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Review

Viral triggers of multiple sclerosis

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ABSTRACT

Genetic and environmental factors jointly determine the susceptibility to develop Multiple Sclerosis (MS). Collaborative efforts during the past years achieved substantial progress in defining the genetic architecture, underlying susceptibility to MS. Similar to other autoimmune diseases, HLA-DR and HLA-DQ alleles within the HLA class II region on chromosome 6p21 are the highest-risk-conferring genes. Less-robust susceptibility effects have been identified for MHC class I alleles and for non-MHC regions. The role of environmental risk factors and their interaction with genetic susceptibility alleles are much less well defined, despite the fact that infections have long been associated with MS development. Current data suggest that infectious triggers are most likely ubiquitous, i.e., highly prevalent in the general population, and that they require a permissive genetic trait which predisposes for MS development. In this review article, we illustrate mechanisms of infection-induced immunopathologies in experimental animal models of autoimmune CNS inflammation, discuss challenges for the translation of these experimental data into human immunology research, and provide future perspectives on how novel model systems could be utilized to better define the role of viral pathogens in MS.

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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) which usually begins in early adulthood and is characterized by tissue inflammation, demyelination and gliosis, various degrees of axonal pathology, and episodic or progressive neurological disability. More than 1.5 million people worldwide and at least 400,000 individuals in Europe alone are affected by MS, which is second only to trauma as a cause of acquired disability in young adults in most Caucasian populations [1].

Genetic and environmental factors jointly determine the susceptibility to develop MS. Collaborative efforts during the past years achieved substantial progress in identifying genetic risk factors that predispose for MS [2]. HLA-DR and HLA-DQ alleles of the HLA class II region on chromosome 6p21 are the highest-risk-conferring genes for most major autoimmune diseases including MS which is particularly associated with the DRB1*1501 allele encoding HLA-DR2b. Less-robust susceptibility effects have been identified for MHC class I alleles and in non-MHC regions. The role of environmental as opposed to genetic risk factors in MS is much less well defined, despite the fact that infections have long been thought to critically contribute to disease development. In 1894, Pierre Marie, a former student of Charcot, argued strongly that infection was the cause of multiple

sclerosis. He felt that infectious pathogens, or more likely combined infections, initiate MS [3]: “These gentlemen, are suppositions, and I put them before you without unreasonably insisting upon them. The one point in this discussion which I would fix in your minds is the following fact, a fact, thank God, has been well established, viz., that the cause of insular sclerosis is intimately connected with infectious diseases.” More than a century later, intriguing epidemiological but weak immunological and virological evidence has resulted in a bewildering list of usual suspects including measles, rabies, scrapie-like agent, Carp agent, paramyxovirus, coronavirus, Epstein–Barr virus, herpes zoster, herpes simplex virus, human herpesvirus 6, rubella, mumps, canine distemper, Marek's Semliki forest virus, animal and human retroviruses, and human T-cell lymphoma virus type I. Almost universally, these associations have later not withstood scrutiny. Such unclarity and vagueness spurred the notion that a pathogenic role of infectious agents in MS cannot be established unless experiments provide unequivocal evidence that the postulates of Koch have been met.

Traditionally, microbiologists have used postulates formulated by Robert Koch and Friedrich Loeffler to demonstrate whether a particular microbe causes a specific disease: a microorganism must be (1) found in abundance in all organisms suffering from the disease, but should not be found in healthy animals; a microorganism must be (2) isolated from the host with the disease and grown in pure culture; (3) the specific disease must be reproduced when a pure culture of the microbe is inoculated into a healthy susceptible host; and (4) the pathogen must be recoverable from the experimentally infected host [4]. These postulates retain historical importance, but fulfillment of all four postulates is not required to

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demonstrate causality, even in infectious diseases. Indeed, Koch applied these criteria in the 19th century to establish the etiology of tuberculosis, but he abandoned the universalist requirement of the first postulate when he discovered asymptomatic carriers of cholera [5]. Likewise, not all individuals exposed to *Mycobacterium tuberculosis* become infected, and progression towards clinical tuberculosis is far from an inevitable consequence of infection with *M. tuberculosis*, since only ~10% of the vast number of infected individuals actually develop clinical disease [6]. Such observations led to the development of the field of human genetics of infectious diseases and the identification of genetic traits that predispose to infection and clinical disease development [7]. The insight that clinical infectious diseases result from complex interactions between the infectious agent, the environment, and host factors rather than following a simple 'one organism–one disease paradigm' has implications for our understanding of how infectious pathogens might trigger complex autoimmune diseases such as MS. Current data suggest that infectious agents that contribute to MS development are most likely ubiquitous and highly prevalent in the general population. Moreover, they require a permissive genetic trait that determines the susceptibility of the host to develop MS. Finally, the distinct conditions, under which primary infection with these pathogens is encountered, might further modulate disease risk. Here, we review new data for an association of certain infectious pathogens with MS and illustrate mechanisms of infection-induced immunopathologies in experimental animal models of autoimmune CNS inflammation.

2. Multiple sclerosis: genes and environment

MS is a relatively common disease in Europe, the United States, Canada, New Zealand, and parts of Australia, and its prevalence generally follows a north to south gradient on the Northern Hemisphere and the opposite on the Southern Hemisphere with very low rates or virtually absence of the disease near the equator. This geographic distribution can be attributed to both genetic effects and environmental influences. Arguing for the genetic hypothesis, the prevalence of MS differs strikingly between geographically close, but genetically distinct populations. Ethnic groups like Lapps in Scandinavia, Gypsies in Hungary, Maoris in New Zealand, or Aborigines in Australia are rarely or virtually not affected by MS, although the disease is otherwise common in these latitudes. Similarly, MS is rare among Japanese, Chinese, African Blacks, North and South Amerindians, and the native population in southern countries of the former Soviet Union, but occurs notably more frequently among Caucasians living in the same area. Further examples are the different prevalence rates in genetically distant populations living on the same island as it has been reported for Sardinia, Cyprus, and Ireland [8].

Familial aggregation studies including twins, siblings, and adoptees demonstrated that the risk to develop MS increases with the degree of relatedness between individuals. For example, monozygotic twins of patients with MS have a more than 100 times higher risk to develop the disease and full-siblings have an approximately 20 times increased lifetime risk compared to the general population [9,10]. While these recurrence risk values are considerably lower than the ones for a Mendelian-dominant disorders such as Huntington's disease (approximately 5000-fold increased risk for siblings), they are similar to the risks that have been reported for other complex polygenic diseases such as type 1 diabetes (approximately times 20 increased risk for siblings).

An elegant approach to dissect the impact of genetic sharing versus a shared family environment on the development of MS are epidemiological studies on adoptees (no shared genes) and half-siblings compared to full-siblings (25% and 50% shared genes, respectively). Adopted relatives, although raised from infancy with

the MS patients, do not develop MS more frequently than it would be expected for the general population [9]. Half-siblings raised apart (different environments) or together (same environment) with MS patients have similar risk to develop the disease and, the recurrence risk for half-siblings is significantly lower than that for full-siblings raised in the same family (1.32% vs. 3.46%) [10].

These studies made clear that the excess of the disease in biological relatives results from the sharing of genetic material, but does not follow a simple Mendelian mode of inheritance. Another important conclusion that can be drawn from these observations is that any nongenetic, environmental factor is likely to be ubiquitous and not confined to the family microenvironment [11].

The concordance rate for MS is approximately 25–30% for monozygotic and 2–5% for dizygotic twins. Although studies on animal models of autoimmune disease demonstrated that the rate of disease expression can be titrated by the number of disease-associated genes under identical environmental influences [12], indicating that a simple concordance rate does not optimally reflect and might, indeed, underestimate the contribution of genetic factors to autoimmune disease development, the fact that most monozygotic twins are discordant for MS clearly points towards an important role of environmental factors in the evolution of MS.

Environmental contributions are further supported by migration studies. The incidence of MS in migrants tends to be intermediate between that of their birthplace and that of their final residence and close to the latter when migration occurs in childhood [13]. As pointed out by Ascherio and Munger [13], the risk to develop MS declines among individuals migrating from high- to low-prevalence areas [14–16] but does not consistently increase with migration in the opposite direction. As an example, first-generation immigrants from the Caribbean and Asia whose genetic susceptibility is proven by the high rates of MS among their UK-born children [17,18] rarely develop MS themselves, suggesting that individuals born in low-risk areas appear to benefit from some long-lasting protection that is, however, not transmitted to their children [13].

Taken together, familial aggregation and migration studies indicate that exposure to environmental factors in childhood, and possibly during adult life, appear to be strong determinants of MS risk. The nature of such trigger factors could be both infectious and noninfectious. In this review, we focus on mechanisms of infection-induced pathology in MS.

3. Mechanisms

Several mechanisms have been proposed to explain how pathogens such as viruses might trigger autoreactive immune responses in MS. These include virus-induced general activation of the immune system and the provision of viral gene products that specifically stimulate immune responses which cross-react with self-antigen (Fig. 1).

3.1. Mechanisms of bystander activation

Infectious agents express specific pathogen-associated molecular patterns (PAMPs). These are recognized by immune cell receptors leading to cellular activation, which increases the antigen-presenting capacity and the expression of costimulatory molecules by antigen-presenting cells (APCs), as well as their production of type I interferons, pro-inflammatory cytokines and chemokines, which in turn initiate and direct the immune response against the invading pathogen. Thus, pathogens are recognized as adjuvants for the immune response against them and could, via pattern recognition receptor (PRR)-mediated activation of APCs that contain self-antigens obtained from dying cells or tissue damage, activate autoreactive T

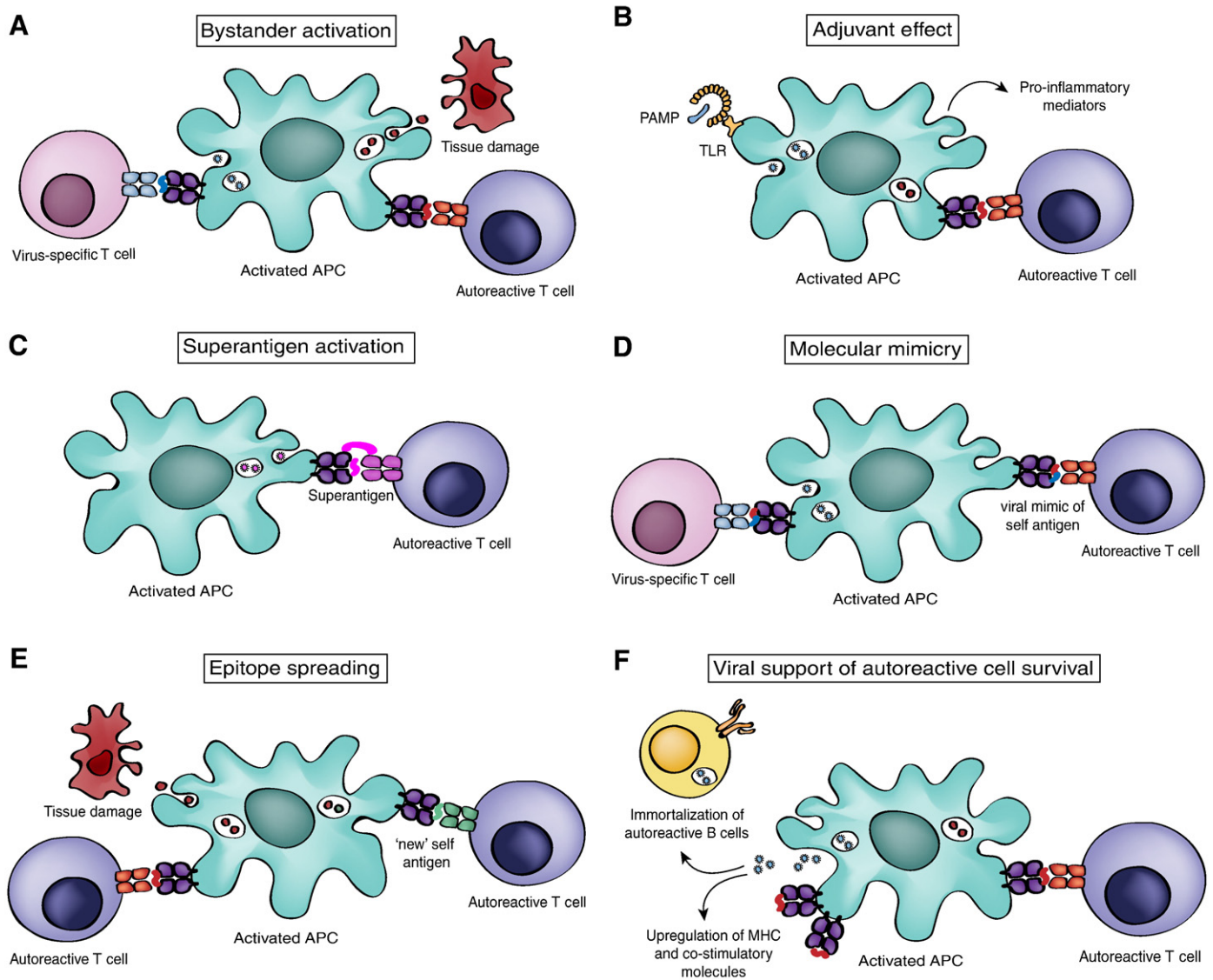


Fig. 1. Molecular mechanisms of pathogen-induced autoimmunity. (A) Pathogen-activated antigen-presenting cells can display self-antigens from dying cells to autoreactive T lymphocytes in a process known as bystander activation. (B) Activation of the immune system resulting from stimulation of pattern recognition receptors by infectious agents can lead to expression of proinflammatory mediators and triggering of autoreactive lymphocytes. (C) Microbial superantigens cross-link MHC class II molecules with TCRs inducing antigen unspecific activation of autoreactive T cells. (D) Certain pathogen-derived antigens share structural similarities with self-peptides causing activation of autoreactive T cells through molecular mimicry. (E) The process of epitope spreading can enhance autoimmune responses by activating autoreactive T cells to 'new' self-antigens during the progression of the disease. (F) Viral agents can enhance the activation state of autoantigen presenting cells and induce the survival of autoreactive lymphocytes. As an example, persistent infection of microglial cells with Theiler's murine encephalomyelitis virus (TMEV) was shown to upregulate expression of MHC and co-stimulatory molecules and enhance the ability of these cells to function as effective APCs [34]. Furthermore, EBV infection could assist in the survival of autoreactive B cells [36]. APC, antigen-presenting cell; MHC, major histocompatibility complex; PAMP, pathogen-associated molecular pattern; TCR, T-cell receptor; TLR, Toll-like receptor.

and B cells. Alternatively, the Th₁-driven environment during viral infection could facilitate activation of autoreactive bystander T and B cells via proinflammatory cytokine production. An even broader form of bystander activation is achieved by microbial superantigens, which cross-link MHC class II molecules with TCRs that contain a certain V β domain, leading to T-cell activation independently of specific antigen recognition. Staphylococcal, mycoplasma, endogenous retrovirus-derived, and enteric microbiota-generated superantigens were suggested to be involved in the disease exacerbation in animal models of MS [19].

3.2. Mechanisms of molecular mimicry

Polyspecific antigen recognition has emerged as a fundamental feature of adaptive cellular immune responses. Mathematical models

indicated that the TCR repertoire is not large enough to give functional protection against all possible foreign epitopes on the basis of a one TCR–one epitope model, and several groups consistently demonstrated that there can be considerable flexibility in TCR recognition of peptide–major histocompatibility complex (MHC) complexes [20–24]. Polyspecific or so-called degenerate TCR recognition is considered to represent a compromise between the need to provide host protection against virtually any pathogen-derived epitope and, at the same time, the need to ensure thymic positive selection and peripheral maintenance of this T-cell repertoire via intermediate affinity recognition of self-peptides that are presented by self-MHC molecules. Such degenerate specificity, however, also carries a certain risk for autoimmunity under special circumstances, e.g., strong innate immune activation. It has previously been shown in a number of animal models transgenic for human autoreactive T-cell receptors

that microbial peptides can induce MS-like disease through mechanisms of molecular mimicry [25–27].

3.3. Mechanisms of epitope spreading

In addition to one TCR being engaged by different MHC/peptide complexes, one TCR specificity can set free epitopes for other TCRs and result in a process called epitope spreading. Epitope spreading describes the phenomenon observed in animal models of autoimmune diseases and cancer patients in which responses to immunodominant epitopes are elicited first, followed by responses to less dominant epitopes [28–32]. Although these examples document epitope spreading within autoantigens and to additional autoantigens, the inflammatory environment of viral infections could also support these immune response cascades by increasing the presentation of autoantigens, thereby spreading immune responses from foreign to self-antigens.

3.4. Emerging mechanisms

The mechanisms of bystander activation, polyspecific antigen recognition/molecular mimicry, and epitope spreading are not the only ways by which pathogens might trigger or accelerate CNS autoimmunity. Viral infections could also directly maintain autoreactive effector T cells or autoantigen-presenting cells. For example, Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease (TMEV-IDD) is a model of MS in which intracerebral TMEV infection of mice leads to an autoimmune demyelinating disorder 30–40 days after infection [33]. Persistent infection of microglial cells with TMEV has been shown to upregulate expression of MHC and costimulatory molecules and to enhance the ability of these cells to function as effective APCs [34]. Furthermore, Epstein–Barr virus (EBV) immortalizes B cells and assists in their differentiation into long-lived memory B cells. These mechanisms could support the survival of autoreactive B cells or of a reservoir of APCs that can present autoantigens to promote autoimmunity [35,36].

The evidence for a biological role of these mechanisms mainly stems from experimental autoimmune disease models. Testing whether these mechanisms are indeed relevant in human autoimmune diseases such as MS is challenging because of a number of reasons including the following:

1. Chronic autoimmune diseases are likely to become clinically apparent only after a considerable period of subclinical autoreactivity, at which time the pathogen might have already been cleared and/or the antiviral immune responses might have subsided.
2. The proposed mechanisms by which a pathogen or a number of pathogens potentially initiate and sustain MS are likely dynamic, not mutually exclusive and might occur simultaneously or sequentially. A simple 'one organism–one disease' or 'one mechanism–one disease' paradigm might not apply to complex and heterogeneous diseases such as MS.
3. The flip side of the idea that autoimmunity is driven by viral infections is that autoreactive immune responses, or even only a predisposition to the development of these responses, might affect the ability of the host to control infections and to regulate antiviral immune responses. The latter probably accounts for the occurrence of polyspecific antiviral humoral immune responses in the cerebrospinal fluid (CSF) compartment in patients with MS, which are characterized by intrathecal synthesis of IgG antibodies towards a variety of viruses including measles, rubella, varicella zoster virus, and the human herpesvirus 6 [37–41]. Intrathecal humoral immune response against measles, rubella, and varicella zoster virus (MRZ reaction, MRZR) is present in 80–100% of patients with MS but less common in acute inflammatory diseases of the CNS [41]. Although these alterations could potentially be

used as surrogate disease markers for diagnostic purposes, there is no evidence that they contribute to the initiation and progression of CNS tissue damage in MS [42].

The argument that infections contribute to disease development is strong for autoimmune conditions associated with one or two specific infectious agents such as Guillain–Barré syndrome which is frequently preceded by *Campylobacter jejuni* infection or rheumatic fever after streptococcal infection. In contrast, MS as well as other major autoimmune diseases has been associated with a number of infectious agents. The ones that have received most attention during the past years are discussed below (Table 1).

4. Herpesviruses

Herpesviruses represent a group of large DNA viruses that are capable of establishing latency with potential for reactivation after primary infection, which typically occurs in childhood. Upon transmission to a naive host, the virus first amplifies the viral load through replicative (lytic) infection, then persists for the life of the host as an asymptomatic latent infection with occasional reactivations into lytic cycle, producing infectious virions transmissible to a new host. The mutual coexistence of human herpesviruses whether of the alpha [herpes simplex virus (HSV) types 1 and 2, varicella–zoster virus], beta [cytomegalovirus (CMV) and human herpesviruses 6 and 7], or gamma [Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV)] subfamilies with their host depends on its ability to mount an appropriate virus-specific immune response, since most herpesvirus-associated diseases involve situations in which host responses either have been seriously compromised or have been unusually hyperactivated by the viral challenge [43]. Infection with VZV, HHV-6, and EBV has particularly been associated with MS.

Table 1
Viral agents suspected as triggers of multiple sclerosis.

Virus	Evidence for association	Reference
Herpesviruses		
VZV	Acquired earlier in life by MS patients	[107]
	Reactivation linked to MS exacerbation	[108]
	Viral DNA isolated from blood and CSF	[48]
	Virions observed by electron microscopy in CSF (inconsistent observations)	[49,50]
HHV-6	Isolated from blood, CSF, and brain tissue	[54,55]
	Presence of antiviral antibodies in blood and CSF (inconsistent observations)	[56,57]
	Increased viral loads linked to MS exacerbation	[58,59]
	Cross-reactivity between virus-specific T cells and myelin antigens	[62,63]
EBV	Near-absolute seropositivity in children and adults with MS	[71–73]
	Increased risk of MS in individuals with history of infectious mononucleosis	[65,66,69]
	Virus reactivation linked to disease activity in early MS	[109]
	Increased EBNA1-specific antibodies before MS onset	[75,74,76]
	Cross-reactivity of clonally expanded EBNA1-specific T cells with myelin antigens	[80,81]
	Enrichment of EBV-infected B cells in MS brain tissues	[83]
Retroviruses		
HERV-W (MSRV)	Isolated from blood and CSF of MS patients	[90,91]
	Encode for proteins with superantigenic activity	[93]
	Chromosomal loci identified as MS-susceptibility regions	[95,96]
	Virions trigger acute neurological symptoms in mice	[94]
Other agents		
Torque	Clonally expanded T cells from CSF of MS patients	[103]
Teno virus	recognize viral motifs	

5. Varicella–zoster virus

VZV is the causative agent of chickenpox, acquired by 95% of adults in the developed world [44]. The virus establishes latency in the dorsal ganglia of most healthy people [45], while zoster is the result of symptomatic viral reactivation that affects about 1% of the general population [46]. Although many epidemiological studies link VZV to MS, a report evaluating 40 studies in the period 1965–1999 indicated that there is insufficient evidence to support the association of MS with varicella or zoster infections [47]. Recent studies conducted by Sotelo et al. [48] indicated the presence of VZV DNA in CSF and mononuclear blood cells of MS patients in relapse, while VZV viral particles were observed by electron microscopy in patients' CSF [49]. Conversely, another study failed to show the presence of VZV virions or DNA in the CSF or in the acute plaques of MS patients [50]. Furthermore, recombinant antibodies prepared from clonally expanded plasma cells in MS CSF, which are thought to represent the intrathecally synthesized oligoclonal IgG, did not bind to VZV-infected cells [50]. Therefore, the role of VZV in MS remains controversial, and further studies with more rigorous methodologies are required to support the environmental role of VZV as a trigger of MS.

6. Human herpesvirus-6

Research on HHV-6 involvement in MS has also been contradictory. This lymphotropic herpesvirus, isolated in 1986 from patients with lymphoproliferative disorders [51], exhibits predominantly CD4⁺ T-lymphocyte tropism but has a tendency to infect neural cells, which renders it a potential suspect in the pathology of many neurological disorders [52]. Two virus variants have been identified, HHV-6B, associated with mesial temporal lobe epilepsy (MTLE), and HHV-6A, which has primarily been associated with MS [53].

DNA from HHV-6A was detected in brain tissue, serum, and CSF of some MS patients [54–57]. Exacerbation of relapsing-remitting MS has been linked to higher viral loads in serum [58,59] and in peripheral blood mononuclear cells (PBMCs) [60], suggesting association of HHV-6 reactivation with disease relapses. This hypothesis is supported by observations of increased viral DNA and antigen levels in MS brain tissues [58]. Other reports suggested that constitutive presence of active HHV-6 infection in glial cells in inflamed CNS tissue could result in virus-triggered immunopathologies in MS [61].

A mechanism of molecular mimicry of the virus-encoded U24 protein and myelin basic protein (MBP), a putative MS-associated autoantigen, has been proposed based on amino acid sequence homology [62]. Although cross-reactive T cells were identified in MS patients, they were also observed to a lower frequency in controls [63]. Taken together, epidemiological data and the presence of active HHV-6 infection in some MS brain samples suggest a possible role for HHV-6 in perpetuating tissue damage in MS.

7. Epstein–Barr virus

Another widespread herpesvirus is EBV (or HHV-4), which causes asymptomatic primary infection in early childhood or if acquired later in life, leads to infectious mononucleosis (IM) in up to 25% of cases [64]. The virus establishes latency in B cells and is notorious for its tumorigenic potential. However, since the immune system constrains viral replication, malignancies occur mostly in cases of immunosuppression or of immunodeficiencies [43].

Evidence for a potential role of EBV in the development of MS arises from reports on the positive correlation between clinical history of IM and MS occurrence [65,66]. The risk of MS has been suggested to increase after IM and to persist for at least 30 years after infection [67]. A recent meta-analysis reviewed 14 studies, 11 case-control and 3 cohort studies, which investigated the association of IM and MS. The analysis concluded that the combined relative risk for development of MS after

IM was 2.3 and in HLA-DR2-positive individuals even 7 [68], indicating that symptomatic EBV infection is a risk factor for MS [69]. These observations were confirmed by Ramagopalan et al. who compared more than 14,000 MS cases and 7000 spouse controls. Their study found a positive correlation of MS disease with history of IM, while no such association was observed for history of symptomatic measles, mumps, rubella, and varicella infections, or with history of measles, mumps, rubella, hepatitis B, and influenza vaccination [70].

Serological studies have demonstrated almost absolute, close to 100%, EBV seropositivity in MS patients. However, high seropositivity, ranging between 90% and 95%, is also detected in the healthy adult population. A more prominent difference in seropositivity was observed in children with MS, 83% of which were reported to be seropositive for EBV, compared to 42% of healthy age-matched controls [71]. Moreover, no significant difference in seropositivity was observed between the two groups for cytomegalovirus, parvovirus B19, and VZV. These results were confirmed by a German study, showing 98.6% EBV seropositivity in children with MS in contrast to 72.1% in age-matched healthy controls [72]. Comparable results were observed by a more recent study that identified broadened and augmented recognition of the latency-associated EBV nuclear antigen 1 (EBNA1), suggesting dysregulation of EBV-specific immune responses in pediatric MS [73].

An age-dependent relationship was suggested between alterations in EBV-specific immune responses and clinical manifestation of MS [74]. A longitudinal study in 69 matched case-control sets of US military personnel investigated the presence of EBV antibodies before MS onset. While EBV-specific antibody titers were similar between cases and people who developed MS before the age of 20, a two- to threefold increase in EBV-specific antibody titers was observed in MS cases after the age of 25. The strongest risk factor, rising MS susceptibility tenfold, were increased titers of serum antibodies to EBV-derived nuclear antigens (EBNA) and, in particular, to the EBV nuclear antigen-1 (EBNA1) [74,75]. A recent study confirmed these results by reporting that EBNA1-specific antibody responses occurred 15 to 20 years before onset of symptoms in MS patients [76]. Although these observations suggest an EBV-specific immune dysregulation preceding MS onset, a limitation of the abovementioned studies is that they compared EBV-specific responses only to HCMV responses and not to those towards other viruses, suspected in MS association.

A more recent study determined immune responses to EBV, HHV-6, human cytomegalovirus (HCMV), influenza virus, and measles virus antigens in a cohort of 147 patients with clinically isolated syndromes suggestive of MS (CIS) with a mean follow-up of 7 years compared to 50 demographically matched controls [77]. CIS patients showed increased humoral and cellular immune responses to EBNA1 but not to other EBV-derived proteins. IgG responses to other viral antigens and frequencies of T cells specific for HCMV and influenza virus gene products were unchanged in CIS patients. Furthermore, EBNA1 was the only viral antigen with which immune responses correlated with number of clinical disability and MRI metrics during the follow-up period and increased EBNA1-specific IgG responses in CIS patients predicted conversion to clinically definite MS. Higher IgG responses to EBNA1 in patients with CIS and relapsing-remitting MS (RRMS) were also reported to correlate with increased frequencies of EBV-specific CD8⁺ T cells [78]. In line with the results of the former study, Farrell et al. [79] found higher immunoglobulin G (IgG) responses to EBNA1 in CIS and RRMS patients, whereas responses to lytic EBV capsid antigens, HCMV, and measles-virus encoded proteins were unchanged compared to healthy blood donors. In these patients, elevated EBNA1-specific IgG responses were associated with the development of gadolinium-enhancing (Gd⁺) lesions and predictive for T2 lesion volume change and clinical disability for a period of 5 years [79].

EBNA1 is the most consistently recognized EBV-specific antigen, which stimulates CD4⁺ T-cell responses in healthy virus carriers.

Selective expansion of T cells specific for EBNA1 was observed in MS patients [80,81]. Moreover, a small subset of them have been shown to cross-react with myelin antigens, supporting the hypothesis that clonally expanded EBNA1-specific T cells could be actively involved in MS immunopathology by stimulating cross-recognition through molecular mimicry [81].

Molecular mimicry is one of the classical paradigms for infection-induced autoimmunity and there is solid evidence from a number of animal models transgenic for human autoreactive T-cell receptors that microbial peptides can induce MS-like disease through mechanisms of molecular mimicry [25–27]. An alternative hypothesis for the association of EBV infection and autoimmune diseases is based on the virus' ability to immortalize B cells and to assist in their differentiation into long-lived memory B cells. Indeed, there is evidence that the EBV-encoded proteins LMP1 and LMP2 mimic signals of T-cell help and B-cell receptor engagement, respectively, possibly rendering autoreactive B cells less susceptible to tolerance control in the periphery [82]. These mechanisms could support the survival of autoreactive B cells or of a reservoir of APCs that can present autoantigens to promote autoimmunity [35,36].

B cells are now recognized to play a critical role in the pathogenesis of MS, and an increasing number of compounds that deplete B cells or target pathways essential for B-cell development and function are currently being tested for their potential use as MS therapeutics. Serafini et al. [83] found that EBV-infected B cells expressing viral antigens are significantly enriched in postmortem brain samples from patients with MS, but not in brain samples from patients with other inflammatory CNS diseases. Activated CD8⁺ T cells were also present close to EBV-infected B-cell foci, suggesting that EBV-specific lymphocyte responses may be involved in MS immunopathologies. However, these findings could not be reproduced in several subsequent studies [84–86]. Further research should clarify the source of this discrepancy. At present, the aforementioned conflicting data on the presence of EBV in MS brain tissue do not allow to draw definite conclusions on the frequency and potential function of EBV-infected B cells in the CNS of patients with MS.

Taken together, there is strong epidemiological evidence for a link between symptomatic EBV infection and MS development. The immune-modifying function of EBV suggests that this virus is, indeed, a major candidate for triggering MS. The mechanisms responsible for this association are, however, far from understood.

8. Human endogenous retroviruses

Human endogenous retroviruses (HERVs), comprising about 8% of the human genome, are remnants of ancestral germ line infections by retroviruses [87]. A founding member of the HERV-W family, known as MS-associated retroviral agent (MSRV), is presumably a complete replication-incompetent virus capable of forming extracellular infectious virions [88,89]. MSRV has been repeatedly isolated from CSF and blood of MS patients [90,91], as well as from body fluids of individuals with other neuroinflammatory disorders, but is less frequently observed in healthy controls [92]. Notably, MSRV has been shown to encode a protein that displays pro-inflammatory and superantigenic activity for CD4⁺ T cells [93]. The immunopathogenicity of activated T cells was confirmed in an *in vivo* study, in which severe combined immunodeficiency (SCID) mice with engrafted human immune system components were injected with MSRV virions and presented with acute neurological symptoms [94].

Interestingly, the HERV-W provirus is located on chromosome 14q11.2 region within a T-cell α/β -receptor, whereas a different HERV-H family provirus, also expressed in cells of MS patients [95], is located on chromosome 7q21-22 region. These two chromosomal regions correspond to genetic loci that have been associated with predisposition to MS [96].

In addition, a study by Sutkowski et al. [97,98] reported that the *env* gene of HERV-K18, possessing superantigenic activity, was transcriptionally activated by EBV. These observations suggest that superantigen-stimulated T-cell activation could potentially have a role in EBV infection and associated diseases. There is increasing interest in the study of HERVs as causal factors in MS. Recent findings identify herpesviruses, including EBV and HHV-6 [99,100], as potential inducers of HERV activation, alluding to the involvement of more than one infectious agent as triggers of MS. However, more studies are needed to confirm this hypothesis.

9. Torque Teno virus

Not only pathogenic but also nonpathogenic infectious agents have been suggested to be involved in exacerbation and/or induction of MS. The Torque Teno virus (TTV), claimed to have a prevalence rate of 72–100% in the general population [101], has been shown to establish persistent infection without significant clinical phenotype [102]. A study by Sospedra et al. [103] determined the specificity of clonally expanded T cells from CSF of MS patients during disease exacerbation. These T cells were shown to recognize poly-arginine regions of TTV as well as evolutionary conserved motifs of other common viruses and prokaryotes, suggesting a mechanism of misdirected autoantigen response as a result of molecular mimicry. However, due to the paucity of data, the relation of TTV infection and MS remains ill defined.

10. Conclusions and future perspectives

The relationship between infections and autoimmune diseases is complex and the mechanisms by which infectious pathogens could trigger MS are likely dynamic, i.e., they might change over time and not be mutually exclusive. Epidemiological observations indicate that viral infections could contribute to MS development not only as triggers of disease exacerbations but also as etiological agents, i.e., long before the disease becomes clinically apparent. The two- to three-folds increased risk of developing MS among individuals with history of IM compared with subjects who acquired EBV without symptoms, the almost universal seropositivity for EBV in adults and children with MS, and the steep and monotonic increase in MS risk with increasing titers of antibodies to EBV in apparently healthy adults could suggest that EBV infection is causally linked to MS development. The mechanisms responsible for this association are far from understood. Moreover, the incidence of IM in Western countries ($\geq 5\%$) [64] exceeds the prevalence of MS in comparable populations (0.1%) by far (more than 50-fold) suggesting that yet unidentified genetic and/or additional environmental factors determine whether symptomatic EBV infection indeed predisposes to MS.

Although one particular MS-causing agent might still be discovered, current data suggest that multiple infections along with noninfectious environmental factors trigger the development of MS. These factors are likely ubiquitous, i.e., highly prevalent in the general population, and they require a permissive genetic background that predisposes for MS development. Future studies investigating infectious pathogens in a complex and heterogeneous disease such as MS will benefit from careful and detailed clinical, pathological, and neuroimaging-based patient characterizations and from reproducibility in different study populations. In addition, novel humanized animal models of autoimmune diseases that are simultaneously permissive for viral pathogens which usually infect only humans [104,105] should allow investigation of specific aspects of host–pathogen interactions during autoimmune CNS inflammation *in vivo*. The integration of these data might eventually allow us to better define the role of viruses in the etiology and pathogenesis of MS and how virus–host interactions could be targeted for MS therapy [106].

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