An integrated approach to design novel therapeutic interventions for demyelinating disorders

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Abstract

Therapeutic strategies are often based on two general principles: interference with the pathogenic process and repair the damaged tissues. Recent studies, however, have suggested that several pathological conditions may result from the interplay between genetic susceptibility traits and environmental influences that, by modulating the epigenome, also affect disease onset and progression. Based on lessons learned from neural development, it is conceivable that new lines of preventive and possibly therapeutic intervention might be developed in the future in order to modulate disease onset or decrease the severity of the symptoms. This review will discuss these concepts within the context of multiple sclerosis (MS), the most common demyelinating disease of the central nervous system (CNS), and the leading cause of progressive neurological disability in young adults.

Keywords
multiple sclerosis; demyelination; genetics; pharmacogenomics; epigenetics

INTRODUCTION

Multiple sclerosis is defined as an inflammatory demyelinating disorder affecting the young adult population (Compston & Coles, 2008), typically affecting individuals between the age
of 20 and 45 years of age, although the occurrence of pediatric MS is on the rise. Clinical symptoms are variable, depending on the involvement of visual, sensory or motor pathways, and include but are not limited to: ataxia, fatigue, cognitive impairment or autonomic manifestations. A large proportion of patients (~80%) develops a relapsing-remitting (RRMS) course of the disease, characterized by acute relapses and long stretches of symptom-free periods (called “remissions”), while a smaller proportion (~10–15%) of the patients do not return to baseline conditions and accumulate disability over time (primary progressive form of the disease). Eventually, the relapsing-remitting course of the disease will enter a phase of incomplete recovery, when the symptoms persist and neurological function deteriorates over time, defining the secondary progressive (SPMS) phase of the disease (Thompson et al., 1997; Rovaris et al., 2006). The challenge of this manuscript is to review the current studies available in the literature and propose an integrated view of disease pathogenesis, with the aim of opening a debate regarding the feasibility of future potential integrated approaches to therapy.

1. Multiple Sclerosis as an autoimmune disease

Most of the current knowledge on the etio-pathogenesis of MS is derived from a large number of epidemiological, histopathological, immunological and genetic studies. Inflammation, demyelination, and axonal degeneration are the major pathologic features of MS (Compston & Coles, 2008). It is well accepted that MS prevalence increases with latitude (Alonso & Hernan, 2008) and that its incidence is higher in people with low levels of vitamin D and low sun exposure (Ascherio & Munger, 2007). Its onset has also been associated with viral infections by Epstein-Barr virus and possibly related to additional strains of herpesviruses (Kakalacheva et al., 2011). However, there has been no conclusive evidence that any of these factors, per se, is capable of inducing the disease. Rather, viral infections, low vitamin D levels, stress, diet and other life style changes have been proposed to act as co-factors to trigger disease onset in genetically susceptible individuals.

Identifying susceptibility genes for MS has been a subject of intense research since early 1970s when genes encoding for class II alleles of the major histocompatibility complex (MHC) were first identified (Jersild et al., 1973; Compston et al., 1976; Terasaki et al., 1976). Consequent gene linkage studies in multiplex families and genetic association studies, fine-mapped this region to the HLA-DR15 haplotype (Fogdell et al., 1995) and suggested the existence of a gradient on susceptibility to MS depending on the specific combination of allelic variants within this locus (Rasmussen et al., 2001; Dyment et al., 2005). In addition, specific MHC class I alleles showed an independent association and modulation of the risk on HLA-DR15 haplotype carriers, with HLA-A3 allele as risk-increasing and HLA-A2 as risk-decreasing allele (Fogdell-Hahn et al., 2000; Harbo et al., 2004; Friese et al., 2008). Further genome-wide association studies (GWAS), analyzing >100,000 single nucleotide polymorphism (SNP) markers, identified additional non-MHC polymorphisms related to MS risk and highlighted the importance of the MHC class II region on disease susceptibility (Lincoln et al., 2005; Sawcer et al., 2005; Burton et al., 2007; Hafler et al., 2007; Friese et al., 2008; Baranzini et al., 2009; The Australian and New Zealand Multiple Sclerosis Genetics Consortium 2009; De Jager et al., 2009a). Most of the non-MHC genes identified by these studies related to the immune system and had been previously associated with increased susceptibility to autoimmune disorders. Among them, interleukin-7 receptor alpha chain (IL7RA) and other genes in the IL7/IL7R axis showed high association to MS (Hafler et al., 2007; De Jager et al., 2009b; Zuvich et al., 2010), and therefore were identified as potential therapeutic targets (Sasson et al., 2006). Similarly, the association of the interleukin-2 receptor alpha chain (IL2RA) to MS (Hafler et al., 2007; The Australian and New Zealand Multiple Sclerosis Genetics Consortium 2009; De Jager et al., 2009b) led to the development of monoclonal antibodies against this protein which are
Currently being assessed in small clinical trials (Schippling & Martin, 2008). An additional polymorphism associated with MS includes the SNP rs2300747 in the CD58 gene (Hafler et al., 2007; The Australian and New Zealand Multiple Sclerosis Genetics Consortium, 2009; De Jager et al., 2009b), which is critical for regulation of T-cell receptor signaling. Higher levels of CD58 transcripts were detected in remitting MS patients and this allele was associated with a protective effect on MS progression (De Jager et al., 2009a).

While a thorough review of the genetic studies is above and beyond the scope of this article, we would like to mention that a recent collaborative study including 9,772 cases recruited by 23 Centers worldwide, including the US, Australia and several European countries (Sawcer et al., 2011) confirmed the critical importance of HLA-DRB1 as risk alleles and validated several genes involved in cytokine signaling, including IL2, IFN gamma, IL-12, TNF alpha and IL6 pathways as genes associated with the disease.

Together, the vast literature on the GWAS studies identified the immune system as the primary system modulated by susceptibility genes. We believe that an integrated review of the distinct loci might lead to the development of panels of candidate susceptibility genes that may contribute to a better diagnostic classification of patients and potentially identify individuals that are more likely to benefit from therapies targeting a specific pathway, such as the IL7 (Sasson et al., 2006) or IL2 (Schippling & Martin, 2008) pathway.

### 2. Immune-based animal models of demyelination to test therapeutic approaches in MS

The rodent model for MS, experimental autoimmune encephalomyelitis (EAE), is one of the most extensively studied animal models of immune disease to identify the molecular mechanisms involved in the inflammatory response and to assess the validity of new therapies for MS. The origins of this model go back to 1885 when Louis Pasteur's rabies vaccine was first used clinically and some cases of paralysis were reported subsequently (reviewed in (Baxter, 2007)). Since the first studies, it has evolved with current immunizations with whole brain emulsions and myelin proteins or peptides. Immunization of the animals with these compounds leads to a disease that shares clinical and neuropathological changes with MS (Steinman, 1999). Most therapies tested in MS patients are based on concepts derived from studies in EAE, but unfortunately an important number of potential therapies that showed effectiveness in this model, failed to demonstrate a positive effect in patients. This can be explained by the fact that the artificial immunization of the animals may not necessarily reproduce all the pathogenic mechanisms in human disease so animal models should be adapted to account for the genetic variability shown in MS. It has been noted that the genetic background of different mouse strains influences the disease course in the EAE model (Miller et al., 2010). Based on these considerations, the development of partially humanized mouse models represents a promising tool to address the influence of the genetic component in disease onset and progression (Gregersen et al., 2004). Transgenic mice expressing MHC-class II molecules and T-cell receptor variants from MS patients may better represent many of the various clinical manifestations and corresponding CNS lesions of MS (Madsen et al., 1999; Quandt et al., 2004). While we appreciate the effort in the generation of humanized transgenic mice, we recognize that this is a very powerful and promising strategy for testing and propose that it would be fully exploited to test proof-of-principle concepts for the involvement of pathways identified by the large GWAS studies.

### 3. Pharmacogenomic approaches to “personalized” medicine

Growing evidence shows that the clinical response to MS therapies varies widely among patients (Miller et al., 2008) and thus the paradigm of “one drug fits all” has been shifted towards the search for a more “personalized” therapy. Pharmacogenomic studies focus on
the identification of genetic variations that have an influence on drug response, in the end aiming at the identification of the patients who will show a more effective response to therapy and will present less adverse side effects. This area has already gained great importance since many FDA-approved drugs currently show pharmacogenomic information in its labels (http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm). Some of these drugs are used in cancer, viral infections, neurologic and cardiovascular diseases. In many cases the genetic markers identified belong to the cytochrome P450 family, related to the pharmacokinetics (metabolism and clearance) of the drugs, and thus individual variations could affect the effectiveness, adverse events and drug interactions of these treatments. Other examples of markers are different receptors whose expression in several types of tumors is assessed in the clinical practice in order to prescribe antagonists that will be effective in the treatment of the disease. Among them are the detection of estrogen receptors (ER) in breast cancer and epidermal growth factor receptor (EGFR) for colorectal cancer. In other cases, the expression of certain variants of genes will confer great response to certain drugs, such as the allele rs12979860 of interferon-lambda-3 gene (IL28B) for the treatment of hepatitis C. Regarding MS treatment, Azathioprine (AZA) is an immunosuppressive drug used as an alternative to interferon-beta and also indicated in other neuroimmunological diseases. The most severe adverse effect is hematological toxicity that can be fatal in about 0.3% of cases (Connell et al., 1993). Methylation by thiopurine-methyl transferase (TPMT) is the limiting step for the inactivation of AZA. Therefore, the FDA recommends TPMT genotyping and/or phenotyping prior prescribing AZA treatment, being contraindicated in patients with low or absent TPMT activity (0.3% of the cases). Dose escalation is recommended for intermediate metabolizing enzymes (10%). Several pharmacogenomic studies have identified diverse genetic variants for other disease-modifying therapies for MS but further investigations needs to be addressed in order to find strong evidence for these observations. Among them, several polymorphisms in interferon (IFN) pathway and IFN-beta responsive genes (e.g. interleukin-17) have been associated with response to this drug, as well as other neurogenesis (GPC5, glypican 5; NPAS3, neuronal PAS domain protein 3) and neurotransmission related genes (AMPA type glutamate receptor GRIA3) (Cunningham et al., 2005; Martinez et al., 2006; Byun et al., 2008; Comabella et al., 2009; Axtell et al., 2010). In addition, responsiveness to glatiramer acetate (GA) treatment has been linked to HLA-DRB1*1501, rs71878 for T-cell receptor-beta and rs2275235 for cathepsin S (CTSS) (Fusco et al., 2001; Grossman et al., 2007). Finally, several polymorphisms in the adenosine triphosphate (ATP)-binding cassette (ABC)-transporter genes have also been identified as potential predictors for clinical response and toxicity of the immunosuppressant mitoxantrone (Cotte et al., 2009; Dorr et al., 2009).

In conclusion, pharmacogenomic studies are contributing to the achievement of a “personalized” therapy for different disorders. The hope is that more data will be available for MS and lead to the creation of platforms that could be used to better stratify the patient population and possibly inform therapeutic decisions.

4. Environmental effects on individuals with genetic traits induce disease onset and related animal models

While the genetic evidence implying an effect of the immune system in disease onset, is irrefutable, it is undoubtedly significant to note that not all the individuals with a given risk allele, develop the disease. In this respect, it is worth noting that even identical twins are only 30% concordant for the development of the disease (Hawkes & Macgregor, 2009). What are the factors that allow individuals with the same genetic risk factor to develop or not develop the disease? One of the potential explanations, based on the epidemiological
data, is that additional environmental components are needed in order to develop the disease. The concept of genetic susceptibility, was described by Poser as “the MS trait” that would manifest itself only if additional events would take place (Poser, 2006). This is an attractive hypothesis that is quite reminiscent of the two-hits cancer model proposed by Knudson (Knudson, 2001). Low sun exposure, low vitamin D levels, stressful situations, viral infections, they could all contribute to trigger the disease in predisposed individuals (Lauer, 2010). A very interesting correlation between MS and diet has also been proposed (Ghadirian et al., 1998) and related to the consumption of cow milk products in countries with high incidence of MS (Guggenmos et al., 2004). The effect of diet on influencing disease onset in a group of genetically susceptible individuals has been reported in children with type I diabetes, where those that were fed an omega 6 rich diet developed the disease, while the group that was fed an omega 3 rich diet was protected (Norris et al., 2007).

An interesting model to test the hypothesis of the link between diet and demyelination is provided by the cuprizone model. Feeding mice with a diet containing 0.2% of the copper chelators cuprizone, results in oligodendrogliopathy followed by myelin loss, microglial infiltration and astrogliosis (Matsushima & Morell, 2001). Furthermore, removal of the toxicant from the diet results in recovery of the lesions due to spontaneous remyelination. Aged mice are more prone to axonal degeneration upon cuprizone-induced demyelination (Irvine & Blakemore, 2006). Previous studies in our laboratory have used this model to test the effect of age in modulating myelin repair (Shen et al., 2008). Some studies reported a lower extent of cuprizone-induced demyelination in SJL mice compared to C57BL/6 strain (Taylor et al., 2009), highlighting the importance of this model to study the role of genetic susceptibility in the development of a pathology induced by dietary intake of the copper chelator. To further address the interplay between genetic traits and external dietary factors, we fed eight distinct mouse strains with the same diet for the same period of time and then assessed the levels of demyelination and microglial activation. Quantitative RT-PCR and immunohistochemistry analyses of corpus callosum showed that two mouse strains (i.e. DBA and A/J mice) were highly sensitive to cuprizone, as evidenced by decreased levels of the myelin protein CNPase in the corpus callosum (Figure 1A and B). Consistent with the specific effect of cuprizone in the corpus callosum, the effect was only observed in this area and not in the spinal cord (Figure 1C). In contrast, NOD mice did not respond to the cuprizone diet evidenced by no decrease in CNPase, indicating that different genetic backgrounds affect the susceptibility to develop a demyelinating pathology in response to dietary manipulations. Previous studies also reported differences in microglia infiltration in the corpus callosum of BALB and C57BL/6 mice, even in the presence of similar demyelination patterns in this specific region (Skripuletz et al., 2008). In order to determine if microglial activation could account for the differences observed in our experiments, we examined mRNA levels of microglial activators, including chemokine L3 (Chem3), lysozyme, beta2-microglobulin, TNFalpha and cathepsin Z in the strains showing greater differences in CNPase levels (Figure 1D–H). In general, a greater activation was observed in A/J mice, which could partially explain the higher sensitivity of this strain to cuprizone action. Strikingly however, DBA mice showed a milder microglial activation pattern, thereby revealing a pattern of extreme sensitivity to cuprizone diet in this strain. In support of a dissociation between microglial activation and demyelination, the NOD mice which represented the most resistant strain to cuprizone diet, also had a significant microglial activation (Figure 1D and E). Together, these experiments reveal a highly heterogeneous response to dietary cuprizone that depends on the individual genetic background. In connection with the findings mentioned previously in this section these data support the importance of the interplay between intrinsic (i.e. genetic) and extrinsic (i.e. diet) factors in the onset of demyelinating diseases.
5. Importance of early intervention to prevent disease onset

The geographic distribution of the incidence and prevalence of MS varies with increasing risk at higher latitudes (Alonso & Hernan, 2008). Interestingly, adults migrating from a high to low risk area are thought to carry their former high risk with them, whereas people who migrate during childhood seem to have the risk associated to the new area to which they migrated (Dean, 1971; Alter et al., 1978; Elian et al., 1990; Cabre et al., 2005). These observations implicate an environmental event involved in MS pathogenesis which acts sometime between birth and young adulthood but does not act thereafter. It is also believed that even environmental factors during preborn life can influence MS risk. There is evidence of a month-of-birth effect on MS reported in Canada and northern Europe, with more patients born in May and fewer in November (Templer et al., 1992; Willer et al., 2005; Sadovnick et al., 2007). Many environmental factors have been proposed to be linked to MS pathogenesis including Epstein-Barr virus (EBV) and other virus infection, vitamin D deficiency, low-sunlight, occupational hazards, living with domesticated animals, dietary habits, trauma, stress and toxic exposures (Compston et al., 2005). Among them, however, vitamin D deficiency and EBV infection have been gaining the greatest support for their potential role in MS pathogenesis. A careful control of these variables in childhood, for instance by providing adequate vitamin D3 supplementation and monitoring its levels in individuals at high genetic risk, is crucial in the determination of the disease course (Figure 2).

6. Epigenetic changes detected during neural development may inform therapies later in life

Our laboratory has previously characterized important mechanisms of regulation of developmental myelination that depend on the modification of chromatin components. We have shown that the activity of chromatin-modifying enzymes called HDACs is essential for oligodendrocyte differentiation in vitro (Marin-Husstege et al., 2002) and in vivo (Shen et al., 2005). We have then extended this work to the analysis of mechanisms of remyelination in the adult brain (Shen et al., 2008). We previously demonstrated that HDAC enzymatic activity is necessary for oligodendrocyte differentiation because it decreases the levels of inhibitors of oligodendrocyte process outgrowth (i.e. stathmin) (Liu et al., 2003; Liu et al., 2005) and of myelin gene expression (i.e. Id4, Hes5) (Gokhan et al., 2005; Marin-Husstege et al., 2006). The use of HDAC inhibitors (HDACi) for MS treatment has been proposed based on their approved use as anti-cancer agents (Marks et al., 2001). However, the use of HDACi for treatment of MS is more controversial since studies on the animal EAE model of demyelination have shown results in both directions (Natarajan & Bright, 2002; Camelo et al., 2005). We also reported the negative effects of treatment with pharmacological blockers of HDAC on oligodendrocyte progenitor differentiation in vitro (Marin-Husstege et al., 2002) and on developmental myelination in vivo (Shen et al., 2005) and during myelin repair after cuprizone-induced demyelination (Shen et al., 2008). We also described the occurrence of similar mechanisms in the adult MS human brain (Pedre et al., 2011) and therefore would like to caution against the use of HDAC inhibitors during a specific time period, which coincides with the early stages of oligodendrocyte differentiation and myelin repair. The involvement of epigenetic changes, particularly in terms of chromatin modifications, is exciting for two reasons. First, it sheds light on the etiology of the early aspects of the disease process. Second, and perhaps more importantly, it may provide insight to understand how environmental factors can influence disease development and acquisition even in genetically identical patients. This insight comes from the fact that epigenetic changes in the brain have been documented to change significantly over the lifetime of individuals (Hernandez et al., 2011), and to diverge significantly in identical twins (Fraga et al., 2005).
As a summary, in this article we have reviewed the different factors that contribute to MS susceptibility, including genetic variants and environmental factors, and have pointed that populations which build up several of these risk factors could be eligible for early therapeutic interventions in order to prevent the onset or lessen the severity of the disease.

We now propose to integrate the currently available information, into the development of two stage-treatment platforms. The first stage would include a careful stratification of patients, based on vitamin D3 levels and based on the results of genetic screens, designed on the basis of the currently available GWAS data sets. The second stage would include pharmacological and environmental intervention, aimed at promoting repair. This would be best achieved by taking into account genotypes associated with greater responsiveness or resistance to specific treatments, while awaiting for the development of targeted epigenomic approaches.

Acknowledgments

This work is supported by grants from the National Institute of Health (NINDS-1R01NS069835-01; R01 NS42925-10) and from National Multiple Sclerosis Society (RG 4134A9/1) to PC and by a postdoctoral fellowship from the National Multiple Sclerosis Society to JL (FG1874-A-1) and from the National Multiple Sclerosis of Canada the Fonds de la Recherche en Santé du Québec. to J.H.

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Eur J Neurosci. Author manuscript; available in PMC 2013 June 01.


Eur J Neurosci. Author manuscript; available in PMC 2013 June 01.


Eur J Neurosci. Author manuscript; available in PMC 2013 June 01.
Figure 1. Different susceptibility of mouse strains to cuprizone-induced demyelination

Eight different mouse strains were fed a diet containing 0.2% cuprizone during 4 weeks. Total RNA from freshly dissected corpus callosum (A) and spinal cord (C) was isolated and CNP levels analyzed by quantitative RT-PCR. Values obtained were normalized to GAPDH transcript levels and the bar graphs represent the normalized values relative to those measured in the corpora callosa of C57BL/6 mice. (B) Immunofluorescence micrograph of coronal brain sections of DBA, A/J and NOD mice, immunostained with the antibody against the myelin protein CNPase (red) and the nuclear stain DAPI (blue). Images show the corpus callosum. V=ventricle; scale bar =100 μm. (D-H) Quantitative PCR analyses of the same three mouse strains for different microglial activators normalized to GAPDH transcript levels and expressed relative to the values measured in DBA controls. Statistical differences for all qRT-PCR analyses were determined using two-tailed independent t-tests with Bonferroni correction (*P < 0.05, **P < 0.01, ***P < 0.001).
Figure 2. Multiple sclerosis is a complex disease with cumulative risk factors leading to its onset. In addition to genetic predisposition, one or more environmental events need to occur in order for MS to develop. The time sequence and age of incidence of each factor appears to play a role in disease development. There is growing evidence of an epigenetic mechanism driven by the different environmental risk factors, so it is conceivable that epigenetic modulators could be used during early stages of life to prevent MS onset or during the early stages of the pathology to decrease the severity of the symptoms.