The Human Microbiome in Multiple Sclerosis: Pathogenic or Protective Constituents?

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ABSTRACT: The human microbiome is comprised of commensal and pathogenic microorganisms, which exert diverse effects in close proximity to the site of intection as well as in remote tissues through immune-mediated mechanisms. Multiple infectious agents have been implicated in the pathogenesis of multiple sclerosis (MS) with variable findings depending on the agent, techniques, and disease phenotype. Herein, the contributions of individual infectious agents to MS and their effects on the immune and nervous systems are reviewed, focusing on herpes viruses, coronaviruses, retroviruses, and synchronic infections. While infectious agents are often assumed to be pathogenic, their effects might also be beneficial to the host in the long-term, depending on age and the type of immunogen/pathogen exposure, as proposed by the hygiene hypothesis. The human microbiome has potential impact on future diagnostic and therapeutic issues in MS.

RÉSUMÉ: Le microbiome humain dans la sclérose en plaques : des constituants pathogènes ou protecteurs? Le microbiome humain est composé d'agents microbiens commensaux et pathogènes, qui produisent différents effets tant à proximité de leur lieu d'infection qu'à distance, au moyen de mécanismes à médiation immunitaire. Plusieurs agents infectieux ont été impliqués dans la pathogenèse de la sclérose en plaques (SP). Les observations sont différentes selon l'agent, les techniques utilisées et le phénotype de la maladie. Nous revoyons ici la contribution de certains agents infectieux dans la SP et leurs effets sur le système immunitaire et le système nerveux, particulièrement les virus de l'herpès, les coronavirus, les rétrovirus et les infections synchrones. Bien qu'on présume souvent que les agents infectieux soient pathogènes, leurs effets pourraient également être bénéfiques à l'hôte à long terme, selon l'âge et le type d'exposition à un agent immunogène/pathogène, tel que proposé par "l'hypothèse de l'hygiène". À l'avenir, le microbiome humain pourrait avoir un impact sur certains aspects diagnostiques et thérapeutiques dans la SP.

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Infectious agents as commensal or pathogenic microorganisms live within and outside of all mammals including humans[1], thus raising the possibility that resident microbes might contribute to the pathogenesis of immune-mediated diseases such as multiple sclerosis (MS). Resident microbes have the capacity to overwhelm the host or be displaced by other invading virulent microbes, which is not evolutionarily advantageous. However, these agents can also influence the host's immune response in both the short and long terms depending on the balance amongst the competing microbial populations and the host background genotype[2]. Several herpes viruses have utilized humans solely as hosts for millions of years and thus constitute part of the human microbiome without integrating into the human genome. Conversely, some retroviruses can be expressed millions of years after the initial integration event and remain part of the human metagenome through their ability to influence host responses[3,4].

The association of infectious agents with autoimmune diseases has been challenging because it is difficult to identify an infectious pathogen as an etiologic factor amidst heterogeneous clinical phenotypes and background genotypes together with geographic, socio-economic, and other demographic variables[5]. Moreover, many infectious agents are difficult to culture or detect precluding fulfillment of Koch's postulates[6]. Koch originally proposed: (A) an infectious agent must be consistently

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found in the disease lesions; (B) the agent must be isolated in pure culture; (C) *in vivo* inoculation of infectious material derived from the culture should cause disease; and (D) the agent must be recoverable from the infected host's ensuing lesions. However, it is clear that these circumstances do not apply to all infectious agents including viruses or bacteria. Although it might be evident that there is a cause-and-effect relationship between a pathogen and a disease, Koch's postulates are not always fulfilled; a prime example of this direct etiologic relationship is the discovery of *Helicobacter pylori* as a cause of peptic ulcers.

Host (immune) perturbations arising during "synchronic" (or time-linked) infections, usually prior to the disease onset, can exert immunopathogenic effects causing cellular injury or death. For example, group A beta-hemolytic *Streptococci* infection causing acute rheumatic fever in childhood can result in the development of Sydenham's chorea up to six months post-infection, presumably through the induction of pathogenic autoimmune responses,[7] while other synchronic infections including sepsis or pneumonia appear to exacerbate the severity of dementia[8]. Conversely, exposure to commensal microbes in the gut can influence the production and type of regulatory T cells, which might also influence the severity of neurological disease[9].

Two principal hypotheses link autoimmunity and infections: the "prevalence" or "polio" hypothesis states that the causative agent is more common in high risk areas. In contrast the "hygiene hypothesis" suggests that early infection confers protection against ensuing autoimmune disease while delayed or absent infection may increase the likelihood of autoimmunity. Several infections and vaccines in early life appear to protect against the development of multiple sclerosis, which might explain why MS is rare in developing countries[10]. Though various infectious agents have been linked to MS, their presence may simply reflect the appropriate environment for the development of an autoreactive immune response directed against central nervous system (CNS) antigens[11]. Application of sophisticated bio-chemical and molecular tools has led to identification of several viruses that exhibit an association with MS, although no pathogen has been accepted as the causal agent of MS. Mere association of a pathogen with a disease entity does not constitute causality –a fact that is often overlooked in studies of disease for which the underlying cause is enigmatic. In the present review, we focus on the contributions of exogenous and endogenous viruses to MS pathogenesis because of their apparent ubiquity and potential for pathogenic consequences.

Clinical and neuropathological features of MS

Multiple sclerosis is a common and usually progressive neurological disease, defined by several phenotypes (relapsing-remitting, primary progressive, and secondary progressive), which differentially affect multiple populations worldwide. It is usually diagnosed in the prime of life, between the ages of 20 and 40 years, and is associated with marked physical and cognitive disabilities[12]. The recent establishment of specific criteria for the diagnosis of MS has improved the inclusion requirements for clinical trials[13]. The neuropathological changes accompanying MS are diverse and have been characterized into different subtypes[14]. Focal demyelination and inflammation are the signature features of MS and involve the CNS at all levels. Accompanying this inflammation and demyelination, is the rediscovery of axonal and neuronal perikaryal injury[15]. Lesions in relapsing-remitting MS patients are usually found in the white matter and are characterized by disruption of the blood-brain barrier, as well as local edema and demyelination typical of inflammatory processes[16]. Conversely, in primary-progressive MS, inflammatory processes are less dominant but progression to disability and brain atrophy may evolve quickly[17]. Recent studies have highlighted the importance of cortical lesions in MS, including demyelination and neuronal loss[18]. Interest in the mechanisms by which demyelination and inflammation-related damage to axons and neurons occurs[19] has been complemented by neuro-imaging studies that suggesting axonal injury accompanied by cerebral atrophy constitutes the major mechanism by which physical disability progresses during MS[20,21].

MS pathogenesis

The contribution of immunopathogenic mechanisms as an etiologic determinant of MS is supported by large-scale genetic studies showing linkages to multiple immune genes, especially to the MHC loci on chromesome 6[22,23], and MS neuropathology, which implicates multiple immune cell types within the CNS and immune activation[24]. Much of the understanding of MS immunopathogenesis has been influenced by the use of different animal models, especially experimental autoimmune encephalomyelitis (EAE)[25]. Innate and adaptive immune mechanisms have been shown to participate in the immunopathogenesis of MS[26] although adaptive immunity may predominate early in disease, as reflected by selective T and B cell activation during clinical relapses. The most widely supported view of MS pathogenesis is that it is a CD4+ T cell-driven autoimmune disorder, albeit with diverse phenotypes[20]. Several groups have demonstrated that enhanced MHC Class II expression on myeloid cells and accompanying innate immune activation are associated with damaged myelin and failure of re-myelination[27]. Th1-associated innate immunity is also activated throughout the different phases of disease, as indicated by persistently increased cytokine and chemokine production by macrophages, microglia and astrocytes[28], even into the later or secondary progressive stages of disease[29]. The mechanisms underlying demyelination and axonal damage remain uncertain although inflammatory molecules including cytokines, chemokines, prostaglandins, reactive oxygen species, and proteases have consistently been demonstrated to contribute to demyelination and axonal/neuronal injury associated with MS[21,30,31]. These inflammatory molecules belong to a complex cascade of signaling events that originate with activation of lymphocytes, macrophages or astrocytes[24]. Activation of these cell types results in the release of soluble intercellular signaling molecules that subsequently provide feedback to the activated cells or induce cellular changes, usually through receptor mediated mechanisms causing damage to myelin, oligo-dendrocytes, axons and perhaps neuronal cell bodies[19,32,33]. The modest clinical benefits of immuno-suppressive and -modulatory therapies have prompted investigators to re-examine potential pathogenic mechanisms in MS with an increased focus on regulation of neurodegenerative disease mechanisms[34].

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Environmental factors including lifestyle (tobacco smoking), nutrition (Vitamin D depletion, dairy products), geography (latitude-dependence, UV exposure) and infectious agents might also contribute to MS development and progression[35]. Transmissibility of MS is not supported by epidemiological studies; in fact, the risk of conjugal MS is no higher than in the general population[36]. There is abundant evidence supporting the contention that genetics has a pivotal role in an individual's vulnerability to MS, perhaps in conjunction with activating factors such as infections. Although MS is not inherited in a Mendelian manner, concordance rates of approximately 15-30% in homozygotic twins and 3% in dizygotic twins are typically seen[37]. There is a strong genetic association of MS in Caucasians with the HLA class II *DRB1*1501* allele. To date, no specific genome locus has been linked definitively to MS onset or progression; several chromosomal loci and multiple polymorphisms have been associated with MS[38], particularly among genes associated with immune mechanisms.

There is diversity in the evidence indicating MS occurrence and progression might be influenced by conventional exogenous infectious agents, which has prompted numerous studies in this area (Table,[39]. Among viruses, epidemiological studies based on serology favor Epstein Barr Virus (EBV) while neuropathological studies support roles for human herpes virus (HHV)-6, human endogenous retroviruses (HERVs), and coronaviruses[10]. Each of these infectious agents are reviewed below with respect to their pathogenic contributions to MS although many other agents have also been proposed[40].

Table: Putative infectious agents associated with MS

Rabies Virus	1946, 1964
Herpes simplex virus, type 2q	1964
Scrapie agent	1965
Varicella zoster virus	1969
MS-associated agent	1972
Parafluenza virus 1	1972
Measles virus	1972
Epstein-Barr virus	1975
Simian virus 5	1978
Chimpanzee cytomegalovirus	1979
Coronavirus	1980/2000
Tick-borne encephalitis flavivirus	1982
HTLV-1	1986
Herpes simplex virus, type 1	1989
Human herpesvirus-6	1997
HERVs (MSRV/HERV-K/HERV-W 7q)	1997/2001/2004

Adapted from: R.T. Johnson. 1998. Viral infections of the nervous system. 2nd Ed.[39]

Herpes viruses and MS

Herpes viruses are large double-stranded DNA viruses, found ubiquitously (and globally) in humans, and for which established diagnostic assays are well developed including serological- and polymerase chain reaction (PCR)-based clinical tests.[41] Most human herpes viruses establish early chronic infection of select cell populations including leukocytes, epithelial cells, and neurons, often resulting in life-long immunity. However, under specific conditions, neurological disease resulting from herpes virus infections can erupt following reactivation or *de novo* infections, causing substantial neurological disabilities. The preeminent herpes virus infections of the central nervous system include herpes simplex virus (HSV)-1 and -2, varicella zoster virus (VZV), cytomegalovirus (CMV), human herpes virus (HHV)-6, and Epstein-Barr virus (EBV) infections all well-recognized to cause meningoencephalitis and myelitis[41]. In acute and subacute herpes virus infections of the nervous system, each of these viruses exhibit distinct pathologies defined by intense inflammation, occasional hemorrhage, and neuronal injury albeit with diffuse blood-brain barrier disruption but rarely focal demyelination[42]. These infections are accompanied by neurological disabilities including seizures, motor and cognitive impairments, aseptic meningitis, myelopathy, and stroke-like events. Infections with these viruses are usually apparent based on clinical presentation including fever with distinct neuroimaging, cerebrospinal fluid, and electroencephalographic findings in conjunction with specific co-morbidities such as HIV/AIDS or transplantation depending on the individual virus. However, chronic herpes virus infections represent more challenging clinical phenotypes, which are not as well recognized clinically or demographically. Indeed, the clinical manifestations of chronic VZV infection of the nervous system are broad and likely under-recognized in some instances[43]. Likewise, specific co-morbidities such as post-transplant lymphoproliferative disease involving the central nervous system are associated with high levels of EBV-encoded DNA in cerebrospinal fluid that persist over time but are not necessarily correlated with neurological disability[44].

Several herpes viruses have been implicated in the pathogenesis of MS. Herpes viruses are obvious candidates as causative agents in MS because of their ubiquity, robust immune responses, and established capacities to infect the CNS and cause acute neurological diseases. HHV-6A serology has been variably detected in MS patients over the past decade[45,46]. Several groups have detected HHV-6 encoded DNA and proteins in brains from MS patients using immunohistochemistry, *in situ* hybridization, PCR, and *in situ* PCR although these latter methods are also associated with high detection rates in non-MS control individuals,[47] (reviewed in[48]). The immune response to human herpes virus (HHV)-6 has been difficult to predict; serum anti-HHV-6 IgM levels were increased in RR-MS compared with CP-MS while HHV-6 DNA was detected during exacerbations, with 15% of MS patients showing presence of viral DNA. Several cells, including oligodendrocytes in MS brains showed the

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presence of HHV-6 antigen or genome, though not exclusively associated with MS.

Likewise, VZV serology was apparently greater in some groups with MS (reviewed in[49]) leading to a clinical trial using a VZV vaccine, which failed to show any clinical benefit[50]. A recent study reported increased detection of VZV genome in the cerebrospinal fluid (CSF) of MS patients[51] but these findings could not be replicated[52]. Most recently, EBV infection has garnered immense attention as a direct etiologic or synchronic infection in MS[53]. Large epidemiological studies have repeatedly shown associations between EBV serological detection or titer coupled with a risk of MS in both adults and children. Indeed, the likelihood of MS is also greater amongst those individuals with a history of infectious mononucleosis as children[54]. While most human adults are infected with EBV, a substantial proportion of children are EBV seronegative[10]. Meta-analysis studies of EBV serology in MS suggest there is a role for EBV in MS, particularly with a higher number of patients showing antibodies to EBV nuclear antigen than EBV seropositive controls; but whether the oligoclonal IgG from MS brain and cerebrospinal CSF is specific to EBV remains uncertain[55]. A large Danish study reported that MS risk increases soon after infectious mononucleosis which persists for at least 30 years. Though HHV-6 and EBV are ubiquitous viruses, seroconversion happens usually before or during puberty into adult life, matching epidemiological evidence for time of exposure to the infectious agent causing MS[55]. Oligoclonal antibodies in CSF of some MS patients were determined to target the EBV-encoded proteins BRRF2 and EBNA-1. A T cell-mediated immune response to EBV-infected cells leads to damage of neurons and oligodendrocytes through bystander activation. Conversely, detection of EBV genome or proteins in brain has been more challenging; indeed, several groups, over the past decade, have failed to detect EBV genome in the central nervous system of MS patients[56] using sensitive molecular tools. However, recent studies from the same laboratory suggest that EBV genome and antigens are readily detectable in the meninges from MS patients[57]; these findings await replication. Given the apparent paucity or lack of striking differences in viral detection among brains from MS and non-MS control patients in terms of direct viral detection for HHV-6, VZV or EBV, it is plausible that these agents represent synchronic infections in MS pathogenesis. Heightened immune responses to a given virus might reflect the individual host's immune ability to respond to specific viruses. Further definitive analyses are required to clarify the guise of these viruses in MS pathogenesis.

Coronaviruses and MS

Coronaviruses are positive-strand RNA viruses that infect humans as well as other mammals, usually causing respiratory and enteric diseases. These large enveloped viruses have remarkable capacity for molecular variation; they chiefly infect cells of epithelial origin. One of the more reproducible models for MS is based on the infection of mice with a specific strain of the murine coronavirus, mouse hepatitis virus, which causes autoimmune inflammatory demyelination[58]. The severity of neurological disease is predicted by the molecular diversity within the spike protein. Complementing this finding, several reports show that the human coronaviruses, HCoV-229E and -OC43 are detected in the brains of both non-MS control and MS patients, with higher detection rates in MS brains for HCoV-OC43[59]. Moreover, T cell responses to human coronaviruses among MS patients were accentuated compared with controls[60]. Intracerebral inoculation of this latter virus into mice was associated with neuroinflammation[61]. Similar to the links between herpes viruses and MS, coronaviruses are ubiquitous in humans and thus might prompt distinct immune responses contributing to MS immunopathogenesis of MS despite not being specific etiological agents; nonetheless, the development of a neurovirulent strain of HCoV-OC43 with experimental features resembling MS is a compelling observation that requires further investigation.

Retroviruses and MS

Retroviruses are single strand RNA viruses defined by their expression of reverse transcriptase and best exemplified by the exogenous retroviruses, HIV-1/2, HTLV-1/2, murine leukemia virus (MuLV) and Visna-Maedi virus, an early animal (sheep) model for MS[62]. Of interest, several retroviral infections in humans exhibit clinical and pathological nervous system-associated phenotypes resembling MS including human T cell lymphotrophic virus (HTLV)-1-associated myelopathy and human immunodeficiency virus (HIV)-associated leuco-encephalopathy[63]. A retroviral cause for MS was postulated in a study showing 70% of MS patients displayed cross-reactive antibodies to HTLV-1/2 and HIV-1 antigens[64]; these observations were disproven, underlining the danger of relying on serology for viral detection. Jocher and coworkers found no HTLV-1 sequences in leucocytes or brain tissues from MS patients[55].

Retroviruses become integrated into the host genome and continue to express proteins for years after infection, often in the absence of substantial viral replication[62]. Thus, retroviruses have the capacity to exert pathogenic effects many years after initial infection. In some instances retroviruses are transmitted vertically or horizontally after integration, depending on the individual retrovirus, thereby perpetuating its proliferation. Human DNA contains ~3,100,000,000 bp comprising ~20,000-25,000 genes, with many RNAs that are non-protein encoding[65]. All humans carry human endogenous retrovirus (HERV) sequences or retroelements as an integral part of human genomes, encompassing 5-8% of the entire genome[33], which likely are vestiges of retroviral infections during primate evolution. Integration of ancestor retroviruses into the human germ line occurred 5 to 70 million years ago depending on the individual retrovirus. Some HERVs can infect human cells but do not replicate, likely due to mutations in the pol and gag genes, although these mutations do not preclude reverse transcription, protein expression, and the release of non-infectious viral particles. Thus, HERVs retain the capacity to influence cellular functions and perhaps survival while also remaining part of the human metagenome.

Human endogenous retrovirus are expressed in healthy normal tissues, such as placenta, where they are assumed to exert beneficial effects[66,67]. However, HERVs are also associated with a range of disease processes including neoplasia, auto-immunity, encephalitis, and fetal malformations[68,69]. Human endogenous retrovirus have also been proposed to exert their pathogenic effects by expressing superantigens[70]. Some groups have

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reported specific HERVs to be associated with pathogenic autoimmune responses depending on the individual HERV and disease context (reviewed in[71]). Human endogenous retrovirus might participate in pathogenesis through cytokine/host gene modulation with neurotoxin production/release, molecular mimicry, or by insertional mutagenesis[72,73]. We showed that induction of individual HERV expression was neural cell type- and stimulus-specific, but more importantly, was significantly associated with neuroinflammation[73].

HERVs and MS pathogenesis

A plausible explanation for HERV-mediated effects in MS is that they act as amplifiers of the inflammatory cascade of events underlying MS. A full length copy of the HERV-W genome is located on chromosome 7q21 (ERVWE1)[74] and the HERV-W7q envelope-encoded protein, Syncytin-1, was shown to be expressed in astrocytes, perivascular macrophages, and activated parenchymal microglia, in acute and chronic demyelinated lesions from MS patients[75] (Figure A). Syncytin-1 over-

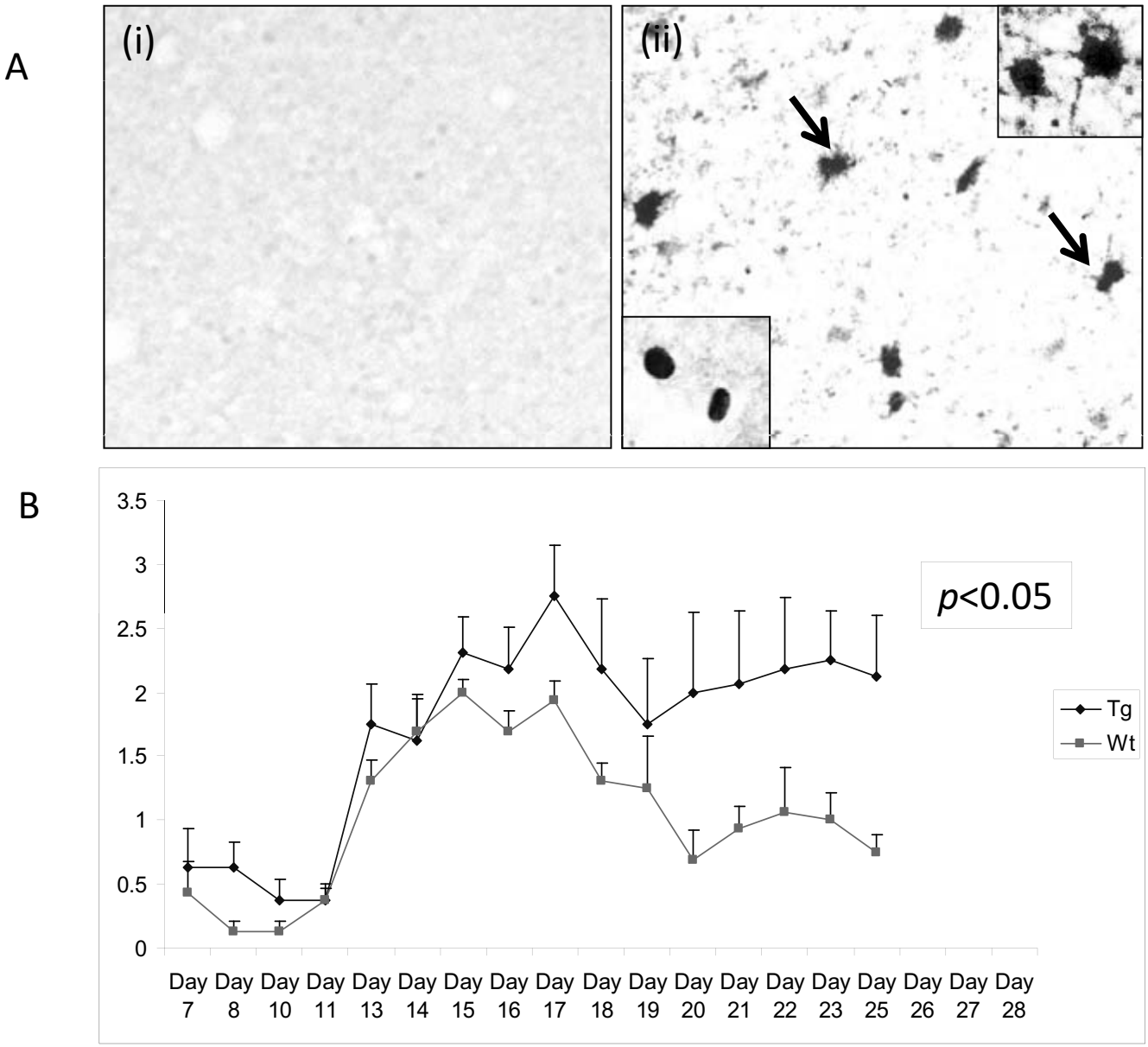

Figure: *(A) Immunocytochemistry showing Syncytin-1 immunoreactivity in (i) normal appearing white matter and (ii) within the margin of a plaque in brain sections from a MS patient. Insets display cells resembling activated microglia within a plaque (lower left hand) and astrocytes at the edge of a plaque (upper right hand). (B) EAE disease course in wildtype (Wt) and Syncytin-1 transgenic (Tg) littermate mice; transgenic animals exhibit worsened disease.*

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expression in astrocytes resulted in cytokine and reactive oxygen species production with ensuing *in vitro* and *in vivo* oligodendrocyte injury.

The MS-associated retrovirus (MSRV) is also a member of the HERV-W family[76] and the two retroelements share approximately 88% identity within their envelope sequences. Using degenerate PCR primers, the MSRV pol was also upregulated in neuroinflammatory conditions[73] but its DNA copy number does not differ in blood-derived leucocytes between MS and age-/sex-matched controls[77]. The MSRV pol is detectable in other neuropsychiatric disorders[68,78]. MS-associated retrovirus expression is induced by inflammatory agents as well[79,80] and might induce potent inflammatory responses[81]. MS-associated retrovirus expression is likely activated in response to specific inflammatory products or infectious agents, particularly viruses (HSV-1, influenza virus). In fact, the expression of MSRV was enhanced in the presence of HHV-6 in MS patients[82]. It has been reported that MSRV is replication-competent and behaves like an exogenous retrovirus, which has prompted substantial interest in MSRV[83], especially with recent data generated using MSRV envelope-specific PCR primers that did not demonstrate up-regulation of MSRV in MS brains[84,85]. There are multiple HERV-W members, with varying sequences encoded by different chromosomal loci. Recent work also indicates that among several HERV envelopes, only Syncytin-1 RNA was upregulated in MS brains[84,75]. Syncytin-1 transgenic mice under the control of the GFAP promoter, exhibited more severe clinical disease after the induction of EAE (Figure B). However, anti-Syncytin-1 antibodies or T cell responses were not detected in blood from MS patients[86], as expected for a host-encoded protein expressed during growth and development.

Other human retroelements have also been posited to participate in MS pathogenesis but a full understanding of their pathogenic role(s) and underlying mechanisms remains to be defined[83]. HERVs might also act as genetic markers for polymorphisms related to MS or as markers of environmental/endogenous stress[87,88]. Induction of HERV-H/RGH, HERV-W and ERV-9 expression was reported for specific cell types (mainly B cells) from MS patients after in vitro culturing[89]. Several studies suggest that specific HERVs, which occur in low copy numbers in the genome (e.g. ERV-3, HERV-R and HRES-1), might show polymorphic patterns in MS and act as auto-, super-, or neoantigens with the potential to enhance inflammatory responses or induce autoimmune reactions[87]. RNA encoded by these HERVs has been detected by reverse-transcriptase polymerase chain reaction (RT-PCR) with degenerate primers in sera/plasma and brain tissues from MS patients, although not exclusively from this patient group[83]. Other methods employed for detecting evidence of HERVs in MS and other diseases include electron microscopic identification of 'virus-like' particles, reverse transcriptase activity, and autoantibodies in blood and CSF, albeit with limited specificity or sensitivity[86].

HERV-H has also been studied in MS patients, particularly in Scandinavian populations[83]. The *in vitro* production of retrovirus-like particles (RVLPs) in cell cultures from MS patients but not healthy controls may be enhanced or activated by infectious triggers such as herpesviruses, e.g. *herpes simplex* virus (HSV), EBV. Independent molecular analysis of retroviral RNA associated with RVLP revealed two different genetic families of endogenous retroviral elements: MSRV/HERV-W and RGH/HERV-H. Retroviral particles of the latter were reported to be transmitted to mitogen-stimulated lymphocytes from healthy donors[90,91]. Though this study was not confirmed, it suggests that one or more HERVs may be associated with MS. In a recent study from Spain of 48 MS patients studied within a patient population of 92, CSF samples showed no HERV-H RNA[92]. In a Danish study, using a well controlled study group, seroreactivity to select HERV-H peptides was determined by a sensitive time resolved immunofluorometric assay and, in general, MS patients with an active disease status had antibodies to several HERV-H peptides compared to non-active disease as well as unaffected relatives. At least 50% of the MS patients' sera exhibited this response, and there was a positive correlation with lower levels of an innate molecule, MASP3[93]. In synergy with other agents associated with MS, HERV-H showed a marked increase in cell-mediated immune responses to peptides corresponding to the gag and env regions by eliciting proliferative effects when combined with HSV and HHV-6A[37]. Indeed, splice variants of HERV-H env were detected in about 40% of MS patients and ascribed to the high transcription and translation of HERV-H env-encoded protein, which also elicited a strong serological activity. Interestingly, the splice variants associated with MS did not have any known homology with a host gene and thus assigning chromosomal loci was not possible[90]. Cultured leucocytes from MS patients produced RVLPs, which varied in size from 70-100 nm and appear morphologically similar to Type C retroviruses[83].

Due to the presence of several exogenous viruses in MS patient tissues, synergy between some of these viruses and HERVs has been examined in various studies. Using the sensitive PERT assay, Brudek et al,[93] examined reverse transcriptase in MS patient lymphocytes and determined that UV-inactivated HSV-1, HHV-6A and VZV could induce HERV reverse transcriptase (RT) activity in PBMCs, suggesting that viral proteins are sufficient to induce RT activity and a productive infection is not necessary[93]. In a follow up study, the same group examined the synergism between herpesvirus antigens and HERVs in the release of pro-inflammatory cytokines in PBMCs[94]. Assuming that HERV-H was the retrovirus used for the assay due to their longstanding interest in the same, RVLPs were isolated from a B lymphoblastoid cell culture. HHV-6A also induced reverse transcriptase activity as well as a proliferative response[94]. An allelic variant member of the HERV-K family, K18, was recently found to be a risk factor for MS[88]. Though the small sample size was a limitation, there was a significant association between HERV-K18 env genotype and risk of MS[88]. HERV-K18 env has previously been described as a super-antigen transactivated by EBV in the pathogenesis of type 1 diabetes[70]. Another member of the HERV-K family, K113, with open reading frame (ORF)s in all its genes, has a widespread geographic as well as ethnic variation in 0-28% of humans, increasing the possibility of its association with MS since the disease appears to be geographically and ethnically restricted. However, using a large population of MS patients (n=951) and unaffected parents (n=1902), there was no overrepresentation of the HERV-K113 provirus allele[95]. Thus, while HERV-K18 shows association in a small subset of MS

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patients, a larger study with another HERV-K member, K113, showed no significant differences. HERV-K is a relatively new HERV and was present in allelic form in 15% (115 HML-2) to 30% (113 HML-2) of DNA of known and unknown ethnic origins. Although a clear association of a HERV haplotype with host genotype has not been made to date in MS patients, there is evidence depending on the population; the LTR sequences of HERVs such as HRES-1 show polymorphisms within MS patient haplotypes, while those from non-MS patients were identical without geographic restrictions[87].

Synchronic infections in MS

For many years, it has been recognized that intercurrent events including infections, other systemic illnesses, vaccinations, and perhaps psychological stress might affect the disease course of MS[96]. The mechanisms by which these events mediate MS relapses remain unclear and in some instances may result in a pseudo-relapse without sustained worsening in disability. Multiple small studies indicate that infections appear to influence the risk and severity of MS relapses with the relative risk[97] of relapse ranging from 1.3 to 2.9 depending on the study[98,99]. Of interest, corresponding neuroradiological changes were not observed in studies of infections in MS during which serial MRIs were performed. Indeed, in one study, the duration and severity of the relapse positively predicted the likelihood of a concurrent infection but only 28% of overall infections were associated with a relapse. In studies in which viral (EBV, HSV, RSV, adenovirus, reovirus, Coxsackie virus B1-6, influenza A, B, C) serology was performed, viral titres were not correlated with relapses. In contrast, EBV reactivation in terms of serological activity and viral DNA detection was evident in a subset of MS patients during relapses[100]. Both the seasonal relapse rate and MRI lesion accumulation has been investigated in multiple studies[101-103]; the results are conflicting although the risk of optic neuritis onset might be increased in the spring months[104].

Multiple sclerosis onset and relapse rate in relation to vaccination have also been studied; onset does not appear to be associated with vaccination based on several studies although these were retrospective analyses[105,106]. Similarly, influenza vaccination did not increase the risk of MS relapse[107,108]. Despite worries regarding vaccination for Hepatitis B virus and MS induction, particularly in France, these concerns seem ill-founded based on several prospective studies which did not find any association between hepatitis virus vaccination and MS. Other synchronic infections or immune events have been associated with the onset of acute demyelinating encephalomyelitis, which resembles aspects of MS and can be difficult to distinguish from MS, particularly in children or in the context of a clinically-isolated syndrome.

In contrast to these apparent adverse effects on MS onset and relapse, there are also reports synchronic infections or vaccinations might be protective in MS. Studies examining the use of Calmette-Guerin bacillus (BCG) vaccination reported a 57% reduction in MRI activity[109] and a 54% reduction in hypodense T1 lesions in MS patients[110]. Moreover tetanus vaccination was associated with both a reduced risk of MS onset and relapse[108]. Recent studies suggest that individual infections might influence the MS disease course, including helminth infections[111]. There are multiple experimental studies suggesting that parasitic infections alter the course of the MS model, EAE[112]. Other bacterial or viral products also attenuate or potentiate the severity of EAE[113]; studies from our group suggest that the bacterial product, lipopolysaccharide, when injected into neonatal mice, reduced the severity of EAE disease through alteration of dendritic cell function together with subsequently enhanced regulatory T cells production[114].

Future perspectives

Given the diversity of findings regarding the role of infectious agents in MS and the substantial number of conflicting reports, several issues require further attention in this field including standardization of procedures for detecting and defining infectious agents in well defined cohorts with controls and blinded analyses. Moreover, these studies need to be extended to different populations in which MS is common together with analyses of infectious pathogen molecular diversity; different viral or bacterial strains might exert differential actions on MS onset, progression and response to therapy, depending on host genetic background or demographic variables. A complete understanding of the dynamic interplay within the human microbiome, including the brain, will facilitate a better grasp of the role of susceptibility to MS in terms of commensal microbial populations as well as synchronic infections. The application of high throughput technologies including metagenomic, proteomic, transcriptomic, and microRNA arrays using well defined tissue samples will illuminate the contribution of infectious agents to the development and progression of MS. In fact, it might be useful to define the potential risks of developing MS, not in terms of specific pathogens, but in combinatorial approaches based on high throughput analyses of the relative expression of a range of different microbes using systems biology approaches. Given both commensal and pathogenic agents are implicated in the induction of immune regulatory processes, their relative expression and actions are imperative to understand in any disease. Finally, select immunogens derived from different infectious agents might serve as new therapeutic strategies for MS through modulation of the innate or adaptive immune responses; there is no doubt that any regulation of immunity as a treatment for MS will require insight into the individual specific host/patient genetic background and perhaps his/her metagenome before effective and safe therapies are initiated. With increasing availability of the human metagenome and personalized medicine, these potential therapeutic strategies will be realities in the near future.

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