Chapter 8
Multiple Sclerosis and Microbes

8.1 Introduction

Multiple sclerosis [MS] is a chronic demyelinating, immune-mediated disorder of the brain and spinal cord of unknown etiology. Although MS is not considered a common disease as such, it is not a rare disorder and it is estimated to be the second commonest cause of neurological disability after traumatic injuries. There is a marked geographical variation in the incidence of the disease, more prevalent in temperate regions and rare in tropical and subtropical countries. In the United States MS affects about 35,000 persons and worldwide 2.5 million people are afflicted [1]. In most temperate regions of the world [North America, Northern Europe, Southern Australia, and South New Zealand] the prevalence of MS is 0.1–0.2 % of the population, whereas in the tropics and Middle East the prevalence is 10- to 20-fold less [1]. The age of onset of MS is typically between 20 and 40 years, about 5–10 % occurs in children less than 18 years, and women are affected threefold more common than men.

Based on the geographical variation it has been proposed that sunlight exposure is protective through the production of vitamin D. Low serum vitamin D levels are common in the population of temperate zones, and prospective studies show that vitamin D deficiency is associated with a greater risk of MS and for relapses [1]. The clinical and pathological extent of MS is very variable and heterogenous, and the course is often characterized by spontaneous relapses and remissions early in the disease.

8.2 Pathobiology of Multiple Sclerosis

Initially in the early stages of MS, patchy inflammation with focal lymphocytic infiltration is the primary pathological feature in the brain, which leads to damage of the myelin and axons [2]. The inflammation is often transient with remyelination and recovery of neurological dysfunction early in the course. Over time the predominant

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pathological changes consist of widespread microglial activation with extensive chronic neurodegeneration and plaques of demyelination [2]. Normally myelin is produced by mature oligodendrocytes which are adjacent to axons of the white matter tracts in the central nervous system [CNS].

It is believed that MS is initiated by some environmental factor that stimulates autoreactive lymphocytes in the CNS. Although MS is considered an autoimmune disease, transfer of antibodies directed against self-antigens [identified in MS] failed to cause MS-like disease in animals [3]. Despite that, T-cells reactive to myelin components, especially myelin basic protein [MBP], are activated in MS patients but not in controls [4]. There is some evidence that uncontrolled autoreactive lymphocytes may induce inflammation [predominantly by perivascular CD8+ cells] to cause neuronal damage due to dysfunction of regulatory lymphocytes and regulatory mechanisms in the CNS of MS patients [5]. Failure of regulatory lymphocytes to suppress autoreactive T-cells appears to be related to overexpression of β-arrestin1, which is a promoter of naïve and activated CD4+ T-cell survival [6]. Previous animal models of experimental allergic encephalomyelitis had supported a critical role of Th1-type γ secreting cells [3], but recent studies indicate that the inflammation in MS is driven by T-lymphocyte subtype that secretes interleukin [IL]-17 under IL-23 control [7].

Myelin proteins may not be the only target of autoreactive lymphocytes, and there is evidence that antibodies against neurofascin may mediate axonal injury in MS [8]; and autoimmune response against αB-crystalline prevents counter-regulatory suppression of inflammation [9]. Cortical biopsies of brain lesions in early MS have revealed perivascular inflammation with CD3+ and CD8+ T cells in the majority of cases [which were highly inflammatory], and 27 % of cortical plaques also contained B-cells [10]. In this study [10] cortical demyelination was present in 40 % of patients and 66 % of the lesions contained foamy macrophages, and all had activated microglia, indicating ongoing demyelination.

8.2.1 Pathogenesis of Multiple Sclerosis

The pathogenic mechanisms of MS are complex and involve multiple genetic and environmental factors. Epidemiological studies have implicated increased risk of MS by gender, sex hormones, ethnic origin, geographical location/latitude/distance from the equator, smoking, viral exposure, and vitamin D status [2, 11–13]. In family studies, first-degree relatives showed a 20- to 40-fold increased risk for MS, and identical twins display 300-fold increased risk over the general population [14]. Genetic studies have reported that genes in the major histocompatibility complex [HLA] region are associated with MS [15]. The primary association was with DRB1 gene in African Americans and individuals of European descent [16, 17]. Further genome-wide association studies have reported about 50 genes associated with MS [18]. However, MS concordance rate in monozygotic twins is only approximately 30 %

and this suggests that environmental factors have a major influence on genetic trait. This would be consistent with epistatic interaction, where two or more independent factors promote disease only when combined [19]. In a large genome-wide association study, DRB1 risk alleles had the strongest association with MS, and HLA-A gene variation had a protective effect [18]. A multitude of genes encoding cytokine pathways and immune related mechanisms were overrepresented, particularly those implicated in T-helper cell differentiation, and acting on cell surface receptors. This study also implicated genes encoding pathways for vitamin D function and targets for therapies for MS, such as VCAM1 [natalizumab] and IL2RA [daclizumab] [18]. Several of the genes are also associated with other autoimmune diseases [IL2RA and IL7RA], and these pathways are involved in regulation of autoimmunity in animal models [20, 21]. It has also been suggested that several genetic variants [IL7RA, IL2RA, MGAT1, and CTLA-4] lead to dysregulation of N-glycosylation that cause pathogenesis in MS [22]. Faulty N-glycosylation of cytotoxic T lymphocyte antigen 4 [CTLA-4] and T-cell receptor [TCR] generates T-cell hyperactivity and promotes autoimmunity in mice, which induces a spontaneous MS-like disease [23, 24].

It has also been proposed that environmental factors may regulate disease manifestation by modulating the epigenome in MS to promote changes in the immune system and brain [25]. These epigenetic mechanisms include DNA methylation, regulation of noncoding miRNAs, and post-translational modification of histone that can be affected by smoking, diet, exercise, and possible previous infection.

8.2.2 Hypovitaminosis D in MS

Geographical regions of the world with limited sunshine for 4–6 months of the year, beyond the 40th parallels North or South, are those with the highest prevalence of MS [26]. Several epidemiological studies have found a vitamin D insufficiency in the great majority of MS patients, including in the early stages [27]. Vitamin D receptors are present in numerous tissues and cells, including circulating immunity cells [lymphocytes, macrophages, and monocytes], brain [microglia], intestine, bone, kidney, gonads, breast, pancreas, and cardiovascular tissues. Besides its classic role in calcium homeostasis and related metabolic functions, vitamin D and its active metabolite 1,25-dihydroxy vitamin D have other important functions: anti-inflammatory, anti-infective, immunomodulatory, antiproliferative, and neurotransmitter, which may be involved to prevent many autoimmune diseases, including MS [27]. The major action of vitamin D that may be important in the pathogenesis of MS is through immunomodulation. Hypovitaminosis D may affect cell proliferation of CD4+ T cells, the proportion and function of regulatory T lymphocytes [28–30]. Thus, in the final global analysis, the current data is supportive of hypovitaminosis D as a risk factor for MS, but acting in combination with other environmental factors in the genetically predisposed individuals.

8.3 Role of Microbes in Multiple Sclerosis

Viruses have been suggested to play a role in the development of MS since the early 1990s, and this debate still continues today [31]. There are several lines of evidence that link viral infections with MS and these include: (1) epidemiological evidence which consistently demonstrate increased risk of MS with some past infections; (2) the CD8+ lymphocyte infiltration in MS lesions is consistent with a viral-immune response; (3) and the CSF oligoclonal IgG bands typically present in MS are also present in CNS viral infections. Moreover, many naturally occurring demyelinating CNS diseases of animals and humans are of known viral origin. This topic of virus-induced demyelination was previously reviewed in 2003 [32]. There are two primary mechanisms by which viruses produce demyelinating CNS disease. The first of these is by an autoimmune process, as exemplified by postinfectious [also postvaccination] encephalomyelitis, which is preceded by a viral infection [i.e., an exanthema] with no evidence of direct invasion of the CNS [33]. For some viral demyelinating diseases there is direct CNS invasion and replication of the agent, with neuronal and axonal pathology. A very rare but well described example is subacute sclerosing panencephalitis due to chronic measles infection of the brain at an early age, <2 years old [33]. In this condition the measles virus can be recovered from the brain and histology demonstrates cytoplasmic and nuclear inclusion bodies, with signs of astrocytes and microglia activation and neuronal loss. Measles is also one of several viruses that can present more acutely with postinfectious encephalomyelitis.

In postinfectious encephalomyelitis the hypersensitivity reaction may occur both against viral and host antigen. In a study of measles postinfectious encephalitis, immune response was demonstrated to MBP with early destruction of myelin in about 50 % [34]. Other viral infections associated with demyelination include JC-virus in progressive multifocal leucoencephalopathy, seen mainly in immunosuppression, human immunodeficiency virus [HIV] itself in subacute HIV encephalopathy, and the human T-cell lymphotropic virus type 1 [HTLV-1]-associated myelopathy affecting the spinal cord [32]. There are also several naturally occurring animal viruses that produce demyelinating CNS disease, some of which are used as animal models to study the biology of MS. These include Theiler's virus, neurotropic strain of mouse hepatitis virus, and Semliki Forest virus [32].

Exposure to microbes in early childhood has also been proposed to influence the development of autoimmune disorders such as MS, the hygiene hypothesis. This could explain the geographical differences in incidence of MS, greater in developed nations of the temperate zones with advanced hygienic communities compared to poorer countries in tropical and subtropical regions with substandard hygiene, but lower rates of MS. There are also marked differences in the incidence of MS in persons migrating from one country to another in which the rates are different. In Israel, MS is common in immigrants from Europe and rare among immigrants from Africa or Asia, whereas in native born Israelis of African or Asian descent have similar rates of MS as the European migrants [35]. These differences cannot be

explained by sunlight exposure or genetic factors. Similar trends have been reported in US migrant studies with a large number of MS cases [>5,000] [36]. It is postulated that multiple infectious exposures [even carriage of parasites in the gut] could reduce the risk of MS by modulating the immunity toward helper T cells [Th]2 and regulatory T cells, with attenuation of the proinflammatory Th-1 cellular immunity [37, 38]. Decreased antigenic stimulation from low childhood infections and reduced microbial exposure burden may result in decreased levels of regulatory cytokines, Il-10, and transforming growth factor B[TGF-B], which are produced by CD 25+ T cells and other regulatory T-cells, to downregulate both Th-1 and Th-2 mediated immune responses [39]. Intestinal helminth, which induces predominantly Th-2 response, has been reported to produce a beneficial effect in patients with MS [40, 41].

8.3.1 Specific Microbes

The role of microbes in autoimmune disorders is complex, some infections can trigger autoimmune responses and others may prevent these reactions [37]. This could be related to the microorganism itself, host genetic trait, age of onset, and epistatic effect with other environmental factors.

8.3.2 Epstein–Barr Virus

Epstein–Barr virus [EBV] is strongly implicated as playing an important role in the pathogenesis of MS. EBV is a human herpesvirus that infects B-cells in nearly 95 % of the population and persists latently in the memory B-cell pool for life. It was proposed more than 50 years ago that MS may be caused by infection that is harmless to the host and confers protective immunity when acquired in early childhood, but become pathogenic later in life [42]. This could apply to EBV infection and the pattern of infection between resource endowed and resource deprived countries of the world could explain the discrepancy in geographic distribution of MS. Almost all children are infected with EBV at an early age in developing countries of tropical and subtropical regions of the world, with a seropositivity of >90 % by 4 years of age, whereas in Europe and the United States only 30–40 % are infected at the same age [42]. The prevalence of age-related EBV infection is also increased with lower socioeconomic status and overcrowding. Infection in later years is more commonly manifested by symptomatic disease and pathology, such as clinical infectious mononucleosis, peak incidence 15–25 years of age in developed countries, whereas infection at a very young age is largely asymptomatic [43]. The epidemiology of MS in developed countries is strikingly similar to that of infectious mononucleosis with respect to age of onset [44]. MS risk in EBV-negative adults is extremely low but is increased in those with previous infectious mononucleosis. There is a 20-fold increased risk of MS in adolescents

and young adults with a history of infectious mononucleosis compared to those who are EBV negative, even for similar childhood hygienic environment [45]. In a meta-analysis of 13 case–control studies comparing MS patients and matched controls, 99.5 % of MS patients were EBV seropositive compared to 94 % of controls, but the risk of MS in EBV-seronegative subjects was extremely low, odds ratio 0.6, highly significant [46]. The mean interval between primary EBV infection and onset of MS [during the vulnerable age of 15–40 years] is estimated to be 5.6 years [47]. A clinical history of infectious mononucleosis increases the risk of MS more than twofold with a relative risk of 2.3 [48].

A few prospective studies have reported that elevated IgG antibodies to EBV nuclear antigen-1 [EBNA1] were found to increase the risk of MS [49–51]. In a study involving US military personnel before onset of MS, high serum titers to EBNA1 increased the risk 36-fold for developing the disease later [51]. Seroepidemiological studies of EBV in children with MS and matched controls have shown a similar pattern as in adults but somewhat less robust. EBV-seropositivity rate in MS children varies from 86 to 94 %, compared to 64–72 % in age-matched controls [52–54]. It can be argued that EBV infection is not essential for development of MS, as 14 % of children with this disorder were EBV seronegative [53]. However, in a recent meta-analysis of 22 adult and 3 pediatric studies on the risk of development of MS in EBV seronegative individuals, it was concluded that EBV appears to be present in almost 100 % of MS patients [55].

The increased synthesis of antibodies in the CSF as reflected by the corrected Antibody Index [56], also support a role of EBV in the pathogenesis of MS [57–59]. CSF oligoclonal IgG bands in MS patients predominantly consist of antibodies against EBV proteins, EBNA1 and BRRF2 [58]. However, increase in CSF antibodies to other viruses [measles and rubella] has also been reported [56, 57]. It is proposed that EBV infection of the CNS could stimulate dominant anti-EBV antibody response and promote synthesis of other viral antibodies by infecting B-cells [60].

A key issue in determining the pathogenic role of viruses in MS is the presence of the microorganism in the CNS and demyelinating plaques. Various studies have examined brain tissue samples for EBV by different methods, such as in situ hybridization, immunohistochemistry, and PCR, in subjects with MS with conflicting results. Serafini et al. [61] previously reported that B-cells and plasma cells of 21 of 22 MS brain sections had detectable EBV by in situ hybridization and immunohistochemical stains. However, Willis et al. [62] detected EBV in only 2 of 24 MS brains by real-time [RT] PCR and none by in situ hybridization nor immunohistochemistry. A subsequent focused workshop was held in Vienna in 2011 to review the data of EBV in MS brain [63]. Overall most studies using PCR for EBV detection from brain sections or CSF failed to detect the virus, except on rare occasions. The data on detection of EBV by in situ hybridization and immunohistochemistry were more mixed with varying results [63]. Hence, unequivocal proof that EBV exist in the brain lesions of MS patients, compared to EBV-related tumors, is still lacking.

The detection of EBV DNA in the blood of MS patients and matched controls by PCR methods also had been reported. In one study from Spain, EBV was detected in the blood of 70/75 [93.3 %] MS patients versus 123/186 [66.1 %] of controls,

$p<0.001$ [64]. Moreover, dual infection with types 1 and 2 EBV was detected in 63 [90 %] of MS patients and only 37 [30 %] of controls, $p<0.001$. In contrast, in a nested study from Australia with 215 MS cases and 216 controls, detectable EBV in blood was similar [55.8 and 50.5 %], and there was no difference between the two groups in EBV DNA load [65]. However, similar to other reports, past history of infectious mononucleosis, high anti-EBV titers, and HLA-DR B1 status had additive risk for MS.

8.3.3 Human Herpesvirus-6 in Multiple Sclerosis

Human herpesvirus-6 [HHV-6], which causes roseola in early childhood, infects >90 % of the population and remain latent probably in lymphocytes. The virus has been associated with meningoencephalitis in immunocompromised hosts on occasion [66] and also has been associated with MS. There is evidence that there is cross reactivity with MBP and HHV-6 in MS patients, which could activate autoimmune reactivity through molecular mimicry [67]. Phosphorylation of HHV-6 protein U24 may confound signaling and other pathways normally utilized by phosphorylated MBP that could precipitate the pathological process in MS [68]. Some studies have reported evidence of HHV-6 reactivation with MS activity but others have not. In one study measuring viral mRNA in peripheral blood mononuclear cells [PBMC] by RT-PCR, and plasma IgG and IgM antibodies, the prevalence of HHV-6 active infection was significantly higher in MS patients than other neurological diseases and in blood donors [69]. Moreover, there was correlation with reactivation of HHV-6 and with relapsing and progressive MS. In another study with 1 year of follow-up, serum samples were analyzed by quantitative PCR to assess HHV-6 prevalence and viral load. Among 63 patients with relapsing–remitting MS only 19.1 % of samples in relapse had active infection compared to 7.9 % of samples in remission [70]. HHV-6 DNA was found in 16 of 64 [25.4 %] MS patients at least once but in none of 63 healthy blood donors, $p=0.04$ [71]. In another report from the same group of investigators, only 16 % of 105 MS patients had active HHV-6 infection versus 0 of 49 healthy controls, but the viral load was higher during attacks than during remission, $p=0.04$ [71]. A subsequent study of 57 MS patients and 57 controls followed for a year also found that reactivation or new infection with HHV-6 variant A was related to relapse of symptoms, with a prevalence of 80.7 % in MS cases and 29.8 % in controls [72]. These investigators also assessed the effect of beta-interferon [IFN-β] treatment on HHV-6 viral load in MS subjects. Treatment with IFN-β was given to 105 patients and 84 were untreated; the viral load of HHV-6 was twice as high in untreated than treated cases in relapse [73]. IFN-β treatment reduced HHV-6 viral burden in patients in relapse but not in remission.

Other investigators have reported discrepant results of the presence of HHV-6 in MS. In the Finnish twin study of 17 MS twin pairs, serum and CSF were analyzed for HHV-6 DNA by PCR and for IgG and IgM antibodies [74]. The prevalence of antibodies was similar between twins with MS and healthy twin siblings, 88 and 86 %;

and there were no detectable antibodies in any CSF sample and no HHV-6 DNA was found in serum or CSF. Other negative studies for HHV-6 DNA in serum or CSF in MS patients have been reported by several groups [75–77].

Several studies have been performed to detect the presence of HHV-6 genome or antigen in brain lesions of MS patients with varying results. HHV-6 genome was reported in acute brain lesions of all five MS cases [78] and in 58 % of established plaques [79]. However, HHV-6 viral mRNA can be detected in both MS plaques and normal appearing white matter, although at higher levels than normal control samples [80]. In another report HHV-6 DNA was detected at similar rates, 41 and 44 %, and quantity in MS and control samples [81].

A previous systematic review of the association of HHV-6 and MS was reported in 2010. Overall, 25 of 61 [41 %] studies showed a significant positive correlation but only 15 of the studies were considered of high [A] quality [82]. Thus, the role of HHV-6 in the pathogenesis of MS remains unclear and correlation with disease activity is not robust.

8.3.4 Human Retrovirus in Multiple Sclerosis

Human endogenous retrovirus [HERV] genetic elements comprise about 1–8 % of the human genome and are believed to be remnants of ancestral infections of exogenous retroviruses during our evolution [83, 84]. HERVs are divided into specific families and may occur in up to 1,000 copies distributed throughout the human genome and inherited by a Mendelian pattern [85]. Although not replication competent, HERV genes may be intact and encode functional proteins [86]. HERV have been implicated in carcinogenesis and autoimmune diseases in both animals and humans [84]. In the late 1980s a novel retrovirus element was isolated from cells derived from CSF of a MS patient and was named MS-associated retrovirus [MSRV], and later was incorporated in the HERV-W family [87]. Although HERV elements are considered normal constituents of the human genome which are rarely expressed in cells, activation in cell culture to develop viral bodies [and possibly in humans] may be precipitated by environmental and endogenous stress. Activation could be an epiphenomenon after flares of inflammatory cytokines, but there is some evidence that specific HERVs may act as auto-, super-, or neoantigen that could enhance inflammation or induce autoimmune reactions [88]. In a humanized SCID mouse model, MSRV retroviral particles injection caused acute neuropathological changes with multifocal brain hemorrhages, mediated by the expression of inflammatory cytokines through T-cell stimulation [89].

Viral RNA from HERVs has been detected by reverse-transcriptase [RT]-PCR in blood and brain of MS patients, but not exclusively [88]. It has been postulated that herpesviruses may activate [transactivation] HERV-W particles and enhance immunopathological reactions in MS [90, 91]. In a recent in vitro study, EBV activated the potentially neuropathogenic HERV-W/MSRV/syncytin-1 in cells derived from blood and brain [92]. The authors proposed a model that include EBV as initial

trigger of future MS, and years later interaction of HERV-W/MSRV/syncytin-1 contributes to MS pathogenicity, paralleling the observed relationship of EBV infection in MS patients. Several studies have found elements of HERV-W family in blood, CSF, and brain lesions of MS subjects and significantly less in controls [93–95]. HERV particles in CSF of early MS cases, followed for 10 years, were associated with greater risk of disability and progression of disease in a small number of subjects [96]. Also B-cells and monocytes from patients with active MS exhibit increased surface expression and high antibody reactivities in sera to HERV epitopes, more than stable MS cases and controls [97].

However, other studies have failed to confirm the association of MS and HERV elements. In a study of 92 CSF samples, 48 from MS patients and 23 from other inflammatory neurological diseases, and 21 from patients with non-inflammatory CNS diseases no HERV sequences were found in any sample [98]. Analysis of humoral and cellular immune responses against MSRV/HERV-W antigens in 50 MS cases and 59 controls, in another study failed to detect any appreciable immune responses [99]. The majority of HERVs are present in 100 % of healthy humans and the paucity of functional genes argues against a causative role in disease. However, recently a new class of polymorphic HERVs has been described with widespread differences in geographic and racial distribution that could explain the geographic variation in MS distribution, if implicated in disease pathogenesis. A subtype, HERV-K113 is present in 0–28 % of humans and could be a disease causing HERV [100]. In a large family-based study, genomic DNA samples from 951 MS patients and 1,902 unaffected parents were tested for the presence of HERV-K113 allele by PCR [101]. HERV-K113 provirus was detected in only 70 of 951 [7.3 %] MS patients and 6.5 % in the parents, which did not support a role in MS. To cloud the issue further, another member of the HERV-K family has been reported to be associated with MS in a large study population. HERV-K18 is considered an EBV-associated superantigen and is a plausible candidate to influence the genetic susceptibility to MS. In a nested case–control study of 207 MS cases and 403 matched controls, with analysis replicated in 909 MS patients and 339 controls, risk of MS was threefold higher in individuals with HERV-K18 env alleles [102].

8.4 Conclusion

Microbes, especially viruses that remain latent in the host for life, are biological plausible triggers or key factors in the development and pathogenesis of MS. This maybe through molecular mimicry, alteration of the immune response to other antigens, and by genetic influence [through HERV elements], acting in a manner similar to gene variation such as with single nucleotide polymorphism. However, it is difficult to explain the discrepancies noted with different viruses in various studies and their findings on the relationship with MS. The most likely explanation is the difference in methods of detection used, which are not standardized or commercialized but locally developed without independent validation.

The present data indicate that the timing of primary EBV infection at a certain age or period in life [teenage to young adulthood] in those genetically susceptible plays an important role in the development of MS. The exact mechanism of this relationship remains elusive. The hypothesis that genetic influence of ancestral endogenous retrovirus interaction with latent EBV is an attractive paradigm but remain unproven.

8.5 Future Direction

Further studies on the role of microbes in MS need to concentrate on the most attractive theory with the best data available, in order to expend valuable resources on a large, prospective cohort with observation over several years. This would preferably be implemented by an international collection of interested investigators, with different interest and expertise, using standardized or validated methods of investigation, repeated at intervals over the years from blood, CSF, and brain samples where feasible. Such a study would be best performed in subjects with strong family history or evidence of genetic predisposition to MS.

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