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Elucidating the Molecular Basis of Multiple Sclerosis and Understanding the Disease Pathophysiology

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Abstract

Multiple sclerosis (MS) is a complex, multifactorial autoimmune disorder of the central nervous system (CNS) that causes inflammation, demyelination and neurodegeneration. The increased prevalence of this disease in Arabian Gulf Countries (AGCs) has captivated the author. The following is a deliberative review of the disease with respect to its molecular basis. Briefly, it considers disease pathophysiology though the molecular composition of the cell; the genome, epigenome and mitochondrial genome; and relates these factors to environmental, etiological factors, including: vitamin D, UVR, EBV infection, smoking and obesity. All in all, this review aims to explain the reasons underlying the increasing prevalence of MS in AGCs.

Keywords: Multiple sclerosis; Molecular biology; Molecular basis of Multiple sclerosis; Multiple sclerosis Pathogenesis; Multiple sclerosis Aetiology

Introduction

MS is a complex, multifactorial autoimmune disorder of the CNS that causes inflammation, demyelination and neuro degeneration. As a result, neurological conductions are blocked and symptoms develop. Neurological disruptions can cause the appearance of a wide range of symptoms, including: fatigue, cognitive dysfunction, paresthesias or numbness, motor weakness (mostly in the lower extremities), monocular visual disturbances (optic neuritis), ataxia and vertigo. Remyelination and neurogeneration occur during the remission phase, whereupon patients show signs of recovery. In the chronic phase of MS, however, neurological disabilities are mostly irreversible due to axonal loss [1,2].

This disease afflicts more patients every day. According to the Atlas of MS, the number of diagnosed patients reached 2.3 million in 2013, in comparison to 2.1 million back in 2008. More so, the global median prevalence increased from 30 to 33 per 100,000 between 2008 and 2013. Considering the AGCs, data compiled based on Kurtzke's classification system have shown that Dubai (U.A.E), Saudi Arabia, Qatar and Kuwait are considered hotspots of MS (≥ 30/100,000) [1].

The increased prevalence of MS in the AGCs has raised plenty of concern. As these countries share common geographical, ethnical and latitudinal factors, exploring the molecular basis of MS in relation to disease pathophysiology would facilitate the search for susceptibility factors within the AGCs. Previously, the author pointed some of the general molecular factors that have been associated with MS; however, more deliberation on this topic will be the scope of this article [1].

Materials and methods

A literature review was performed to identify the molecular factors associated with MS pathophysiology. Only peer-reviewed, full-text articles published in English were included. Keywords and phrases entered onto database browsers included, but not exclusively: multiple sclerosis molecular, multiple sclerosis genetics, multiple sclerosis pathogenesis, multiple sclerosis aetiology, multiple sclerosis genetic study, multiple sclerosis association study, multiple sclerosis genetic susceptibility factors, multiple sclerosis epigenetic factors, multiple sclerosis microRNA and multiple sclerosis mitochondrial genome. Data were then subtracted according to their significance and thoroughly reviewed.

Molecular pathophysiology of MS

Despite extensive research on MS, it remains elusive for many people. A plethora of molecular factors have been associated with this disease (Figure 1), only some of which will be the focus of this article. When related to their physiological functions, molecular factors can give indications of the reasons underlying their association with complex diseases, including MS. This report first discusses the pathophysiological process of MS, and subsequently focuses on the molecular and environmental factors that could trigger each step.

Immune triggering in the periphery

It is believed that the inflammatory events of MS start in the periphery, where T- and B-cells are activated and then shuttled into the CNS.

Dendritic cells (DC)

Dendritic cells (DC) play a major role in immune provoking in MS. Immature DC present processed antigens to naive T-cells, whereas mature DC, promoted by inflammatory cytokines, express major histocompatibility complex (MHC) class I and II and B7 molecules to induce T-cell polarization and B-cell activation. Moreover, immature and regulatory DC can induce peripheral tolerance and regulate T-cell development [3].

In MS patients, DC is recruited to the CNS and undergo maturation in MS lesions. Being near to autoantigens, they thus activate pathogenic T- and B-cells [4]. Actually, several MHC molecules have been associated with MS susceptibility. Important examples include: HLA-DRB1*15:01 (OR=5.8), HLA-A*02:01 (OR=0.63), HLA-B*38:01 (OR=0.36), HLA-DRB1*13:03 (OR=1.96), HLA-DRB1*02/HLA-DQB1*06:02 (OR=4.6), HLA-C*05, HLA-DRB1*03:01 (OR=2.01), HLA-DPB1*03:01 (OR=1.33), and HLA-DRB1*08:01 (OR=1.63) [5-10].

Literally, the most common MS susceptibility allele is the HLA-DRB1*15:01, which encodes HLA-DR2B. When inherited in trans to HLA-DRB1*08:01, this allele can result in a higher MS risk. In contrast, if inherited in trans to HLA-DRB1*14:01, it can result in a lower MS risk [5,11]. Intriguingly, inheritance of HLA-A*02:01 was reported to protect against MS, possibly through negative selection of pathogenic T-cells in the thymus [11]. One interesting study done on a Kuwaiti population suggested a trend where HLA Class II antigens (DR4, DQ6, DQ7 and DQ8) correlate with MS. However, the study was performed on a small number of subjects using a low resolution methodology, and thus remains to be validated [12].

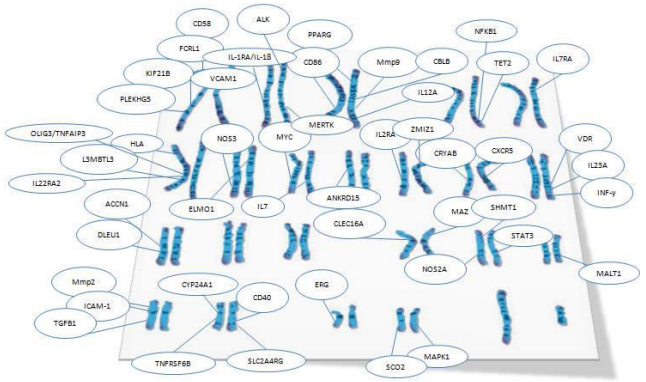

Figure 1: Graphic illustration of some of the MS associated genes with respect to their chromosomal locations. Several genes were found to associate with MS and affect vital cellular functions. (The picture illustrating the human chromosomes is a copyright of Sven Kamin.

Epigenetic studies have revealed a lot of information regarding HLA loci in MS patients. One of such studies was found in 19 differently methylated CpG sites in the MHC regions and 10 sites in the HLA-DRB1 region alone [13,14].

Another interesting molecule that co-localized with MHC class II on DC and was found to be associated with MS is CLEC16A ($p<6.30 \times 10^{-14}$, OR>1.15) [5,15]. This molecule has been recently found to regulate the pathway of MHC class II endosome formation and trafficking in DC [16].

Smoking, a known effector of MS pathogenesis, was found to correlate with increased susceptibility to MS in the presence of HLA-DRB1*15 and absence of HLA-A*02 (from OR=4.9 to OR=13.5) [17]. In the brain of Lewis rats, cigarette smoke can up-regulate expression of MHC class II, induce inflammation and pro-oxidant markers. Also, it raises Th1, Th17 and Treg cell-associated cytokine responses and increased expression levels of Nrf2. The latter is an oxidative stress-induced transcription factor that regulates the expression of a number of antioxidant genes [18]. Not surprisingly, smoking has for a long time raised concern in the AGCs [19-21].

DC secrete cytokines into the bloodstream, where they enter lymph nodes and stimulate CD4+ T-cell differentiation into effector T-helper cells. The secretion of IFN-γ and IL-12 mediates T-bet activation and promote Th1 differentiation. In contrast, secretion of IL-4 promotes Th2 cell differentiation by activating the transacting T cell–specific transcription factor GATA-3 (GATA-3). IL-1β and IL-23, on the other hand, stimulate Th17 cell differentiation. Finally, FOXP3+ Treg cell differentiation is promoted by TGF-β [3].

Notably, the vitamin D receptor (VDR) can regulate DC maturation, differentiation and proliferation. The VDR reduces stimulation of effector cells by down-regulating inflammatory cytokines (IL-2, IL-6, IL-17, INF-γ and TNFα), and induces stimulation of Treg cells by up-regulating anti-inflammatory cytokines (CD46, IL-10 and TGFβ1) [22-25]. More so, vitamin D/ VDR regulate the transcription of the HLA-DRB1*15 allele and several other non-HLA MS susceptibility loci [26-29]. In addition, vitamin D can down-regulate CLEC16A, consequently suppressing MHC class II expression [16]. Also, VDR regulates the cathelicidin antimicrobial peptide (CAMP), which induces the expression of cathelicidin LL-37. The latter down-regulates the expression of monocyte Toll-like receptors (TLRs) that induce inflammation and trigger sepsis and autoimmune disease exacerbation [30]. On the genetic level, associations were found between MS and SNP rs731236 that lay in the Taq1 region of the *VDR* gene ($p = 0.004$, OR = 0.53), indicating a genetic predisposition of VDR misregulation in MS [26]. Evidently, vitamin D deficiency has been a general health problem for AGC populations [1].

Another vitamin of concern in MS pathogenesis is vitamin A. Supplementation of such a vitamin in EAE models inhibits IL-1β, IL-12, TNF-α, and nitric oxide (NO) generation and decreases the proliferation of myelin-basic protein-reactive cells. In MS cases, such supplementation leads to decreased myelin oligodendrocytes glycoprotein-reactive cells, reduces IL-17 levels, promotes TGF-β production as well as elevates Foxp3 expression [31].

Regulatory T cells (Treg)

As is already known, regulatory T cells (Treg) are a lymphocytic subclass that regulate immune responses and maintain of self-tolerance [32]. As such, it is intriguing to contemplate why these are defective in MS.

Two types of regulatory T-cell are present in the body. Naturally occurring Foxp3+ Tregs arise from the thymus, whereas adaptive Tregs arise from immune responses extrathymically. Both natural and adaptive Treg cells maintain immune homeostasis and regulate autoimmune inflammation in MS and EAE. Differentiation of Treg precursor cells into effector Tregs is mediated by TGF-β [3]. Suppressive activity of Tregs is actually mediated by IL-10 [33]. In fact, Treg cells inhibit IFN-γ and IL-17 secretion and down-regulate the expression of costimulatory molecules on DC. Consequently, antigen presentation is down-regulated [3].

In contrast to pro-inflammatory T-cells, Treg cells express high levels of Foxp3. Three separate Foxp3+CD4+ Treg cell populations can be distinguished: CD45RA+Foxp3low, CD45RA−Foxp3high, and CD45RA-Foxp3low. Activated Foxp3+CD4+ T-cells (CD45RA-Foxp3low), however, have no suppressive properties. Only cells within CD45RA+Foxp3low and CD45RA−Foxp3high populations have suppressive activities. CD45RA−Foxp3high Treg cells suppress target

cells and then undergo apoptosis, while CD45RA+Foxp3low differentiate and proliferate to maintain the CD45RA−Foxp3high line. Moreover, CD8+ T-cells can express Foxp3 upon activation by antigens and act as suppressor cells [3].

Significant down-regulation of Treg cells in MS has been reported, while appearances of these cells in the CNS correlate with disease recovery in EAE. Interestingly, some studies have suggested that most Treg cells in MS are in fact memory Treg cells that had diminished suppression of mean Ca2+ influx and lost their inherent suppressive capacity [3,34,35].

T-cells autoreactive to myelin antigens are present in healthy individuals, suggesting that Tregs are clearing these cells continuously. However, several studies have pointed to either depletion of Tregs in the MS CNS, dysregulation, or their ineffectiveness on effector T-cells. The deficit in such regulation is thought to be a response to the elevated levels of IL-17 and IL-6 in MS. Several MS drugs, including glatiramer acetate (GA), IFN-β and glucocorticoids have been of reported to increase the levels of Foxp3+ Tregs [extensively reviewed by 2; 3].

Interestingly, miR-223, which modulates the NF-κB pathway, was found to be highly up-regulated in the blood and Treg cells of MS cases [36,37]. Elevation of miR-223 has previously been correlated with lower Treg cell counts in maternal and cord blood of pregnant women [38]. If anything, this could explain the lower number of Tregs in MS, a result of an miR-223-NF-κB mis-regulated pathway. Recently, DLEU1, another regulator of the NF-κB pathway, has been associated with MS susceptibility ($p=9.95 \times 10^{-9}$, OR=0.86) [5].

Another mediator of NF-κB that has been associated with MS is ultra-violet radiation (UVR). UVR induces prostaglandin E2 (PGE2) synthesis. PGE2 in turn binds prostaglandin E receptor subtype 4, thereby signaling an increase in the expression level of receptor activator of NF-κB ligand (RANKL) in the epidermis and an elevated influx of Treg cells into the draining lymph nodes [39].

More so, UVR mediates the conversion of cis-urocanic acid, an immunomodulator known to increase IL-10 and CD4+CD25+FoxP3+ Treg cell percentages and reduce IFN-γ production and antigen presentation capacity [40]. Importantly, CD25 [also known as IL-2 receptor-α chain (IL-2RA)], was previously associated with an increased risk of MS (OR> 1.12). In actual fact, one of the associated SNPs (rs2104286) was correlated with decreased serum IL-2RA in both healthy controls and MS patients. The soluble form of the receptor functions in inhibiting the pro-inflammatory effects of IL-2; however, it simultaneously induces T-cell proliferation and expansion. Therefore, this can potentially regulate MS pathogenesis [6,11,15].

CD58 is another noteworthy factor that plays a role in enhancing the expression of Foxp3 in Tregs through binding to CD2. It seems that patients express higher levels of CD58 during the remission phase of the disease. Looking at the molecular level, three variants of the *CD58* gene (rs12044852, rs1335532 and rs2300747) have been associated with an increased risk of MS ($p<9.71 \times 10^{-4}$, OR>1.18) [5-6,11,15,41]. The rs2300747 AA was reported to correlate with lower CD58 expression in the relapsing phases of the disease [42]. However, in independent replication studies, no significant differences were found with expression, disease activity, or progression. As such, thoughts have been directed to hsa-mir-548ac, an miRNA present in the first intron of CD58 that functions as a post-transcriptional regulator of gene expression. This miRNA actually lies between the two MS associated SNPs rs1335532 and rs2300747. Additionally, SNP rs1414273, located between the associated SNPs, was found to be in perfect linkage disequilibrium (D'=1) with rs1335532; indicating that even though not associated with MS previously, it may play a major pathogenic role. The position of this SNP can possibly influence the stability of the hairpin structure of the hsa-mir-548ac and processing by the miRNA biogenesis enzymatic machinery [41].

Activation of T- and B-cells

The inhibitory state of T- and B-cells is thought to be distributed in MS patients; thus, immune cells are more aggressive. Recently, Andlauer et al. found a novel association between the gene *L3MBTL3* and susceptibility of MS in a huge cohort of patients ($p=4.06 \times 10^{-9}$, OR=0.84). The gene encodes a Polycomb group protein that regulates the transcriptional inhibition state of several genes and is found in several forms of acute leukemias. MBT-1, murine ortholog of *L3MBTL3*, maintains the maturation of myeloid progenitor cells [5].

Another MS-associated regulatory gene that was found in the same study is the *MAZ* ($p=4.58 \times 10^{-8}$, OR=1.21). MAZ is up-regulated in chronic myeloid leukemias (CML), wherein it binds to the *MYC* gene promoter and regulates its transcription [5]. MYC has previously been associated with the disease ($p=7.70 \times 10^{-9}$, OR=1.09). This gene regulates cell cycle progression, apoptosis, and cellular transformation [1,15].

Another regulator of the hematopoietic process is the transcription factor ERG. Not only has the gene encoding this protein been associated with MS ($p=2.84 \times 10^{-8}$, OR=1.22), but it has also been associated with acute myeloid leukemia (AML) and acute T-cell lymphoblastic leukemia. In parallel, SHMT1, which plays a critical role in nucleotide and methionine synthesis, was also reported to associate with AML and MS ($p=2.69 \times 10^{-9}$, OR=0.85) [5].

More so, miR-20a and miR-17, which repress transcription of genes involved in T-cell activation in the Jurkat T-cell line, were found to be down-regulated in MS patients. miR-20a inhibits TCR-mediated signaling, CD69 expression, and decreases cytokine production when overexpressed in naive CD4+ T cells [43]. Also, hsa-miR-146a and hsa-miR142-3p were found to be significantly elevated. The former microRNA functions as a regulator of T-cell activation, while the latter functions as an immune modulator [44].

In 2009, a study defined hsa-miR-145 as a biomarker of MS with a specificity of 95%, a sensitivity of 97.6%, and an accuracy of 96.3% [45]. This microRNA was found to induce rearrangement of the actin cytoskeleton and nuclear rotation when overexpressed, affecting vital cellular functions such as cell migration, division and polarity. More importantly, hsa-miR-145 overexpression in MCF-7 cells was found to inhibit rhotekin and result in growth retardation and apoptosis [46]. All in all, the regulation of immune cell hibernation, proliferation and maturation is genetically distributed in MS patients, resulting in disease provocation.

Migration into the CNS

Provoked immune cells are thought to be then directed into the CNS where demyelination and neurodegeneration take place. On one level, sphingosine 1 phosphate (S1P) and sphingosine 1 phosphate receptor subtype 1 (S1P1) are thought to be involved in the migration into the CNS. T-cells express S1P1 on their plasma membrane; however, this receptor undergoes downregulation upon entry into the lymph node. After activation and clonal expansion, S1P1 expression

increases to facilitate T-cell migration from the lymph nodes. In effect, a gradient of S1P expression is established, which, in a way, is sensed by S1P1. Accordingly, activated T-cells can be shuttled back to the circulation and possibly to the CNS [47]. In real life, this theory has been proven with the use of fingolimod, a drug that blocks S1P1 and thereby contributes to the resolution of MS inflammation [48].

So how do such activated cells get across to the CNS? The blood-brain barrier (BBB) is a highly selective membrane that allows only solutes and ions to pass, a phenomenon known as immune-privilege [1]. This phenomenon is stratified for unknown reasons; however, it could be a result of deregulated protein expression patterns [49,50].

Other effectors of leukocyte migration include intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These molecules are members of the Ig superfamily. The latter are a plethora of cell surface sialoglycoproteins expressed by cytokine-activated endothelium; they mediate leukocyte-endothelium adhesion and signal transduction. Further, they bind the integrins leukocyte functional antigen 1 (LFA-1) and very late antigen-4 (VLA-4) and mediate cellular transfer through the BBB [15,49]. The genes encoding this pair of adhesion molecules have been shown to be associated with an increased risk of MS (*ICAM1*: K469; p=0.02, *VCAM1*: rs11581062 and rs12048904; $p=2.50 \times 10^{-10}$ and 4.00×10^{-08}, respectively). Drugs have been developed to target this step of the disease. An important example is natalizumab (aka. tysabri), which targets the α4-chain of α4β1 integrin (VLA-4) and prevents binding to VCAM-1 and migration of leukocytes to the CNS [2,15,50].

Another factor proposed to mediate migration through the CNS has actually been investigated by the author group in a Kuwaiti population. This is the endothelial nitric oxide synthase (eNOS), and is encoded by NOS3. It functions as a vasodilator that modulates BBB permeability and cerebral blood flow (CBF). NOS3 association with MS was limited to one marker known as eNOS-27VNTR (p=0.002, OR=2.6) [51].

The next step in leukocyte homing into the CNS is extravasation from the BBB; this is in part mediated by matrix metalloproteinases (MMPs). Several studies have identified elevated expression levels of both MMPs mRNA and proteins in animal EAE models and MS patients. T-cells, monocytes and dendritic cells express MMP-2 and MMP-9 to facilitate their migration through the barrier. MMP-2 and MMP-9 first tunnel the brain endothelial tight junctions and then cleave the receptor transmembrane β-dystroglycan, which anchors astrocytes' end feet to the basement membrane. Association studies repeatedly reported the present of a significant correlation between MMP-2 and MMP-9 with MS, further implementing the role of these molecules in disease development [52- 56]. Other MMPs, including MMPs-1, 3, 9, 19, have also been implicated in BBB extravasation [52].

Inflammation

The next step in MS pathogenesis is inflammation generated by immune cells. It is believed that immunological pathogenesis of MS is mediated by T-cells, B-cells and monocytes. Both helper CD4+ T-cells and cytotoxic CD8+ T-cells have been correlated with MS plaques. Moreover, several subpopulations of CD4+ T-cells, including Th17, Th1 and Th2 cells, were associated with MS lesions in the past [3]. In fact, some seem to think that CD4+ T-cells suffice to develop the disease, as transgenic mice that express both HLA-DRB*15:01 and human TCR that recognize myelin-basic protein (MBP) spontaneously develop MS-like disease. However, since monoclonal antibodies that target CD4+ T-cells in clinical trials are less effective than are those that target CD8+ T-cells, one must not exclude the pathogenic role of CD8+ T-cells [11].

Th17 cells

The hallmark secretions of Th17 cells; IL-17A, IL-17F, IL-21, IL-9, IL-22 and TNF-α, are considered vicious inflammatory modulators that action results in pathogenic consequences in the CNS. They are able to recruit neutrophils into the CNS and induce further inflammatory reactions. Also, both Th17 cells and IL-17 were found to be elevated in several studies in the active form of MS [2,31]. IL-17 functions in induction of pro-inflammatory cytokines and chemokines, enhancing DC maturation and promoting neutrophil functions [57]. More so, IL-17 increases BBB permeability upon binding to IL-17R and promotes further inflammatory cell migration into the CNS. Notably, MS endothelium showed increased levels of IL-17R expression [31].

Interestingly, patients demonstrating insulin resistance (IR) and obesity had significantly higher EDSS scores (p=0.03, 0.0179; respectively) and those who demonstrated IR had significant elevation of IL-17 (p=0.0006) and inflammatory reactions [58]. Another key modulator of Th17 cells is TNF-α, which interestingly has been associated with MS risk [59,60].

Although still a subject of debate, it is thought that Th17 cells need IL-23 to induce their expansion and TGF-β to induce their differentiation. More so, TGF-β is thought to suppress Th1 and Th2 cells, the cytokines of which inhibit Th17 differentiation [2]. One polymorphism, C2370A, in the 3'-UTR of the *IL-23* gene has been associated with MS in the Hungarian population (p<0.05, OR=2.072); however, no replication studies were found in any other populations [61]. Another polymorphism in the gene encoding *TGFB1*, the T +869C (Leu10Pro), have been associated with MS susceptibility, especially in men (p=0.031, 0.004, respectively). The T+869C have been associated with raised serum TGF-β levels. Accordingly, enhanced TGFβ1 expression could influence the differentiation of Th17 cells in MS patients [62].

Th17 cells were found to induce EAE in mouse models, while models that had IL-17 either neutralized or knocked-out had reduced disease severity, blockage of INF-γ exacerbation, delayed disease onset, reduced disease score and had early recovery and improvement in symptom severity. In concordance, blockage of IL-23 and IL-17 can cause complete and partial depletion of MS symptoms, respectively [2]. Notably, vitamin D seems to suppress the production of IL-17A from CD4+ T-cells by recruiting histone deacetylase, which results in decreased disease severity [14]. IL-9 has also been associated with MS development, whereas IL-21 and IL-22 seem dispensable [2].

More so, it seems that IL-1 is required for Th17 development, as IL-1RI-/- mice have non-functional Th17 cells and cannot induce EAE [2]. Notably, rat models of MS that have been exposed to cigarette smoking demonstrated significantly higher gene expressions of TNF-α, IL-1α, IL-1β, IL-23, IL-17 and TGF-β in contrast to those not exposed [18]. Smoking was thought to cause hypermethylation of vital *DNA* genes and induce imbalance in their expression [44,63]. More so, smoking is thought to affect histone acetylation and miRNA expression patterns [14].

Recruitment of Th17 cells to the CNS is thought to be a function of C-C chemokine receptor type 6 (CCR6), as CCR6-/- mice show resistance to EAE despite unaltered peripheral responses. CCR6 is a chemokine receptor expressed on Th17 cells that binds to chemokine

(C-C motif) ligand 20 (CCL20). The expression of CCR6 in Th17 cells is mediated by TGF-β and two nuclear receptors: RORα and RORγ. The latter two also bind CCL20 expressed on Th17 cells in response to TGF-β and IL-6 and in the presence of STAT3, RORγ and IL-21. Thus, polymorphisms noted in *TGFB1*, the T+869C (Leu10Pro), and in *STAT3*, rs9891119, could induce MS not only by inducing Th17 differentiation but also by inducing their migration [2,3,15, 62,64,65].

Subsequent to Th17 cell migration to the CNS, these cells stimulate Th1 passage through the BBB in a second inflammatory stage. Several pieces of evidence have supported the role of Th17 cells in the initiation and relapse stages of MS. Th17 cell suppression could in part be mediated by peroxisome proliferator-activated receptor gamma (PPAR-γ) molecules that inhibit TGF-β/IL-6- induced expression of RORγt [3]. In fact, increased expression of PPAR-γ in MS patients has already been reported [66]. This suggests that during inflammatory processes although PPAR-γ is up-regulated, for unknown reasons it either does not effectively mediate Th17 cell suppression or is perhaps not expressed highly enough to suppress all Th17 cells. Association signs were found between Pro12Ala in *PPARG* and the delayed onset of MS (p = 0.006), implicating these molecules in MS pathogenesis [67].

Moreover, hsa-miR-326 and hsa-miR-155, which induce T-cell differentiation along the Th17 axis, were found to be up-regulated in RRMS cases [44]. Also, miR-20b, which negatively regulates Th17 differentiation by targeting RORγt and STAT3, was down-regulated in MS patients and correlated with EAE pathogenesis [68].

Th1 cells

Initial data showed that only Th1 cells infiltrate the BBB and subsequently recruit Th17 cells. Th1 cells secrete chemokines that attract monocytes and macrophages and facilitate their infiltration into the spinal cord [2]. Observation of activated Th1 cells in the periphery, their recognition of MBP, their allocation to MS lesions, and their secretion of pro-inflammatory cytokines have all implicated these cells in MS. Th1 cells function in cellular immunity and antigen presentation through the secretion of INF-γ and TNF-α. After passing to the CNS, they are further activated by microglia and subsequently activate macrophages to mediate myelin destruction by action of Fc-R-mediated phagocytosis and secretion of toxic mediators. Examples of the latter include NO, proteases, free oxygen radicals, INF-γ and TNF-α [3,31,65].

Guerau-de-Arellano et al. demonstrated significant increases in the expression of miR-128 and miR-27b in MS CD4+ T cells. These miRNAs inhibit the differentiation of CD4+ T-cells into Th2 cells. Thus, CD4+ T-cells are effectively skewed towards the more aggressive Th1 axis of development, which correlates with disease exacerbation [69].

Soleimani et al. also found a correlation between vitamin D3 supplementation and decreased Th1 cells and TNF-α in EAE mice, connecting vitamin D deficiency with MS [70].

Th2 cells

Th2 cells function in humoral and allergic responses by expressing IL-4, IL-5, and IL-13 [3,65]. Recently, evidence for prominent infiltration of Th2 cells into MS lesion of deceased patients has implicated these cells in the disease pathophysiology for the first time [71].

Gamma-Delta T cells (γδ T-cells)

γδ T-cells have also been implicated in EAE and MS pathogenesis. Clones of γδ T-cells were found in MS lesions and CSF of recent disease onset. It is thought that high secretion of IL-17 by this T-cell subpopulation may contribute to MS pathogenesis, as is evidenced by increased γδT-cells IL-17 secretion in EAE brains. To further support this theory, *Tcrd-/-* mice demonstrated a less severe pathogenic course of EAE and reduced MOG-specific IL-17 production by conventional T-cells in contrast to control counterparts [2].

Compellingly, the expression of the TCR γ-chain seems to be affected by signal transduction of the interleukin-7 receptor (IL7R) [72]. IL7R was found to be associated with MS (rs6897932; $p=2.94 \times 10^{-7}$, OR=1.18) [6]. The associated SNP affects the slicing region of exon 6 of the IL7R, resulting in increased genetic expression of soluble IL7R. This in turn may affect T-cell survival, signaling and possibly disease pathogenesis [11].

IL-17 produced by γδ T-cells promotes further IL-17 production by CD4+ T cells [2]. Microglia stimulated by ligands specific for TLR-2, TLR-4, TLR-7, or TLR-9, can produce IL-1β and IL-23 that, in turn, induce γδ T-cell IL-17 secretions. IL-17 derived from γδ T-cells mediates neurotoxicity, but requires direct cell-cell contact between T-cells and neurons. This pathway depends on TLR adaptor myeloid differentiation primary response gene 88 (*MyD88*) expressions by microglia [73].

Notably, the TLR-2 agonist zymosan, was assessed in both naïve and EAE mice. Both groups showed a massive neuro-inflammatory response, with 80% mortality in the EAE group [74]. One must note that TLRs are down-regulated by LL-37, a member of the cathelicidin family. LL-37, in turn, is a vitamin D-dependent immunomodulator [30]. By virtue of its binding to the VDR, vitamin D, forms a transcription complex that affects many vital immune response genes. Within the *VDR* locus, SNPs in the TaqI region (rs731236) were found to be associated with MS (p=0.004, OR=0.53), thereby suggesting reasons for vitamin D association with MS [26].

Vitamin D supplementation in EAE mice models has been correlated with *MyD88* gene suppression and profound decrease in the expression of several TLRs. In effect, this has also correlated with reduced IL-1β. These mice, therefore, showed effective reduction in inflammatory cytokine expression in the spinal cord and ameliorated EAE [75]. As mentioned previously, modulation exerted by vitamin D could be a result of LL-37-mediated down-regulation of TLRs [30].

CD8+ T-cells

Cytotoxic CD8+ T-cells have been repeatedly associated with MS plaques and EAE pathogenesis. These cells express the glycoprotein CD8 and recognize targets through peptide: MHC class I presentation [2]. Notably, associations were found between HLA-A*02 and HLA-C*05 and protection from MS ($p=7 \times 10^{-12}$ and 3.3×10^{-5}, respectively) [8,9].

A couples of studies showed that the presence of HLA-DRB1*15 and absence of HLA-A*02 in obese or smoking patients increases the risk of MS (OR= 16.2 and 13.5, respectively), while the absence of those genotype correlated with a much lower risk (OR=3.7 and 1.4, respectively) [76,77]. If anything, this indicates that the presence of HLA-A*02 may confer resistance to provocative stimulation through obesity or smoking in MS.

HLA-C*05 belongs to group 2 of HLA-C alleles that interact with killer immunoglobulin-like receptors (KIR) [78]. The latter are a family of receptors expressed on NK cells and T-cells and play regulatory roles in their activation [9]. The KIR2DL1 inhibitory receptor and the KIR2DS1 activating receptor identify molecules of the C2 group [79]. Patients with psoriatic arthritis that express either the homozygous HLA-C1 group or homozygous HLA-C2 group show less inhibitory KIR activity. Similarly, patients with type I diabetes that express HLA-C1, but not HLA-C2 or HLA-Bw4, show less inhibitory effect of KIRs [80]. Thus, alleles of the HLA-C2 group may confer a protective effect on MS progression through increasing the inhibitory activity of KIRs. Unfortunately, effects of the HLA-C2 genotype have not been valued in MS [9]. This could be of value, although Björkström et al found the expression of KIRs and its functional modulation on CD8+ T cells are independent of the HLA-class I expression [81].

Macrophages

It has already been established that macrophage and microglia induce inflammation of the CNS. Paradoxically, these cells have also been associated with functional recovery and nerve regeneration. Such debate in macrophage/ microglia function in the CNS was speculated to relate to the way of their activation: classically activated cells promote inflammation and demyelination, whereas alternatively activated cells promote functional recovery and nerve regeneration [82].

Data have demonstrated the presence of significant amounts of myelin and neuro-antigens captured by macrophages in the peripheral cervical lymph nodes of MS patients. Notably, myelin is captured by pro-inflammatory cells and neuro-antigens are captured by anti-inflammatory cells. Thus, both types of macrophage are present in MS [3].

Classical activation results in the secretion of key pro-inflammatory cytokines, such as IL-1β and TNF-α. Important cell surface markers expressed upon such activation include MHC class II, CD80, CD86, CD16/32, CD58 and CD40. More so, this is accompanied by enhanced inducible nitric oxide synthase (iNOS) activity. Th1-derived IFNγ is the primary signal for activation, whilst the secondary signal is transuded upon cognate CD40: CD40L conjugation. As a result, macrophages upregulate CD40 and TNF receptor expression to magnify activation signals. Interestingly, a polymorphism in the gene encoding *CD40*, rs6074022 C, has been shown to enhance CD40 translation, and is associated with MS susceptibility [3,82-84].

More so, several studies have found IL-1β and TNF-α to be significantly elevated in MS patients, especially in progressive forms [85-87]. IL-1β induces astrogliosis in the CNS, which in turn responds by producing IL-6. In the presence of TNF-α, INF-γ, and IL-6 receptors, the effects imparted by IL-6 are magnified. IL-6 synergies with TNF-α and INF-γ to further induce IL-6 production. Collectively, this collaborative activity results in an increased level of inflammatory cytokines to further sustain inflammation [87].

Obese people have decreased serum levels of adiponectin, a cytokine derived from adipocytes. Adipokine (ADPKO)-knockout EAE mouse models had greater levels of TNF-α, INF-γ, and IL-6, indicating a possible inhibitory role for adipokine on the induction of these particular cytokines. Furthermore, these knockout mice had higher inflammation, demyelination, and axonal injury rates [88]. Moreover, obesity has been correlated with vitamin D deficiency, a known modulator of MS [89-91].

Further, a genome-wide association study found several SNPs within the CD86 locus to be associated with MS ($P<5.70 \times 10^{-08}$, OR>1.02) [15]. This particular cell surface protein imparts a co-stimulatory signal vital to T-cell activation and survival [92]. Also, CD58, which strengthens the adhesion between macrophages and T-cells through binding CD2, has been associated with MS ($p=2\times10^{-9}$, OR>1.18) [6,15].

In a separate study, a 14 repeat allele of the (CCTTT) marker (within the *NOS2A* gene) was shown to be associated with protection from the disease. Not surprisingly, this repeat was already known to correlate with protection from retinopathy, reduced prevalence of renal complications, and reduced prevalence of hypertension [93,94].

It has, also, been noted that miR34a, miR155 and miR-326 are up-regulated in MS active lesion targets. Such molecules target CD47, a known inhibitor of macrophage and DC cytokine synthesis. Consequently, up-regulation of these molecules releases macrophages from their inhibitory status and enhances phagocytic activity towards myelin sheaths [14,36,95].

Moreover, microglia and macrophages have been implicated in the initial tissue damage mediated by oxidative bursts. The latter in turn cause mitochondrial injury and disturbance of the oxidative respiratory chain. In concordance, increased expression of the Nox2 complex was noted in acute MS lesions, specifically in the 'pre-phagocytic area'. Also, peroxynitrite, a mediator of oxidative tissue damage, was found in active lesions. Notably, macrophages phagocyting myelin debris show lower expression of p22phox and gp91phox, components of the classical Nox2-dependent oxidative burst. Effectively, this implies that uptake of myelin debris converts macrophages from a pro-inflammatory to an anti-inflammatory status. To an extent, this concurs with earlier findings of both subpopulations of macrophage/microglia in active lesion sites [96].

In another study, COX5b expression was found to be down-regulated in MS patients both at the protein and mRNA levels. This protein is a component of complex IV within the electron transport chain. COX5b protein fractions obtained from MS patients were smaller in molecular weight compared with controls, owing to the loss of the 5b subunit. Such a loss can impair assembly and maintenance of complex IV. Impairment of this complex may lead to dysregulation of cytochrome c oxidase, a component that imparts protection from highly reactive peroxynitrite [97].

In addition, a haplotype that contains A9055 from *complex V*, G10,398 from *ND3*, and C14,798 from *cytochrome c* was found to correlate with an increased risk of MS ($p<0.0001$). The effect of such a haplotype is thought to be mediated by increasing endogenous free radical production, resulting in oxidative damage, impaired oxidative phosphorylation and neuroaxonal loss in MS lesions [98,99].

CNS demyelination and neurodegeneration

B-cells

Several reports have implicated B-cells in the pathology of MS. This is evidenced by the presence of B-cells, plasma cells, Ig, and complement deposition in MS lesions. Further, the effectiveness of Rituximab on MS patients incriminates B-cells. Rituximab is a monoclonal antibody directed against CD20, a marker expressed on almost all B-cell lineages except pro-B cells, plasmablasts, and plasma cells [3].

Activation of naive B-cells occurs via two means: through antigen-specific Ig receptors on B-cells, or CD4+ T-cell antigen presentation mediated by CD40-CD40L. As a result, activated B-cells proliferate and develop into antigen-specific memory B-cells. CD40-CD40L and CD28-B7 conjugation induces B-cell anti-inflammatory cytokine production, namely: IL-10 and TGF-β. The latter inhibit T-cell proliferation. However, IL-10 producing cells were found to be deficient in MS patients [3]. As discussed previously, polymorphisms in the *CD40* gene may enhance CD40 translation, thereby enhancing activation of those cells [83]. More so, Owens et al. found a significant over-representation of the H and L chains of Ig receptors on B-cells of MS patients. Effectively, this may increase the possibility of Ig recombination and antigen identification [100].

CXCL13 acts to recruit B-cells into the CNS. CXCL13 binds CXCR5 on B-cell and T-cell subsets. Notably, CSF from MS cases had significantly higher levels of CXCL13, and the levels correlated with B-cell number and increased disease activity [3]. Additionally, associations were identified between rs630923, located in the *CXCR5* locus, and MS ($p=2.80 \times 10^{-07}$, OR=1.13) [15]. rs630923 is located at a transcription factor binding site for NFκB in a region of DNase I hypersensitivity, and is predicted to alter *CXCR5* transcription [101].

It is not clear what exact antigen drives B-cell activation. It is also unclear whether this activation happens peripherally or in CNS. However, several studies have alluded to the role of Epstein Barr virus (EBV) infected B-cells. Antibodies directed to this virus can persist for years and correlate with raised gray matter atrophy and loss of brain volume in MS. Infected cells can secrete B-cell–activating factor of the TNF family (BAFF), which induces B-cell survival and differentiation. Also, BAFF promotes plasma cell and memory B-cell prolonged survival in meningeal follicle-like structures, and causes T cell–independent B-cell dys-regulatory immune responses. More so, EBV-infected cells produce large numbers of small RNAs called EBERs that induce apoptotic resistance in B-cells. EBERs bind retinoic acid-inducible gene I (*RIG-I*) and *TLR-3*. Upon binding, EBERs induce production of type 1 INFs and other pro-inflammatory cytokines [1,3].

It is also thought that the relapse phase of MS is caused by periodic reactivation of EBV and related CD8+ T-cell responses. Moreover, the pentapeptide sequence of the nuclear antigen is homologous to the MBP epitope. Also, the α-B crystalline surface antigen of EBV is similar to that of an abnormal auto-antigen expressed in the MS brain. Thus, memory B-cells that persist for years and produce anti-EBV antibodies can react with CNS antigens and cause demyelination and tissues damage even after clearance of infection [102-105].

α-B crystallin is a stress protein that accumulates in the oligodendrocyte-myelin unit and induces potent T-cell responses. Significantly elevated levels of this molecule have previously been reported in MS brains. This protein is encoded by the *CRYAB* gene located at 11q22.3-q23.1. Two polymorphic characters of this gene (CRYAB–249*G and CRYAB– 652*A) were associated with more inflammatory responses on MRI, MS early age of onset and slower disease progression. Having these two SNPs in the promoter region of the gene may allude to increased transcription of the gene and increased α-B crystalline levels in CNS of MS patients [105-107].

Moreover, hypomethylation in the peptidyl arginine deaminase 2 (*PAD2*) gene promoter was illustrated in MS white matters. This results in increased citrullination of MBP and myelin instability in MS, and may induce production of anti-MBP in CNS [36].

In Kuwait and the United Arab Emirates (UAE), significant number of patients infected with EBV was found within the MS populations [108,109].

Oligodendrocytes

Myelin production is a function of oligodendrocytes. These large cells have few processes that wrap around nerve axons, producing multiple lipid bilayers with their plasma membranes, known as myelin sheaths [110]. In the case of direct attack against myelin sheaths mediated by active T-cells, demyelinated sites would rapidly remyelinate and some oligodendrocytes are spared from destruction. However, in some cases, severe demyelination may occur with complete loss of oligodendrocytes and lack of remyelination. These cases may result from pathological mechanisms that effectively destroy all oligodendrocytes with their progenitors, due to defects in the oligodendrocytes' progenitors or antigen-mediated immune responses against these cells [111].

Demyelination in the CNS occurs by several means. A well-studied mechanism involves DC-mediated activation of T-cells specific for myelin oligodendrocytes glycoprotein (MOG) autoantibodies [112]. These autoantibodies are directed against myelin sheaths and oligodendrocytes and act directly with activated T-cells. They cause severe demyelination with complete loss of oligodendrocytes and remyelination [111].

Interestingly, studies conducted on *TLR-7-/-* mice have shown a decline in MOG autoantibodies in circulation, MOG- derived T-cell activation, and attenuation of inflammation in CNS in comparison with wild type (WT) mice. More so, Foxp3+ Treg cells numbers were raised in both the periphery and CNS [112].

More so, in attempts of remyelination, some stressed oligodendrocytes are prone to γδ T-cell destruction in association with stress proteins, such as Hsp65 or α-B crystallin. Other studies propose that insufficient growth factors may result in apoptosis. Also, oligodendrocyte destruction could result from viral infections, toxins, or dysregulation of microglia or macrophages. In those cases, primary oligodendrocyte destruction may result in secondary oligodendrocyte destruction [111].

Evidence of up-regulated miR-155, miR-338 and miR-491 expression in cerebral white matter of MS patients can further explain reduced neuronal generation in some patients. These miRNAs suppress essential neurosteroid production [14,113].

Essential to neuronal development, proliferation, survival, differentiation, synaptogenesis and maturation are neuronal growth factors. The latter function through association with cognate receptor tyrosine kinases, one of which is the anaplastic lymphoma kinase (ALK). In fact, an association was found between MS and a genetic polymorphism (rs7577363) in the *ALK* gene locus ($p=1.14 \times 10^{-4}$, OR=2.1) [6,114].

Additionally, observation of high levels of histone acetylation was noticed in NogoA+ oligodendrocytes in concordance with increased levels of transcriptional inhibitors of oligodendrocytes differentiation in aged MS patients and those with chronic MS. In contrast, early MS lesions had clear reduction in oligodendrocyte histone acetylation. In agreement, increased histone acetylation was observed in MS chromatin in close proximity to the *TCF7L2* gene promoter, a key gene in oligodendrocytes differentiation [14,36,63].

Neurons

Axonal viability has been related to *ACCN1* gene expression, as any alteration can result in changing the composition of heteromeric acid-sensing ion channels and further excessive accumulation of ions in axons. As a matter of fact, a SNP (rs28936) located downstream of the regulatory element of *ACCN1* gene that may result in differential expression of the gene was formally associated with MS (p=0.002, OR=2.1) [11,115].

Another potential player in MS neurodegeneration is the *PGC-1α* gene, which was found to be downregulated in MS grey matter. This mitochondrial gene reduces the expression of oxidative phosphorylation subunits and various mitochondrial antioxidants and UCPs 4 and 5. More so, silencing of this gene can result in a decreased mitochondrial membrane potential, increased ROS formation and ROS-induced cell death [116].

Also, UCP2, which prevents neuronal death and injury, has been correlated with MS susceptibility in a German population. The G allele of -866G/A, present in the promoter region, was the associated factor. This allele relates to the lower levels of UCP2 expression. Hence, this can in part explain the resultant enhanced neuronal injury and death [117].

Glial cells

One interesting study performed on mouse models to assess the function of Schwann cells on hematopoietic stem cell (HSC) hibernation in bone marrow (BM) defined the regulatory role of TGF-β. Schwann cells of the periphery nervous system (PNS) are identical to CNS astrocytes or astroglia. Astrocytes function to support the endothelial cells of the BBB. Accordingly, astrocytes have direct contact with immune cells. The respective team found that non-myelinated Schwann cells (also referred to as glial cells) that express GFAP can activate latent TGF-β excreted by various BM cells to the extra-cellular matrix (ECM). Activated TGF-β binds the receptor Smad (R-Smad) 2/3 and subsequently induces the expression of several genes. An important target is a cyclin dependent kinase inhibitor, *p57Kip2*. The latter is highly expressed in HSCs and is thought to maintain their dormant state [118].

Intriguingly, denervation of lumber sympathetic nerves results in a significant decrease in GFAP-glial cells, active TGF-β, and HSCs count (reached 1/5 by day 7); the remaining HSCs were committed to the cell cycle. More so, transplanting these particular HSCs into lethally irradiated CD45-congenic mice resulted in a significant drop in BM HSCs [118]. All in all, one can conclude that denervation of MS can result in the possible loss of CNS GFAP-glial cells and active TGF-β, thus loss of the inhibitory regulation of HSCs hibernation. This means that more HSCs can commit to the cell cycle and differentiate into aggressive immune cells, hence inducing further immune responses towards the CNS.

Attractively, the *ZMIZ1* gene, responsible for the regulation of various transcription factors of which R-Smad 3/ 4, was associated with risk of MS in a couple of studies (OR>1.10) [15,119].

Prospective

MS is a complex disease, the etiology of which is not yet clearly defined yet. This is a concern because many influencing factors have already been shown to correlate with the disease. Genetics, epigenetics, and mitochondrial genome variants can and do affect cellular commitments; however, looking at the molecular level does not suffice to explain the full picture of disease pathogenesis. One must consider molecular, environmental and immunological variables to elucidate the pathogenesis of such a disease (Figure 2).

Environmental modulators like vitamin D, EBV infection, smoking and obesity, influence disease pathophysiology through modulation of gene transcription and various cellular processes, hence influencing susceptibility to MS. In AGCs, vitamin D deficiency, smoking and obesity are shockingly popular, providing insight into the rise in the number of MS cases in these countries [1].

In the last few decades, research has primarily focused on cellular subsets, their components, their effectors and commitment. More so, the focus of association studies has been on particular genetic, epigenetic, and mitochondrial genomic variants. In the future, further research is required into the collective factors behind MS, including environmental triggers, molecular mutations, polymorphisms, and expression defaults. Importantly, it would be critical to elucidate the effects of altered expression on disease pathogenesis with respect to each stage of the disease.

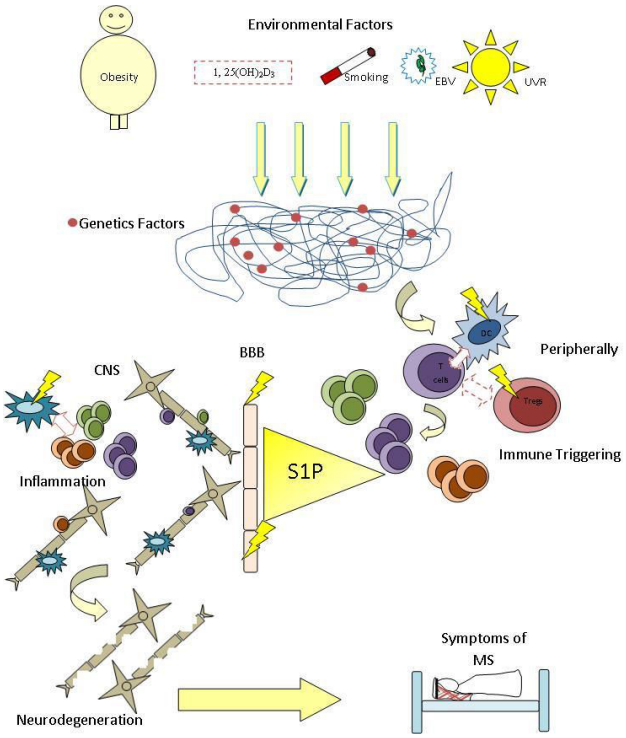

Figure 2: A summary of MS pathophysiological process. Environmental factors; obesity, vitamin D, smoking, EBV, and UVR, all play integral role in MS stimulation, through affecting vital genes regulations; genetic predisposition of patients can further strengthen disease intensity. Immunological factors thereafter are activated and released to attack CNS. Migration of immune cells to CNS through the BBB is facilitated by both S1P gradient and ICAM-1 and VCAM-1. Immune cells in the CNS will trigger further recruitments of attack molecules and results in inflammation and neurodegeneration. Finally, symptoms of MS start to accumulate and threaten patients' life.

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