

## Is the risk of multiple sclerosis related to the ‘biography’ of the immune system?

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**Abstract** Multiple sclerosis (MS) with onset in childhood offers a unique opportunity to study the infectious background of this disease but the immune reactions against infectious agents in such children have only recently been investigated. These and other epidemiological studies strongly implicate involvement of one or more infectious agents in the aetiology of MS, with Epstein-Barr virus (EBV) being the prime candidate. Rather than being the actual cause of MS, it is more probable that these agents are involved in the development of immunoregulatory pathways. These pathways, if disturbed by hygiene-related factors including an altered sequence of infections, may generate and maintain a deficit within the immunological network that facilitates, to particular early events in the development of MS, preceding the onset of MS disease by years or a decade. A framework that can serve as a guide for further epidemiological, immunologic and molecular biologic investigations is formulated. This approach may shed light on the complex natural history of MS and may

lead to rational preventive and therapeutic strategies. It is possible that, in the future, MS could be prevented by vaccination against EBV in early childhood; the framework is of relevance to the design of an appropriate type of vaccine.

**Keywords** Multiple sclerosis · Common infections · Epstein-Barr virus · Hygiene hypothesis · Endogenous retroviruses · Immunologic network

### Introduction

The pathogenesis of multiple sclerosis (MS) is certainly complex and heterogeneous in nature [1], involving an interplay between innate and environmental factors [2–4] and genetic factors, notably the polymorphism of HLA [5]. The epidemiology of this disease strongly indicates that it has emerged as a major neurological disorder in industrially developed nations over the last 150 years and is likely to be affected by hygiene-related factors [3, 6].

According to the so-called hygiene hypothesis [7], modern living conditions in the industrialised nations isolate infants and children from many infectious challenges that are required for the development of appropriate immunoregulatory networks. This hypothesis has been advanced to explain the rise in incidence of disease associated with immune dysregulation, including asthma, allergy, autoimmunity and at least some forms of cancer, in the industrially developed world [7]. In addition, the hypothesis suggests strategies for interventions for the prevention of such diseases, as illustrated by studies on the epidemiology of malignant melanoma which indicate that certain vaccinations, BCG, vaccinia and yellow fever, can substitute for the significant protective effect of natural

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infections [8–11]. As epidemiological investigations on MS strongly indicate that this disease is likewise affected by hygiene-related factors and by a history of infections [3, 6], the challenge is to determine whether there is an underlying distortion of immune responses in this complex disease that could be prevented or corrected by therapeutic intervention.

### Associations of common infectious agents with MS

Evidence from epidemiological studies of a role for any of the many common infections in the aetiology of MS is largely inconsistent [3, 6]. This could be due to the prolonged silent stage of the disease, but recent studies on MS commencing in childhood might be able to shed light on this issue [6, 12–14]. Although there is no convincing evidence that any specific active infection directly causes MS, at least 14 specified infections have demonstrable associations with this disease on the basis of serological characteristics [2, 3, 6, 12–15], with Epstein-Barr virus (EBV) emerging as the most likely single candidate for a leading aetiological role [12–22]. This infection is unique in that the prevalence of specific antibody is consistently higher in children with MS as compared with healthy age-matched controls [12–14], whereas at the typical age of onset of MS (late 20s to early 30s) nearly all patients and control subjects have already experienced EBV infection. The two key points to emerge from these studies in relation to EBV are that all children with MS had already been infected with EBV [12–14], and that the levels of antibody against the EBV EBNA1 antigen were very significantly higher in both adults and children with MS as compared with controls [13, 14, 16, 23–25]. In this context, infections with EBV usually occur in infancy in the developing nations where MS is rare while in the industrialised nations infection usually occurs later in life, often being delayed until adolescence or early adulthood.

At the time of diagnosis of MS in children, the earlier EBV infection had become latent and there was no serological sign of reactivation (IgM or anti-early antigen titres  $\geq 80$  as measured in an indirect immuno fluorescent assay) [12, 13]. Moreover, in adult patients, the MS-associated serological EBV pattern was probably established many years before the onset of clinically evident MS as prior infectious mononucleosis is a strong risk factor for MS manifesting 2–20 years (mean around 10–12 years) later [18, 25]. Taken together, these studies strongly indicate that latent EBV infection is an essential predisposing condition for the development of MS but other genetic, environmental and hygiene-related conditions appear necessary for the actual expression of the disease and the unifying condition might be a dysregulated immune response.

A distinct possibility is that other infections synergise with EBV in the aetiology of the disease and the timing of infections might be important. In this context, *Chlamydia pneumoniae* (CP) infection may play a special role [26], as in a recent study on children with MS, CP-specific IgM antibody points to a high frequency of fresh, recent or reactivated infection with this pathogen at the onset of the disease [12]. A final key point is the demonstration that slight, though statistically significant, elevated levels of antibody to certain common infectious agents other than EBV and CP occur in children and adults with MS compared with age matched control subjects [12, 15, 27]. This finding does not, however, imply a direct causative relation of any infection to MS as it could reflect a more general dysregulation of immune function as a cause or consequence of the development of the disease. It is indeed likely that the elevated antibody concentrations have no significance per se for the development of MS but reflect a shift in patterns of immune reactivity away from a protection and towards enhancement of the risk of disease. Nevertheless, studies on these MS-associated infectious agents could lead to the identification of specific antigenic determinants involved in the generation and maintenance of immune dysregulation.

### A hypothesis which relates MS to the ‘biography’ of the immune system

On the basis of the available epidemiological evidence it may be postulated that MS is dependent on an infection with EBV which, owing to hygiene-related factors, occurs later than usual in life. Under such circumstances, the EBV infection might encounter patterns of immune responsiveness generated by prior infections with certain other microorganisms. In this paper we suggest a scenario in which the sequence of certain common infections results in immune dysregulation favouring the onset of MS and in the following sections this hypothesis is elaborated.

### The ‘biography hypothesis’ and regulatory T-cells

Recent studies have shown that the immune system contains a very complex network of regulatory pathways. To some extent these pathways are genetically determined, but there is growing evidence that they are critically determined by the nature and timing of infections and other immune challenges that an individual experiences earlier in life. This could be termed the ‘biography’ of the immune system.

The immunoregulatory pathways are based on populations of lymphocytes, termed regulatory T cells ( $T_{reg}$ s), in

which there is currently considerable interest. In the case of infectious disease, such populations may lead to rapid resolution, the establishment of latent or persistent infection or to tissue damage by autoimmune processes [28]. Accordingly,  $T_{\text{regS}}$  have been termed ‘a dangerous necessity’ [29]. This term implies that  $T_{\text{regS}}$  are neither ‘good’ or ‘bad’ per se but may, according to the overall pattern of responsiveness, participate in appropriate immune reactions leading to resolution of disease or in inappropriate ones resulting in immunopathology.

The temporal sequence of infections, especially initial and early ones, is crucial to the development of patterns of immune reactivity as prior contacts with other antigens may have induced cross-reactive T-helper cells competing with  $T_{\text{regS}}$ . As a consequence  $T_{\text{regS}}$  normally induced by the second pathogen may be marginalized or even eclipsed. The latter phenomenon, also known as lateral inhibition, has many parallels in biology, particularly in neurology. The locking of an immune response into an eclipsed state seems to involve an active deletion of clones of T-cells occurring as a result of reinfections or reactivations [28]. In the case of MS, infections such as those with HHV-6 [30, 31] and, possibly, with CP [12, 26] occurring before or at the time of initial or reactivated EBV infection could have such an effect.

Thus these prior contacts could have induced populations of  $T_{\text{regS}}$  that have a crucial role in protection against MS but also cross-react with an epitope on EBV. A subsequent infection by EBV could therefore generate a dominant population of cross-reactive T-helper-cells which could suppress or delete the  $T_{\text{regS}}$ .

Under normal physiological conditions, these  $T_{\text{regS}}$  could either suppress other populations of T-cells which would otherwise be able to induce autoimmune processes, including those involved in the pathogenesis of MS, or they could cause the expansion of a population of specific  $CD8^+$  T-cells which would have an immune repair function. The nature of the epitope or epitopes involved in this postulated process remains unknown but, by analogy with the parallel studies on melanoma mentioned above [10, 11], it is suggested that key host epitopes involved are coded for by certain human endogenous retroviruses (HERVs) as activation of these has been extensively documented in MS [32, 33].

Accordingly, a challenge in MS research is to delineate patterns of MS-related immune responses [34, 35], and the  $T_{\text{regS}}$  involved, that affect the risk of MS both beneficially and detrimentally and the likely targets of these responses.

The IgG-anti-EBNA1 antibody concentrations are particularly elevated in patients with MS [13, 14, 16, 18, 23–25], and systematic studies on the T-helper cell epitopes in the EBNA1 protein revealed that  $CD4^+$  T-cells from healthy EBV carriers matched for MS-associated HLA-DR alleles recognised several epitopes within the central region

of the C-terminal domain of this protein but not other EBV-encoded proteins [36, 37]. In contrast, those from MS patients recognised many more epitopes spread over the entire domain [36]. Concomitantly, the number of memory T-cells directed against this domain is increased about tenfold in MS and have been shown to be T-helper 1 cell precursors and polarised effector memory cells [36, 37], containing a subfraction of regulatory T-cells ( $T_{\text{regS}}$ ) [38, 39].  $T_{\text{regS}}$  were suppressed in MS [40], and high level of  $CD8^+$  T-cell activation against EBV but not cytomegalovirus was demonstrated early in the course of MS [41].

A search for the possible origin of competing T-helper cells was undertaken with the BLAST analytical program [42, 43]. Sequence homologies were evident over the entire expanded EBNA1 epitope with proteins from CP and HHV-6 (Table 1). This possible involvement of HHV-6 and CP in T cell competition is supported by the observation that the targets of T- and B-cells which have been found to be MS-associated by systematic studies [34, 35] also have homologies in HHV-6 and CP (Table 2).

The central EBNA1 epitope marginalized in MS (amino acids FENIAEGLRALLARSHVER) could well have different functions in health and in MS and is therefore a major putative candidate for generation of  $T_{\text{regS}}$  which control the relevant immune processes. A specific or functional deficiency of  $T_{\text{regS}}$  in MS has only recently been recognised, and the need for a large cohort of  $T_{\text{regS}}$  for the resolution of experimental autoimmune encephalomyelitis has been demonstrated [44]. For the purpose of studying the potential infectious and immunological background of MS, it is relevant to search for homologies to this ‘epitope No. 1’ in the MS-associated pathogens [42, 43]. Interestingly, homologies to the putative epitope were found in all these pathogens (Table 3).

### Epitopes shared by various pathogens and by a HERV peptide

It is likely that a diverse range of MS-associated infectious agents other than EBV and, possibly, CP, is involved in the generation and maintenance of the postulated immune responses associated, beneficially or detrimentally, with MS. By generating populations of  $T_{\text{regS}}$  or of competing T-helper cells, such infectious agents would play a role in the generation of various immunological networks based on  $T_{\text{regS}}$  which, in turn, would facilitate the expansion or suppression of populations of effector T cells including epitope-specific  $CD8^+$  T-cells. The epitope recognised by these T-cells should be common to the MS-associated infectious pathogens and to one or more cellular gene products. The latter was preferentially sought in HERVs since patients with MS expressed HERV-W env at higher

**Table 1** Homologies in proteins of HHV-6 and *Chlamydia pneumoniae* (CP) to the in MS expanded T-helper cell epitope in Epstein-Barr virus EBNA1 (amino acids 400–478)

400	439
P G R R P F F H P V G E A D Y F E Y H Q E G G P D G E P D V P P G A I E Q G P A	
<b>HHV-6:</b>	* * + * Y K * * + * * * D + * * * * * *
<b>CP:</b>	* * * R + E * * + * * *1* * * * * * * *2* * * * * * * * * * * * * *3* * * * *
440	478
D D P G E G P S T G P R G Q G D S G R R K K G G W F G K H R G Q G G S N P K	
<b>HHV-6:</b>	* * * * * * * * + * * * * * * * * * V * * * * * * P A * * * * * N * * *
<b>CP:</b>	* A * φ * * * * * *
* * * * * *	* * * * * *
479	527
F E N I A E G L R A L L A R S H V E R T T D E G T W V A G V F V Y G G S K T S L Y N L	
see Table 3 for this region;	<b>HHV-6:</b> * * * * φ φ * * φ * *
	<b>CP:</b> * * * + + * * * * * * * *

Specific T-cells directed against this region were found to be expanded in MS patients as compared to control individuals [36]; the region with homologies in HHV-6 and CP proteins extends to EBNA1 amino acid position 640

\* for identical amino acid; + = conserved amino acid exchange; φ = missing amino acid; arabic numbers for additional amino acids: 1 = DKK; 2 = PF; 3 = D

copy numbers as compared with controls ( $P = 0.00003$ ) [32, 45] and a HERV-K18 *env* genotype was described as a risk factor for MS [46].

A putative target epitope for effector T-cells in the processes suggested above (‘epitope No. 2’) was identified in a short peptide from the HERV-W *env* gene complex: MPVPSAPST. It is predicted that this peptide is presented, though only subdominantly, by diverse HLA class I molecules including Ld, A\*0201, B\*0702, B\*5101, as determined by reference to the SYFPEIHI database for MHC ligands and peptide motifs [47].

Only three pathogens bearing the two homologies on the same protein, which is postulated to be optimal for a

co-operation of the corresponding T-cells, have been identified, namely, herpes simplex (1 and 2) virus (tegument protein), measles (nucleoprotein), and varicella (tegument). The MS-association of the serology of these pathogens (higher specific antibody concentration) [16] was confirmed in a recent study: herpes simplex-2,  $P < 0.0001$ ; measles,  $P < 0.0001$  and varicella,  $P < 0.0001$  [12]. The epidemiological evidence for the MS-association of the majority of the other pathogens in Table 3 is only weak and inconsistent. Moreover, a simultaneous occurrence of homologies to epitopes 1 and 2 was also found in a diverse range of pathogens causing respiratory and gastrointestinal infections and which have also been associated, beneficially and

**Table 2** Homologies in proteins of sero-epidemiologic MS-associated pathogens to MS associated EBV-epitopes [34, 35]

Type of epitope	T-cell	T-cell	B-cell	B-cell
Microorganism/virus				
Epstein-Barr virus (protein)	EBNA3A	LMP2	BRRF2	EBNA1
Epstein-Barr virus (sequence)	RPPIFIRRL	CLGGLLTMV	SPAASRSK	RGRGGGR
<i>Chlamydia pneumoniae</i>	*Y**** +	*****	**S***	*****
HHV-6	**T*L**	****L	**L*I**	****R**
HSV	****φA**	*****	*****	**P*****
Measles virus	***L*E**	*****	*****	
Mumps virus	**L*S**			****A*

Targets of higher frequent B- and T-cells in MS-patients as present in proteins of Epstein-Barr virus and consensus in proteins of other MS-associated pathogens

Enhanced immune reactivity in MS patients in comparison with healthy control subjects as identified by systematic studies [34, 35]; consensus in other proteins to the EBV sequence given by \* for identical amino acid, + for similar amino acid (conservative exchange), and φ for missing amino acid; one additional homology in vaccinia virus

detrimentally, with the risk of MS [48–50]. Likewise, unknown parasitic infections have recently been found to be associated with a reduced risk of MS [51], and some parasitic organisms share the two homologies (Table 3, footnote). In addition, preliminary studies indicate that parasite infections in MS patients lead to fewer exacerbations and this has been linked to the emergence of T<sub>reg</sub>S [52]. The relatively widespread occurrence of these two homologies explains, at least in part, why the infectious background of MS has proved so complex and difficult to define.

The MPVPSAPST-peptide is the amino-terminal part of a small hypothetical protein of 29 amino acids encoded by the complementary DNA strand of the HERV-WE1 *env* gene which is conserved in all homologous HERV-W sequences in the human genome. Gene transcripts of 21 of 25 open reading frames with an initiating start codon have been found in association with MS according to genetic data bank entries. Moreover, several other HERVs (-H, -K, -L) exhibit this homology. As these HERV peptides are all self-antigens, they could serve as targets, but not as inducers, of the postulated MS-protective immune response. The main HLA class I molecule A\*0201 for the presentation of the peptide, with a frequency of about 30% in a European population, was shown to be associated with

a significantly reduced MS risk (OR = 0.52,  $P = 0.0015$ ) [53].

### A view on early events in the natural history of MS

Immune dysregulation in MS is likely to be an early event [18, 22, 25, 41] preceding the onset of MS disease by many years or a decade [18, 22, 25]. It should thus be emphasized that the epidemiologic observations on the possible infectious background of MS partly, if not predominantly, reflect the earliest stage in the natural history of MS. A situation similar to that postulated here has been described in malignant melanoma in which, as discussed above, cross-reactive protective immune responses are induced by homologous epitopes in BCG, vaccinia and yellow fever vaccines given at least 10 years before the onset of disease [8–11]. It was suggested that melanocytes in the early stages of malignant transformation, may be eliminated or repaired by CD8<sup>+</sup> T-cells which recognise cells expressing a HERV peptide. This immune reaction seems to suppress the genetic activity of HERV proviruses (*env* genes) associated with malignant transformation [10, 54, 55]. The HERV *env* proteins probably impair the redox

**Table 3** MS-associated pathogens and homologies in proteins thereof to candidate epitopes No. 1 (EBV EBNA1, putative T<sub>reg</sub>) and No. 2 (HERV-W)

Microorganism/ virus	Candidate epitope No. 1 F E N I A E G L R A L L A R S H V E R	Candidate epitope No. 2 M P V P S A P S T
Adeno virus	* * L *1* * * * ϕ. *2* * * +	* * A *6A * * * * +
<i>Chlamydia pneumoniae</i>	* H * E *3* * * * * +	* * * * * * *7*
Epstein-Barr virus	* * * * * * * * * * * * * * * *	* * V N * * * * +
Herpes simplex-1	* V * * * * * D * +	* * T * * * *
Herpes simplex-2	* V * * * * * G * +	* * * * A * * +
HHV-6	* * Y I N * * * * * *	* * L * L * * * +
Influenza A virus H <sub>1</sub> N <sub>1</sub>	* * * * * K * +	* * * * * *
Measles virus	* * * * *	* * L * T * *
<i>Mycoplasma pneumoniae</i>	* * * * * Y * * * *	* ϕ * * * G A * *
Parainfluenza 2	* D P V * * ϕ * * + +	I * D * R * * +
Respiratory syncytial virus	* * V * * ϕ.ϕ * *4* * *	* *8* * * E * *
Rubella virus	* * S * * * * A * *5* * *	* * T * * * +
Vaccinia virus	* * H * * * T * * *	* * S * * * +
Varicella virus	* * E T * S R * * * * I E * +	* * * * E *

\* for identical amino acid; + = conserved amino acid exchange; ϕ = missing amino acid; arabic numbers for additional amino acids: 1 = TEE; 2 = AG; 3 = QKE; 4 = YCK; 5 = AT; 6 = V; 7 = V; 8 = LAT

Pathogens against which higher antibody concentrations were observed as compared with control individuals. Homologies to both epitopes were also found in the following pathogens causing respiratory and gastrointestinal diseases: *Bordetella parapertussis*, *Corynebacterium diphtheriae*, cytomegalo virus, *Haemophilus influenzae*, human corona virus, human rota virus, *Mycobacterium tuberculosis* (also *M. bovis*, strain BCG), *Salmonella enterica*, *Pseudomonas aeruginosa*, *Serratia marcescens*, A and in two parasites causing gastrointestinal infections: Entamoeba histolytica, Giardia lamblia

Proteins with homologies to epitope 1/epitope 2, respectively: adeno virus: DNA stabilization protein/protein V; *Chlamydia pneumoniae*: hypothetical protein cpB U609/hypothetical protein; Epstein-Barr virus: EBNA1/BBLF2/BBLF3; herpes simplex-1: tegument/tegument; herpes simplex-2: tegument/tegument; HHV-6: tegument/major capsid; measles virus: nucleoprotein/nucleoprotein; *Mycoplasma pneumoniae*: phosphate import ATP-binding protein pstB/enolase; parainfluenza-2: large protein/nucleocapsid; RSV: fusion protein precursor/glycoprotein; rubella: RNA-directed RNA polymerase/E1; vaccinia: 14K membrane protein/putative DNA-binding core; varicella virus: tegument/tegument

regulation within the cells via reduced levels of glutathione peroxidase [10]. In MS there is still another environmental risk factor, namely, suboptimal levels of

bio-active vitamin D [4], which, as demonstrated in rat astrocytes, may impair via γ-glutamyl transpeptidase intra-cellular glutathione levels [56].



In cell culture experiments, gangliosides of the neolacto series, such as LM1, were identified as possible relevant mediators as they suppress HERV genes [10] and induce lytic replication cycles in cells latently infected with EBV [57, 58], thereby releasing a viral antigen which would be readily recognisable by the specific immune system. The failure of such protective mechanisms would facilitate an uncontrolled establishment of the observed extensive EBV infection of brain lymphatic tissue in MS, albeit only at a low level of virus activity [59–62]. The specificity of the process for the brain may be associated with the high content of gangliosides in nervous tissue, as other gangliosides may antagonize the activity of those that possibly protect against MS.

Multiple sclerosis is certainly a complex disease entity and the pathogenic process involves more than just increased neuro-degeneration. In particular, reduced remyelination contributes to disease progression. Thus, relevant targets in addition to the HERV peptide might exist, possibly host proteins with a homology to the HERV peptide. One such candidate is neuron-specific ankyrin-2 which, owing to complementary binding sites, forms a complex with spectrin. In an animal model of remyelination of axons the latter is the target of an immune repair mechanism mediated by a monoclonal antibody [63].

## Conclusion

Based on epidemiological considerations, it is postulated that the relatively high risk of MS in the industrialised nations is due to hygiene-related changes in the sequence of common infections, resulting in the emergence of patterns of immune reactivity that either cause, or fail to protect against, the development of MS. Further epidemiological studies are required to determine whether the timing of EBV infection is related to the risk of MS [20]. Based on studies of altered immune responses to certain infectious agents and evidence for the expression of HERVs in MS, epitopes that have a putative role, depending on the appropriate or inappropriate activity of immunoregulatory networks, in protection against or predisposition to MS have been delineated.

Studies based on the above considerations should focus on processes that initiate MS years or a decade before manifestation of the disease. Subsequently, a better understanding of the highly complex immunopathology of MS will, hopefully, lead to the eventual development of vaccination strategies for the prevention or correction of anomalies in immune function. Such vaccines could well work by preventing or correcting hygiene-related immune dysregulation [64]. It has previously been postulated that a vaccine based on EBV should afford a high level of

protection against MS [16], but the exact nature of an efficient and safe vaccine would depend on the nature of the relationship between EBV and MS. If the framework presented in this study is in principle confirmed, the appropriate vaccine is most likely to be a living attenuated one that induces a strong T cell response to EBV epitopes but is not expressing EBV EBNA1 in latency.

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