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Evaluating epigenetic landmarks in the brain of multiple sclerosis patients: A contribution to the current debate on disease pathogenesis

Patrizia Casaccia-Bonnet^{a,*}, Giovanna Pandozy¹, and Fabrizio Mastronardi^b

^a Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, UMDNJ, 675 Hoes Lane, Piscataway, NJ 08854, United States

^b Program in Molecular Structure and Function, The Hospital for Sick Children, Toronto M5G 1X8, Canada

Abstract

The evidence suggesting a role of epigenetics in the definition of complex trait diseases is rapidly increasing. The gender prevalence of multiple sclerosis, the low level concordance in homozygous twins and the linkage to several genetic loci, suggest an epigenetic component to the definition of this demyelinating disorder. While the immune etio-pathogenetic mechanism of disease progression has been well characterized, still relatively little is known about the initial events contributing to onset and progression of the demyelinating lesion. This article addresses the challenging question of whether loss of the mechanisms of epigenetic regulation of gene expression in the myelinating cells may contribute to the pathogenesis of multiple sclerosis, by affecting the repair process and by modulating the levels of enzymes involved in neo-epitope formation. The role of altered post-translational modifications of nucleosomal histones and DNA methylation in white matter oligodendroglial cells are presented in terms of pathogenetic concepts and the relevance to therapeutic intervention is then discussed.

Keywords

Epigenetics; Chromatin; Histone; DNA methylation; Multiple sclerosis

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) which affects about 2.5 million individuals worldwide, and whose cause remains unknown. MS affects mostly young adults with a higher prevalence in developed, industrialized countries. Disease-modifying therapies, such as interferon beta are available on the market and partially effective in slowing down and mitigating the disease course. However, these immunomodulatory or immunosuppressive strategies fall short of success in the long-run, and cannot ward-off indefinitely the accumulation of disability and chronic neurological damage.

In this article we review the current literature suggesting a role for epigenetic regulation of gene expression in MS and contribute etio-pathogenetic concepts of disease progression with the intent to stimulate a dialogue for the development of new therapeutic solutions. Although the etiology of MS remains elusive (Barnett and Sutton, 2006; Sawcer, 2006; Sawcer and

*Corresponding author. Tel.: +1 732 235 4520; fax: +1 732 235 4029. E-mail address: casacpa@umdnj.edu (P. Casaccia-Bonnet).

¹GP is a scientific free-lance writer.

Compston, 2006), drug development has targeted inflammation and autoimmunity in order to manage clinical relapses (Calabresi et al., 2007; Elies, 2007; Koch et al., 2007; Korn et al., 2007; Linker et al., 2007; Vanderlocht et al., 2007). The current immunological hypothesis (as summarized in Fig. 1) advocates a critical role for cellular and humoral immunity in myelin damage and involves the role of cytotoxic cytokines (i.e. IFN-gamma, TNF, Fas and other death receptors; Duddy et al., 2007; Forte et al., 2006; Karni et al., 2006; Lopatinskaya et al., 2006; Xu et al., 2007). This model is supported by a large body of evidence, including genetic linkage data with specific HLA-DR2 loci that are critical for antigen presentation to T cells (Becher et al., 2006; Muller-Hilke and Mitchison, 2006; Olsson, 2006), the detection of high antibody titers and T-cell abnormalities in MS patients (Gestri et al., 2001; Carotenuto et al., 2006) and the neuropathological findings of perivascular plaques characterized by a strong lymphocytic component and myelin damage with sparing of oligodendrocytes (Lassmann et al., 1998).

However the detection of plaques that are distant from blood vessels and are characterized by prevalence of microglial cells and myelin loss associated with significant oligodendrocytic apoptosis suggested the existence of alternative mechanisms of etio-pathogenesis (Barnett and Prineas, 2004; Kutzelnigg et al., 2007; Lucchinetti et al., 2000; Pittock and Lucchinetti, 2007; Roemer et al., 2007). The heterogeneity of the neuropathological findings and the relative lack of response to immunomodulatory therapy has led to the hypothesis that MS is a complex pathology characterized by demyelination as hallmark of the disease, possibly determined by multiple mechanisms of distinct etiology (Kornek and Lassmann, 2003).

The importance of myelin products in the pathogenesis of the disease has been supported by several studies (reviewed in Sospedra and Martin, 2005). Demyelination can be induced in rodents by immunization with myelin proteins or by transfer of T-cells stimulated with myelin peptides (i.e. MOG, PLP, MBP), (Kassiotis et al., 1999; Zargari et al., 2007). In addition, the detection of antibodies directed against myelin proteins in the brain of MS patients (Genain et al., 1999), and the presence of myelin-reactive T-cell and B-cells (McLaurin et al., 1995; Hafler, 2004; Klawiter and Cross, 2007; Nikbin et al., 2007) in the serum and CSF of MS patients (Tomassini et al., 2007; Berger et al., 2003) have suggested the importance of cell-mediated and humoral responses in the pathogenesis of the disease.

The nature of the triggering event leading to exposure of immunogenic myelin-derived peptides however remains unanswered. One hypothesis suggested that autoimmune demyelination is triggered by a mechanism of “molecular mimicry” to viral or bacterial proteins after a non-specific initial infection (Merkler et al., 2006). However, different forms of viral encephalomyelitis trigger an inflammatory response without widespread demyelination (Chen et al., 2005; Komoly, 2005; Mendez-Fernandez et al., 2003; Murray et al., 2001). Alternative hypotheses have implicated mechanisms that are intrinsic to the myelinating cells and depend on post-translational modifications of myelin proteins (Mastronardi and Moscarello, 2005). One of the best characterized molecular changes in a myelin component that has been associated with the exposure of an immunodominant epitope of the molecule is the deimination of myelin basic protein (Moscarello et al., 2007; Musse and Harauz, 2007) and it will be discussed later in this review. An alternative possibility has been suggested by the recent identification of autocatalytic anti-MBP antibodies in the sera of MS patients, characterized by proteolytic activity against the auto-antigen (Belogurov et al., 2008).

In this manuscript we will first review the epidemiological evidence supporting an epigenetic component to MS pathogenesis. We shall then discuss the potential role of epigenetic regulation of gene expression in modulating the ability of endogenous progenitors to repair the demyelinating lesion. We will conclude with a discussion on aberrant epigenetic modulation

of gene expression in regulating the levels of enzymes responsible for the deimination of MBP and the generation of immunodominant neo-epitopes.

2. Evidence supporting the contribution of epigenetics to the pathogenesis of multiple sclerosis

Genetic linkage studies and genome-wide profiling arrays have contributed to the identification of multiple genes that may exert a combinatorial effect in affecting the susceptibility of individuals to develop the disease (Brynedal et al., 2007; Lundmark et al., 2007; Olsson, 2006; Zivadinov et al., 2007). However, it is becoming increasingly evident that gene expression based on DNA sequence alterations or mutations is not sufficient to explain the variety of manifestations observed in disease states (Dai and Rasmussen, 2007; Hake et al., 2007; Lu et al., 2006; Tabancay and Forsburg, 2006). If genetic information would be the sole determinant for susceptibility to MS, then it would be expected that homozygous twins, who are genetically identical, should display the same risk to develop the disease. In contrast, in MS the concordance rate for homozygous twins is only 20–30% (Hansen et al., 2005a,b; Poser, 2006; Willer et al., 2003). Although traditionally these differences have been attributed to exposure to environmental toxins, it is becoming evident that the effect of environmental factors is exerted by modulating the epigenome of the cells (Szyf et al., 2008). In this article we will use the term “epigenome” to define the higher-order, inheritable set of instructions determining gene expression (Weaver et al., 2006; Petronis, 2006), including modifications of chromatin components. We shall refer to “epigenetics” as the study of the “mechanisms modulating gene expression that do not involve mutations of DNA but are nevertheless inherited and persist in the absence of the signal that generated the event” (Ptashne, 2007). Additional support to the importance of epigenetics in MS is the parent-of-origin effect that predicts a higher rate of incidence of the disease in the offspring of parents of a specific sex. This concept was originally described in terms of parental transmission of disease susceptibility to the offspring (Ewen, 1995; Kantarci et al., 2006), although it has been recently reconsidered (Herrera et al., 2007). Finally, although the importance of sex hormones in MS has been well studied (Holmqvist et al., 2006; Tomassini and Pozzilli, 2006; Vukusic and Confavreux, 2006) it is important to stress the ability of sex steroids to epigenetically modulate gene expression by affecting chromatin components and transcriptional complexes (Kaminsky et al., 2006).

This article addresses the challenging question of whether altered control of gene expression, due to loss of mechanisms of epigenetic regulation within the myelinating cells, may contribute to the pathogenesis of multiple sclerosis.

3. Mechanisms of epigenetic regulation of gene expression

Chromatin is made of negatively charged DNA and of histone proteins with a net positive charge, due to the presence of arginine and lysine residues. Histones are disposed in an octamer (2 of each: H2A, H2B, H3 and H4), with globular bodies and tails irradiating from within.

Specific aminoacid residues in the histone tails are subject to post-translational modifications that affect gene expression, by modulating the degree of compactness of chromatin. The modifications include acetylation/deacetylation of lysine residues, methylation of lysine and arginine residues (Kouzarides, 2002), phosphorylation (Cheung et al., 2003), sumoylation (Shio and Eisenman, 2003), ubiquitination (Jason et al., 2002) and citrullination (Mastronardi et al., 2006). The combinatorial arrangements of distinct histone modifications establish a specific “histone code” which, together with DNA methylation, is essential for the induction of specific patterns of epigenetic inheritance (Kouzarides, 2007; Turner, 2000). We shall first

review some of the histone changes and the relative enzymatic activities catalyzing these changes and then discuss their relevance to the oligodendroglial lineage and myelin formation.

3.1. Histone acetylation/deacetylation of lysine residues

Acetylation of selected lysine residues in the tails of nucleosomal histones represents an efficient way to regulate gene expression (Strahl and Allis, 2000; Turner, 2000; Yang, 2004; Yoshida et al., 2003) and is regulated by two families of enzymes: histone acetyltransferases (HATS) and histone deacetylases (HDACs). High levels of acetylation have been correlated with the ultrastructural feature of dispersed euchromatin and the detection of “pale” nuclei. In contrast, histone deacetylation has been morphologically associated with the initiation of hetero-chromatin and it is often detected together with repressive histone methylation in “dense” nuclei, characterized by condensed chromatin (Casaccia-Bonnet and Shen, 2008).

The HAT family of mammalian histone acetyl-transferases has the ability to promote gene expression, by transferring acetyl groups to lysines residues and thereby loosening the histone-DNA contacts, binding to specific transcription factors, as well as with basal components of the transcriptional machinery (Gregory et al., 2001; Kalkhoven, 2004; Roth et al., 2001). The HDAC family is responsible for the removal of acetyl groups from lysine residues in the histone tails and is highly conserved from yeast to human (de Ruijter et al., 2003; Michan and Sinclair, 2007; Thiagalingam et al., 2003). Based on sequence homology, mammalian HDACs can be broadly grouped into four major classes. Class I (HDAC-1, -2, -3 and -8), class II (HDAC-4, -5, -6 -7, -9 and -10), class III (SIRT1–7), which is composed of the NAD-dependent family of sirtuins, and class IV (HDAC11), which has intermediate properties between class I and class II. In general terms, the removal of the negative acetyl groups from the histone tails has been associated with transcriptional repression and indeed class I HDAC family members form repressive complexes with transcription factors and co-repressors (Forsberg and Bresnick, 2001; Wade, 2001). In the oligodendrocyte global histone deacetylation has been defined as a prominent event during the differentiation of progenitors into myelinating oligodendrocytes (Marin-Husstege et al., 2002). This post-translational modification of histones was defined as necessary during a specific temporal window in oligodendrocyte maturation (Marin-Husstege et al., 2002). It was also shown that it is important for *in vivo* myelination because systemic administration of HDAC inhibitors to developing rodents (Shen et al., 2005) or zebrafish (Cunliffe and Casaccia-Bonnet, 2006) arrested progenitors at an immature state. It remains to be determined whether similar events occur during the repair phase in demyelinating disorders.

3.2. Methylation of lysine residues

The functional role of histone methylation is dependent on the position of the modified lysine residue within the histone tail and on the number of methyl groups (one, two or three) added to the epsilon position of lysine residues (Ashraf and Ip, 1998; Bannister et al., 2002; Berger, 2002; Bird and Wolffe, 1999; Goll and Bestor, 2002; Kouzarides, 2002; Lachner and Jenuwein, 2002; Rice and Allis, 2001; Richards, 2002; Spencer and Davie, 1999; Zhang and Reinberg, 2001). The presence of three methyl groups on lysine 4 residue on histone H3 (Me-H3K4), for instance, has been associated with transcriptional activation. In contrast, the presence of three methyl groups on residue 9 or 27 has been associated with transcriptional repression. The enzymes responsible for activating and repressive methylation are also quite distinct. Methylation of K4, for instance, is catalyzed by MLL, a group of enzymes defined by the presence of specific structural motifs (Ruthenburg et al., 2007). Repressive methylation in contrast, is catalyzed by members of the Polycomb family, including EZH2 and other components of the PRC2 complex (Schuettengruber et al., 2007).

Histone methylation was originally considered a permanent modification, until the report of lysine-specific demethylase enzymatic activities, such as the flavin oxidase LSD1, whose substrates are mono- and di-methyl groups on lysine residues, and the Jumonji domain containing enzymes (JmjC), whose substrates are tri-methylated lysine residue (Anand and Marmorstein, 2007; Bannister and Kouzarides, 2005; Klose and Zhang, 2007; Kubicek and Jenuwein, 2004; Stavropoulos and Hoelz, 2007; Takeuchi et al., 2006; Wilson, 2007; Wysocka et al., 2005). Our group has reported the occurrence of global repressive histone methylation during the developmental window following histone deacetylation (Shen et al., 2005). We proposed that this event is critical for the stable down-regulation of stem cell markers and of transcriptional inhibitors of myelin gene expression (Shen and Casaccia-Bonnel, 2008). We also suggested that a deficit of global histone methylation could interfere with the ability of progenitors to form new myelin or with the proper pattern of gene expression characterizing mature oligodendrocytes. Indeed, studies conducted in oligodendrocyte lineage cells in the brain of old mice have indicated the aberrant persistence of global histone acetylation and lack repressive histone methylation (Shen et al., 2008). We therefore hypothesized that an aberrant transcriptional response might occur in old mice during the repair phase following demyelination and lead to the persistence of high levels of transcriptional inhibitors of myelin gene expression and the inefficient differentiation of progenitors into myelin-forming cells (Shen, Sandoval and Casaccia-Bonnel, unpublished).

3.3. Methylation and citrullination of arginine residues

Besides lysines, arginine residues can also be methylated by specific enzymatic activities and play a critical role in dynamic gene regulation (Bedford and Richard, 2005; Boisvert et al., 2005; Davie and Dent, 2002; Denman, 2005; Pahlich et al., 2006; Thompson and Fast, 2006; Wysocka et al., 2006). In mammals, the asymmetric transfer of methyl groups to arginine residues is catalyzed by the protein arginine methyltransferases PRMT1 and by the coactivator associated arginine methyltransferase 1 (CARM1). This asymmetric methylation of arginine residue 3 on histone H4 (H4R3) and arginine 17 on histone H3 (H3R17) results in gene activation (Bauer et al., 2002; Chen et al., 1999; Strahl et al., 2001; Wang et al., 2001). Repressive methylation of arginines is catalyzed by the enzyme PRMT5 that is responsible for the symmetric methylation of arginine 8 in histone H3 (H3R8) and of arginine 3 in histone H4 (H4R3; Pal et al., 2004; Wysocka et al., 2006). Arginine methylation is also reversible and can be removed by specific deiminases, such as human peptidylarginine deiminase 4 (PAD4), which converts arginine into citrulline (Wang et al., 2004). The reversibility of protein arginine methylation by PAD enzymes has more recently been disputed in an extensive *in vitro* biochemical analysis making the reversibility of this post-translational modification equivocal (Raijmakers et al., 2007). The functional significance of increased global histone citrullination in oligodendrocyte lineage cells remains to be clearly defined. However, increased levels of PAD4 and of citrullinated histone H3 have been detected in experimental models of demyelination and in the brain of MS patients compared to controls (Mastronardi et al., 2006).

3.4. DNA methylation

Individuals from any given species, and particularly humans, vary in methylation patterns. These patterns are transmissible and define inheritable patterns of gene expression through cell divisions. Methylation involves several reactions. The methyl group is transferred from tetrahydrofolate to vitamin B12 which in turn methylates homocysteine to form methionine. Methionine is then activated by ATP to form S-adenosylmethionine, which is the methyl donor in all biological methylation reactions (Stubbe, 1994). Methylation is an important regulatory mechanism for gene expression, which occurs on CpG sequences in the immediate 5' promoter regions of genes. These regions have CpG contents greater than 60% compared to 40% for the bulk DNA. Methylation of cytosine in CpG sequences turns off promoter activity and thereby

decreases gene expression, since methylation of DNA affects both DNA structure and the binding of transcription factors (Lamb et al., 1991; Nonkwelo and Long, 1993; Ohtani-Fujita et al., 1993). This event is regulated by the balance between DNA methyltransferase and DNA demethylases (Szyf, 1994). The possible contribution of altered DNA methylation in white matter cells in MS brains will be later discussed for its possible implications in modulating disease progression. Additional studies on methylation of HLA loci (Rakyan et al., 2004) and on the pattern of X-chromosome inactivation in patients with progressive forms of MS (Knudsen et al., 2007) further confirm that epigenetic modifications might be an important component of disease pathogenesis.

4. Epigenetic modulation of gene expression in demyelinating disorders

Disease state implies tissue damage and this local disruption of homeostasis by itself may elicit repair mechanisms recapitulating developmental pathways (Herrero-Herranz et al., 2007; John et al., 2002), a bit like phylogenesis is resumed in ontogenesis and embryology. Molecules and signalling pathways expressed during development are re-expressed in each case of altered tissue homeostasis. In urodeles (salamanders and newts) limb regeneration, for example, one of the few successful cases of actual regenerative ability in the animal kingdom, genes that are essential in embryological development are re-expressed with a modified pattern (Brown and Brockes, 1991; Savard et al., 1988; Savard and Tremblay, 1995). These events might attempt to restore balance through recapitulation (Guimaraes et al., 1975; Wizenmann and Bahr, 1997). Where adult cellular elements need to be replenished, new-born cells, derived from pools of precursors, need to migrate to the proper location and acquire mature phenotypes; once they have reached their destination they must interact with resident tissue cells and respond to local cues.

The existence of repair event in the brain of MS patients is supported by a large body of literature. One of the first reports of newly formed myelin in MS brains was presented by Prineas and Connell (1979) and several studies from other laboratories confirmed these findings (Prineas et al., 1984; Raine and Scheinberg, 1988; Compston, 1993, 1995; Chang et al., 2000).

It was suggested that the extent of remyelination events was affected by the frequency of occurrence of relapses (Prineas et al., 1993) and that overall remyelination was relatively rare within demyelinating lesions (Raine and Wu, 1993). The efficiency of new myelin formation is thought to be dependent on intrinsic signals within oligodendrocyte lineage cells and extrinsic signals from axons (Bruck et al., 2003) and astrocytes (John et al., 2002). Among these signals, it has been shown that developmentally regulated molecules (i.e. Notch) can be detected at the borders of the demyelinated plaques (John et al., 2002). This finding is consistent with the concept that re-activation of developmental programs occurs during remyelination and that these events are localized at the edges of the demyelinated lesions (Albert et al., 2007). Although the extent of spontaneous remyelination is variable, no correlation was found with the gender or age of disease onset (Patrikios et al., 2006), underscoring the functional importance of the intrinsic properties of cells involved in repair. A potential explanation for the inappropriate repair is the persistence of progenitors at an undifferentiated state due to inappropriate epigenetic regulation of a developmental program of gene expression. Further investigation will be necessary to prove the validity of this assumption and to determine whether the persistence of developmental pathways at the lesion site may inhibit further differentiation of oligodendrocyte progenitors (Wang et al., 1998, 2006; Mastronardi et al., 2004).

4.1. Aberrant recapitulation of developmental processes due to epigenetic disequilibrium: attempts to repair or contribution to MS disease progression?

In MS, myelin is lost in multifocal loci following episodes of neuroinflammation (Chaudhuri and Behan, 2004). These foci of demyelination, called “plaques”, are often characterized by the appearance of new myelin at the edge of the lesion (Prineas et al., 1993). However, disease pathology may not be exclusively limited to sites of active demyelination, since also the properties of myelin in the normal appearing white matter of MS brains are substantially different from those of normal myelin (Ludwin, 2006). Pathological studies on post-mortem tissue obtained from MS brains, for instance, revealed that the composition of myelin in the normal appearing white matter is different than the composition in non-MS brains and has the features of developmentally immature myelin (Moscarello et al., 1994). Therefore, the interpretation of biochemical analysis of tissue samples from MS brains needs to take into account the coexistence of multiple populations, including progenitors involved in repair and mature cells with altered properties, in the normal appearing white matter. For this reason it is critical to correlate biochemical studies with immunohistochemistry. Previous studies from our laboratory, for instance, had reported the up-regulation of stathmin, a microtubule destabilizing protein that keeps neural precursors in an immature morphology in the MS brain compared to controls (Liu et al., 2005). The expression of this protein in progenitors could indicate the presence of active repair, since high levels of stathmin promote progenitor migration. However, the persistence of this molecule in mature oligodendrocytes leads to the cytoskeleton destabilization and increased susceptibility to apoptosis (Liu et al., 2005). Together, these data led us to propose a speculative, and yet intriguing, hypothesis of disease progression resulting from the generation of neo-epitopes due to deregulated “epigenetic identity”. We have previously shown that histone deacetylase activity is necessary for the definition of the epigenetic signature of oligodendrocytes in developing rodents (He et al., 2007; Marin-Husstege et al., 2002; Shen et al., 2005). Our gene profiling studies defined the epigenetic down-regulation of genes encoding for cytoskeletal depolymerizing molecules (i.e. stathmin) and transcriptional inhibitors (i.e. Hes5) as pre-requisite for the attainment of a mature myelinating phenotype. We have discussed in Section 3 how transient global histone deacetylation during oligodendroglial development serves the important function of decreasing genes whose expression is characteristic of the progenitor stage (Liu et al., 2006, 2003, 2005). The re-expression of these genes in oligodendrocyte progenitors in the MS brains could be attributed to the occurrence of repair mechanisms. However, the persistence of progenitor markers in mature cells would indicate the defective establishment of the epigenetic identity, as observed in aging (Shen et al., 2008). For this reason, it will be important to experimentally characterize the cell types displaying aberrant epigenetic regulation in MS patients and correlate the findings to specific neuropathological features. It will also be important to correlate specific histone modifications to the developmental stage of oligodendrocyte lineage cells and their localization in reference to the demyelinating plaque. For instance, the persistence of histone acetylation and the inappropriate re-expression of stathmin (Liu et al., 2005) and Hes5 (John et al., 2002) in cells characterized by mature markers, would indicate the loss of the “epigenetic signature” of oligodendrocyte identity. In support of the concept that inappropriate establishment of epigenetic identity occurs during repair, previous studies on the appearance of nuclei of oligodendrocytic cells in chronic multiple sclerosis lesions suggested the predominance of large pale nuclei over the dense nuclei characteristic of mature oligodendrocytes (Wolswijk, 2000). Since the pale or dense appearance of nuclei is dependent on the state of chromatin condensation, the persistence of “pale” nuclei in oligodendrocytes would provide further support to the concept of inappropriate chromatin condensation during the repair phase of demyelinating lesions.

Potential causes for this aberrant regulation of chromatin condensation during disease states may include accelerated senescence due to exposure to proinflammatory cytokines, microglia

activation, neuronal or axonal dysfunction. An additional possibility is that external stimuli, such as oxidative stress (Ceccatelli et al., 2007; Madhavan et al., 2006) or viral infections (Ogle et al., 2005), might induce changes in chromatin conformation that may halt (Odeberg et al., 2006) or prematurely induce differentiation (Covacu et al., 2006; Dietrich et al., 2004). Because the modifications of the epigenome are transmissible to progeny, it is intriguing to envision the propagation of these modifications to several daughter cells thereby resulting in progressively cumulative changes in the properties of the normal appearing white matter.

4.2. The controversy regarding the use of histone deacetylase inhibitors in demyelinating disorders

The immune hypothesis of MS proposes that it is a T-cell driven pathology defining inflammatory demyelinating lesions throughout the brain and spinal cord (Delgado and Sheremata, 2006; Hafler, 2004; Hafler et al., 2005; McDole et al., 2006; Sheremata et al., 2006). Based on this premise, the currently available therapies are mainly focused at controlling the inflammatory components by either preventing the access of activated lymphocytes in the brain or interfering with the production of cytotoxic cytokines by immune cells. Since HDAC inhibitors were shown to inhibit proliferation, activation and cytokine production by activated T cells (Dangond and Gullans, 1998; Moreira et al., 2003; Skov et al., 2003), they have been proposed as alternative immunomodulatory treatment for MS. Given the role of histone deacetylation in decreasing gene expression, it was suggested that favouring HDAC activity promotes an anti-inflammatory effect due to the down-regulation of pro-inflammatory genes. This concept was supported by the finding that the action of the most potent anti-inflammatory agents, the glucocorticoids, is dependent on HDAC activity. Glucocorticoids were shown to recruit HDAC to the promoter of pro-inflammatory genes (Adcock et al., 2005) and knockdown of HDAC2 function selectively impaired glucocorticoid function and rendered the cells insensitive to the action of these anti-inflammatory agents (Ito et al., 2006). Thus, these studies indicated that HDAC inhibitors had a pro-inflammatory effect, by antagonizing the effect of glucocorticoids and favouring the expression of pro-inflammatory molecules. Different conclusions were reached by other laboratories reporting elevated HDAC levels (especially HDAC3) after stimulation of peripheral blood lymphocytes (Dangond and Gullans, 1998). According to these authors, treatment of CD4+ T cells with HDAC inhibitors induced a decrease of the proliferative response and the suppression of the production of the pro-Th1 cytokine IFN-gamma (Dangond and Gullans, 1998; Skov et al., 2003; Su et al., 2008). It was also suggested that HDAC inhibitors favoured the shift from a Th1 to a Th2 phenotype; however, trichostatin A (TSA) attenuated T-cell suppressor effects in a mouse asthma model (Choi et al., 2005) and down-regulated various costimulatory adhesion molecules (Moreira et al., 2003). These studies suggested an anti-inflammatory role of HDAC inhibitors that was also supported by the recent results in the MOG induced model of monophasic, progressive EAE (Camelo et al., 2005).

It is important to mention, that the evidence supporting HDAC inhibitors as anti-inflammatory agents is quite controversial, especially in light of the fact that glucocorticoids, the most potent anti-inflammatory agents, require HDAC activity in order to shut down the synthesis of pro-inflammatory genes (Adcock et al., 2005; Ito et al., 2006). Besides the controversial findings related to the role of HDAC inhibitors as anti-inflammatory or pro-inflammatory agents, additional arguments against the use of these inhibitors in demyelinating disease were provided by studies conducted on microglial cells (Suuronen et al., 2003) and by studies conducted in oligodendrocyte progenitors, (Marin-Husstege et al., 2002) and neural stem cells (Hsieh et al., 2004; Kondo and Raff, 2004). Several studies reported that treatment with HDAC inhibitors has a negative impact not only on oligodendrocyte differentiation (Marin-Husstege et al., 2002) and developmental myelination (Shen et al., 2005), but also on the commitment of stem

cells to the oligodendrocyte lineage (Cunliffe and Casaccia-Bonnel, 2006; Hsieh et al., 2004; Kondo and Raff, 2004; Lyssiotis et al., 2007).

5. Alternative models of pathogenesis of demyelinating disorders: epigenetic deregulation of specific enzymes leads to the accumulation of neo-epitopes affecting the humoral response in MS

According to the autoimmune model of pathogenesis of MS, the cell-mediated and humoral response against myelin products would be initiated by the occurrence of damage following viral infections or myelin damage. We now propose an alternative model relating the formation of immunogenic peptides to the aberrant regulation of gene expression in myelinating oligodendrocytes. More specifically, we shall first review the evidence in support of aberrant methylation of the promoter of PAD2, a specific enzyme involved in deimination of myelin basic protein, and then discuss at least two potential mechanisms affecting the generation of neo-epitopes.

5.1. PAD2 as an example of aberrantly methylated promoter in MS brains

One of the important targets of hypomethylation in MS brain is the activation of the PAD2 locus. PAD2 is a peptidylarginine deiminase enzyme that was originally isolated from bovine brain white matter and that catalyzes the conversion of the guanidino group of arginine to citrulline in myelin basic protein (MBP; Lamensa and Moscarello, 1993). The PAD genes are clustered in a distinct genomic region in humans (1p36.1) and syntenic locations of rodent genomes (Vossenaar et al., 2003). PAD2 levels are elevated in MS (Mastronardi et al., 2007a) and in a transgenic animal model of MS (Moscarello et al., 2002). Its promoter has 74% GC content and studies involving bisulfite treatment followed by sequence analysis have revealed that this region is hypomethylated in MS brains compared to controls. Together with the findings of increased DNA demethylase, these data suggest that specific genes are reactivated in MS brains, due to epigenetic modulation.

5.2. The role of DNA methylation in MS

Studies on DNA isolated from white matter of MS brains contained only about 1/3 of the amount of methyl cytosine found in DNA from normal subjects (Mastronardi et al., 2007a). Decreased methylation of cytosines in CpG islands in white matter homogenates from MS brains was not the result of decreased methyltransferase activity, but rather the result of a two-fold higher DNA demethylase activity than that in homogenates from normal brain (Mastronardi et al., 2007a). It was specific for MS because DNA from thymus gland of the same MS patients and patients with Alzheimer's, Parkinson's and Huntington's diseases were normally methylated and the DNA demethylase activity was unaffected (Mastronardi et al., 2007a). A summary of the hypothesis linking dysregulation of the PAD2 locus to the generation of immunogenic peptides is illustrated in Figs. 2 and 3.

5.3. Consequences of increased PAD2 activity: citrullination of MBP

Protein substrates for PAD include polyarginine and MBP. While in normal brains only a very small proportion of MBP is citrullinated, in chronic MS, citrullinated MBP comprises approximately 40% of the total MBP (Moscarello et al., 1986, 1994). The relationship between the amount of citrullinated MBP and the severity of MS was suggested in a study which documented over 90% citrullination of MBP in a rare acute Marburg's form of MS. In this acute case of MS 18 of 19 arginines were converted to citrulline residues. This dramatic conversion of the majority of arginines into citrullines, resulted in a visible apparent mass change of MBP (Wood et al., 1996) and in a net reduction of its positive charge, thereby affecting the interactions of this protein with other myelin components (Finch and Moscarello,

1972; Wood and Moscarello, 1989). Electron microscopy and immunogold localization of the citrulline containing MBP isomer with specific antibodies revealed aberrant localization of the citrullinated form of MBP in human brain white matter biopsies (McLaurin et al., 1993).

5.4. Citrullinated MBP and neo-epitope generation

Several studies support the importance of citrullination of MBP in modulating the immune response in MS (Tranquill et al., 2000; Zhou et al., 1995). There are at least two possible mechanisms explaining the contribution of citrullinated MBP to the enhanced immune response. Citrullination could increase the generation of immunodominant peptides, due to increased auto-cleavage of the protein (D'Souza et al., 2005). Alternatively, citrullination can disrupt the physical and chemical properties of MBP and thereby affect its localization within the myelin membrane (Musse et al., 2006).

Whereas very little auto-cleavage can be observed with the MBP from normal tissues, it is clear that citrullinated MBP from MS white matter undergoes auto-cleavage at slightly alkaline pH values (D'Souza et al., 2005; D'Souza and Moscarello, 2006). The conversion of arginine residues into citrullines is catalyzed by the enzyme PAD2, and it has been shown to render MBP more susceptible to proteolytic digestion, to compromise its ability to form compact lipid bilayers thereby resulting in a less compact myelin structure and in the generation of immunodominant peptides (Fig. 3). For this reason, we postulate that autocatalysis of citrullinated MBP during the early stages of MS may contribute to the sensitization of T-cells and thereby enhance the autoimmune response (D'Souza et al., 2005). Citrullinated MBP is also more readily digested by myelin associated proteases such as cathepsin D (Cao et al., 1999) and stromelysin compared to non-citrullinated MBP. Both proteases released immunodominant peptide epitopes. Whereas the immunodominant peptide released by cathepsin D is part of a large peptide (residues 40–89), the one released by stromelysin (MMP-3) is only 17 residues long and can be accommodated by MHC class II molecules (Pritzker et al., 2000).

An alternative possibility has been suggested by the work of Musse et al. (2006) who reported the importance of citrullination of MBP in neo-epitope formation. The current view regarding the identity of immunogenic peptides predicts that only molecules with amino acid sequences extrinsic to the myelin membrane (such as MOG) would be able to elicit an immune response. This is true in physiological conditions, when the localization of MBP is within compact myelin. It is important to notice, however, that in pathological conditions MBP is predominantly citrullinated and this modification profoundly affects the physical and chemical properties of the molecule (Musse et al., 2006) likely resulting in membrane redistribution.

In conclusion, we suggest that citrullination of MBP caused by epigenetic deregulation of PAD2, leads to the release of modified and highly immunogenic forms of MBP from myelin which might significantly affect the progression of the demyelinating disorder (Mastronardi and Moscarello, 2005).

6. Future directions and concluding remarks

Disease induction and the onset of immune response in MS has been linked to mechanisms of myelin damage. In this review we discuss alternative models of disease onset suggesting that MS develops as the consequence of changes intrinsic to the whitematter oligodendroglial cells that favour their immunogenicity and promote the establishment of an autoimmune response. Within this review we discussed the evidence supporting the role of epigenetic regulation of gene expression in MS, and defined several relevant landmark changes occurring in the normal appearing white matter of MS brains that might contribute to disease progression.

We also discuss additional potential consequences of deregulated epigenetic changes in MS tissue and suggest that aberrant changes in the chromatin of oligodendroglial cells in the normal appearing white matter might lead to impaired remyelination, besides the formation of neo-epitopes affecting the humoral response. Some of these critical changes have been documented in the normal appearing white matter of individuals with MS and in animal models (Liu et al., 2005; Shen et al., 2008; Mastronardi and Moscarello, 2005; Moscarello et al., 2007). Other changes may be more subtle, they are transmitted to daughter cells that remain functional but keep on accumulating the epigenetic changes within each cell division. This may in turn lead to increased susceptibility of oligodendroglial cells to damaging stimuli (Liu et al., 2005), by favouring the formation of myelin debris and stimulating the autoimmune process and disease progression. Based on the epigenetic hypothesis, focused investigations will need to address early epigenetic changes associated with MS within the white matter.

We also propose here that reprogramming the epigenome for the proper performance of oligodendroglial and immune cells is a potential therapeutic strategy for MS. It is important, however, to consider the potential repercussion that each treatment could have on both compartments. As hematopoietic stem cells have been shown to migrate from niche to niche (Kaplan et al., 2007) and have the potential to generate multiple cell types, and mesenchymal cells are able to instruct neurogenesis under many circumstances (Bai et al., 2007), it is likely that epigenetic interventions in favour of the neural tissue might also have repercussion on the immune system. It would therefore be prudent to target the 2 compartments, in the case of MS the myelin and immune compartment (Mastronardi and Moscarello, 2005), together, or more precisely, to intervene adequately in their cross-talk. A possible way to support this favourable outcome involves favouring methylation through vitamin B12 supplementation (Mastronardi et al., 2004, 2007b) along with administration of NAD, as nicotinamide, which is essential for all cellular metabolic processes and which has been recently shown to have protective and reparative functions in animal models of demyelination (Kaneko et al., 2006).

In conclusion, we suggest that MS is a phenotypically complex disease that may include multiple aetiologies, including epigenetic deregulation of the myelinating cells. We further suggest that therapeutic interventions that take into consideration the disease complexities, and promote epigenetic repression of multiple loci, in combination with other immunomodulatory therapies, may hold promise.

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Abbreviations

EAE	experimental allergic encephalomyelitis
HAT	histone acetyl-transferase
HDAC	histone deacetylase
MBP	myelin basic protein
MS	

multiple sclerosis

PAD

peptidylarginine deiminase

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Fig. 1. Schematic drawing of the current hypotheses for MS etiology. In black is a simplified drawing of the current pathogenetic hypothesis. In red is the hypothesis that is proposed in this review.

Fig. 2.

Aberrant recapitulation of developmental epigenetic landmarks in multiple sclerosis. This drawing illustrates the major epigenetic events occurring in differentiating oligodendroglial cells during development (green) and leading to repression of the differentiation inhibitors Hes5 and stathmin. In the brain of multiple sclerosis patients, in contrast, these repressive epigenetic events are replaced by activating events (red arrow) leading to re-expression of differentiation inhibitors (Hes5) and of molecules destabilizing the cytoskeletal network of oligodendroglial cells (stathmin).

Fig. 3.

A Proposed epigenetic mechanism of PAD2 up-regulation in MS oligodendrocytes. The upstream regulatory region of the PAD2 gene contains a CpG island. In normal individuals the methylation and expression of PAD2 is under homeostatic regulation. However in MS white matter the cytosines in CG dinucleotides in the PAD2 promoter become demethylated via increased DNA demethylase activity. The result of this CpG demethylation might explain why there is hypercitrullination of arginine rich protein substrates like myelin basic protein. Myelin basic protein once citrullinated in the normal appearing white matter loses its interactions with myelin membranes, which fragment thereby leading to autoimmune progression and MS.