Methylation Effects in Multiple Sclerosis

Biological DNA and protein methylation appear to play striking roles in both the immune and neurologic compartments. In the immune compartment, specifically in Th cells, DNA methylation may promote inflammatory T cell responses through hypomethylation of antigen presentation-related genes and hypermethylation of anti-inflammatory gene protein tyrosine phosphatase *SHP1* in MS patients. Arginine methylation is also essential for immune responses as PRMT1 promotes Th1/Th2 cell responses and PRMT5 enhances inflammatory Th1/Th17 cell and suppresses regulatory T cell responses. Lysine methylation, though there is conflicting evidence in the literature, may regulate the maintenance and effector functions of Th cell phenotypes and plasticity. In the neurologic compartment, all three types of methylation are essential for oligodendrocyte progenitor cell (OPC) differentiation into mature oligodendrocytes (OLs) and thus, myelination. In MS patients, oligodendrocyte-specific genes are DNA hypermethylated, thus contributing to decreased expression of oligodendrocyte genes and reduced capacity to re-myelinate axons. Arginine methyltransferses PRMT1 and PRMT5 have been shown to be indispensable for OPC differentiation and methylation. It has also been shown that Arg-107 of myelin basic protein (MBP) is symmetrically dimethylated, which has been proposed to stabilize myelin structure. Additionally, increased H3K9me3 lysine methylation is essential for OPC differentiation. Finally, H3K4me3 lysine methylation has been shown to be reduced in MS patient brain tissue and may play a significant role in mitochondrial respiration in neurons; thus, its decreased may reduce axon integrity and promote neurodegeneration.

Table 1

MS Risk Variants Involved in Human Methylation Pathways. N: non-coding, c: coding

SNP	Gene name	Base pair	Mutation type (amino acid)	Risk allele	Biological effect of risk allele	Reference
rs1801131	*MTHFR*	c.1298A>C	Missense (E/A)	A (protective)	Reduced DNA methylation and protection from MS	[44,45]
rs1801133	*MTHFR*	c.677C>T	Missense (A/V)	T	Reduced MTHFR enzymatic activity/stability	[48]
rs1051266	*SLC19A1*	c.80G>A	Missense (H/R)	A (protective)	Lower plasma folate levels and later age of MS onset	[43]
rs72058776	*CBS*	c.844_855ins68bp	Intronic	Insertion	Higher expression and earlier MS disease onset	[43]
rs2726518	*TET2*	c.7577A>C	Intronic	C	Unknown	[40]
rs4925166	*SHMT1*	c.530G>T	Intronic/eQTL?	T	Increased DNA methylation at SNP locus impacting expression	[39]
rs4410871	*MYC*	n.8049A>G	non-coding	G	Unknown	[41]
rs34286592	*MAZ*	c.418C>T	Intronic	T	Unknown	[39]
rs2836425	*ERG*	c.21281C>T	Intronic	T	Unknown	[39]
rs4364506	*L3MBTL3*	c.374G>A	Intronic	A	Unknown	[39]