Multiple sclerosis: the environment and causation

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Purpose of review

We review current thinking on the aetiology of multiple sclerosis, how genetic susceptibility interacts with environmental risk factors at the population level, multiple sclerosis-associated risk factors and contemporary causation theory.

Recent findings

Two large genomic studies have confirmed the unambiguous associations with the DRB1 and DQB alleles of the human leucocyte antigen class II region. No other region with genome-wide significance has been identified. Family-based genetic epidemiological approaches have found no evidence of nongenetic transmissibility. This indicates that the action of the environment in influencing multiple sclerosis risk is operative at a macroenvironmental or population level, and not within families or the microenvironment. Environmental factors receiving renewed attention include vitamin D status, Epstein–Barr virus infection and smoking. Bradford Hill's criteria for causation have been modified and should be adopted as a framework for demonstrating causation in relationship to multiple sclerosis.

Summary

Multiple sclerosis is a complex disease because of interaction between genes and the environment. Any theory of causation for a specific agent will have to be congruent with the biology of the disease.

Keywords

causation, Epstein–Barr virus, multiple sclerosis, smoking

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Abbreviations

CNS	central nervous system
EAE	experimental allergic encephalomyelitis
EBV	Epstein–Barr virus
HERV	human endogenous retrovirus
HHV	human herpesvirus
HLA	human leucocyte antigen
MHC	major histocompatibility complex

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Introduction

Multiple sclerosis is considered by many to be a complex or multifactorial autoimmune disease that is not ascribable to a single genetic or environmental factor [1•]. There is wide acceptance that interactions between genes and environmental factors lead to tissue injury by autoimmune mechanisms, implicated by strong circumstantial evidence. If multiple sclerosis were associated with various immune disorders (e.g. Hashimoto thyroiditis, psoriasis, inflammatory bowel disease and rheumatoid arthritis), especially in families with several members with multiple sclerosis, then this would suggest that the disease might arise on a background of a susceptibility to autoimmunity in general [2]. Ascertainment was by public appeal, however, and uncontrolled recall bias has not been excluded. Population-based cohorts may be needed to settle this issue.

It has been suggested that genes that contribute to multiple sclerosis susceptibility are difficult to identify because they exert a relatively modest effect on disease risk. Putative linkages have been reported for every chromosomal arm, but the only unambiguous genetic association and linkages identified are with alleles of the human leucocyte antigen (HLA) class II region, which is part of the major histocompatibility complex (MHC); this is particularly the case for HLA-DRB1 and HLA-DQB1 alleles. A large linkage study conducted in 2692 individuals from 730 multiplex families of Northern European descent [3] confirmed linkage to the MHC on chromosome 6p21 and possible linkage on chromosomes 17q23 and 5q33. Importantly, however, when the study population was stratified based on carriage of the multiple sclerosis-associated DRB1*1501 allele, no other region of linkage with genome-wide significance could be identified. A focus on the MHC recently has clarified matters. A large study of Canadian and Finnish families with multiple sclerosis [4] genotyped 4203 individuals with a high-density single nucleotide polymorphism panel spanning the genes encoding the MHC and flanking genomic regions. Again, strong associations were observed with blocks in the HLA class II genomic region, with the strongest association with HLA-DRB1. Importantly, conditioning on either the HLA-DRB1 or the most significant HLA class II haplotype block revealed no additional block or single nucleotide polymorphism association that was independent of the HLA class II genomic region.

In summary, these two large studies [3,4] indicated that MHC-associated susceptibility to multiple sclerosis is

determined by HLA class II alleles, their interactions and possibly closely neighbouring variants, and that other regions in the genome are unlikely to contribute much to disease susceptibility. This latter assertion is based on the assumption that multiple sclerosis is a disease and not a syndrome. At present, multiple sclerosis is defined using a set of polythetic criteria [5], which by their nature cannot be 100% specific. There will always be patients who are diagnosed as having multiple sclerosis but who do not have the 'disease'. Even low rates of misclassification may drastically reduce the power of genomic studies to detect loci associated or linked with the disease [6]. The role of class I alleles has been inconsistent, but recent reports appear to confirm observations reported by by Fogdell-Hahn *et al.* [7]. Nevertheless, accounting for linkage disequilibrium will need to be exhaustive, given the frequency with which type I errors occur in association studies.

In a study designed to examine parent-of-origin effects [8], high-density microsatellite genotyping of several regions of interest identified from previous whole-genome scans in families containing noncolineal affected pairs did not identify any non-MHC linkage. These results further challenge the widely accepted hypothesis that genetic susceptibility to multiple sclerosis is due to multiple genes that exert small individual effects. Similarly, recurrence risks in half-siblings are at odds with a model used to justify genome searches [9]. The nature of complexity at the class II loci appears crucial, and Dyment *et al.* [10] showed that there are multiple epistatic interactions at this locus. Susceptibility and resistance alleles interact, and multiple sclerosis risk is clearly determined by the genotype in this region.

Microenvironment

Migration studies, geographical gradients and high rates of discordancy in identical twins indicate that environment has significant influence on the development of multiple sclerosis. Migration studies suggest that exposure to these environmental factor(s) in early adolescence is associated with development of multiple sclerosis [11].

Whether environmental factors act as a trigger for multiple sclerosis or are involved in the ongoing pathogenesis of the disease has major therapeutic implications [12•]. If they are a trigger, then preventing exposure should prevent or reduce the risk that people will develop multiple sclerosis. On the other hand, if environmental factors are involved in the ongoing pathogenesis of multiple sclerosis, then identifying them may provide a potential therapeutic target for treating multiple sclerosis that is already present.

The 'hygiene' hypothesis has been evoked to explain the apparent increase in incidence of autoimmune diseases in general, including multiple sclerosis [13]. It is based on the theory that the immature immune system needs to be challenged in early life if it is to develop normally. In developed countries increased standards of hygiene, widespread use of antibiotics and vaccines, reduced exposure to siblings and peers with infections, and voluntary quarantine practices reduce the frequency and variety of early childhood infections. As a result of this the immune system fails to develop properly, and when it is challenged later in life it is prone to the development of autoimmunity. A corollary is that with a high incidence of parasitic infections, modulation of the immune system may cause a T-helper-2 T-cell bias that favours multiple sclerosis [13]. To address this issue quantitatively, Fleming and Cook [13] used the global prevalence of *Trichuris trichiura*, a relatively common human helminth, as a surrogate marker for infection with other parasites and low levels of sanitation. They found that the prevalence of multiple sclerosis falls steeply once a critical threshold of *T. trichiura* prevalence (about 10%) is exceeded.

A prospective, double-cohort study [14] was conducted to assess multiple sclerosis disease activity in patients with multiple sclerosis presenting with eosinophilia secondary to parasitic infections. Parasite-infected patients had significantly fewer exacerbations and magnetic resonance imaging changes compared with uninfected multiple sclerosis patients. The investigators ascribed this to increased production of interleukin-10 and transforming growth factor-β and induction of $CD25^{+}CD4^{+}$ $FoxP3^{+}$ T regulatory cells during parasite infections. The findings of this small study including only 12 patients require confirmation.

Within the context of the hygiene hypothesis, sibship is considered a surrogate marker of infectious load during early childhood. In a small population-based case–control study conducted in Tasmania, Australia, from 1999 to 2001, Ponsonby *et al.* [15] reported that increasing duration of contact with a younger sibling, aged under 2 years, during the first 6 years of life was associated with reduced risk for multiple sclerosis. This has not been confirmed. A Danish population-based study of 1036 people diagnosed with multiple sclerosis between 1968 to 1998 [16•] found no association between number of older siblings, number of younger siblings, total number of siblings, age distance from the nearest younger sibling, or exposure to younger siblings under 2 years of age and risk for multiple sclerosis later in life. There was no association of multiple sclerosis risk with multiple birth (versus singleton birth) or with the age of the mother or father at birth. In a Canadian population-based study of individuals with multiple sclerosis and their healthy siblings [17], no relationship between multiple sclerosis risk and birth order position was found. Using a different strategy to assess the impact of intrafamilial,

possibly infectious, factors, Dyment *et al.* [18] re-evaluated multiple sclerosis risk in 687 step-siblings of 19 746 multiple sclerosis index patients. They found the risk for multiple sclerosis to be indistinguishable from that in the general population. These results are concordant with studies of conjugal pairs, adopted children and half-siblings, and show no risk attributable to the familial microenvironment. In conclusion these findings lend no support to predictions of the hygiene hypothesis that the number of older siblings or any of the other sibship characteristics studied is associated with risk for multiple sclerosis. Family-based genetic epidemiological approaches have found no trace of nongenetic transmissibility. The environment appears to influence multiple sclerosis risk at a population level.

Macroenvironment

The rate of multiple sclerosis has been increasing, especially in females [19••], and this increase may also suggest an influence of early life events. Migration studies suggest that exposure to the putative environmental factor occurs in early adolescence [20]. Individuals who migrate from one area of the globe to another at some stage before adolescence are essentially exposed to a level of risk equivalent to that of the area to which they migrate. In comparison, those who migrate after adolescence carry with them the multiple sclerosis incidence of the area they migrated from. Countries such as Israel and South Africa have a much greater incidence than would be expected based on their latitude, presumably because they have such high levels of immigration of first-generation Europeans. Conversely, first-generation Afro-Caribbean immigrants to the UK have a much lower incidence of multiple sclerosis than their second-generation counterparts [21]. Dean and Elian [22] studied the incidence of multiple sclerosis in Caribbeans who migrated to England. They found that Caribbeans acquired the multiple sclerosis rate of Londoners after a latent period of 15–20 years. Indian and Pakistani immigrants to England who entered the country when they were younger than 15 years had a risk for multiple sclerosis that was greater than that of those who entered after this age. A similar observation has been noted in Canada [23]; children with multiple sclerosis are more likely to be of Caribbean, Asian, or Middle Eastern ancestry, and are less likely to have European heritage, as compared with individuals with adult-onset multiple sclerosis.

Vitamin D and sunlight

Two associated factors that have been recognized as potentially accounting for the link between geography, in particular latitude, and the incidence of multiple sclerosis are sunlight exposure and vitamin D status. Experimental and epidemiological data suggest that high levels of vitamin D decrease the risk for multiple sclerosis. A prospective, cohort study found that the taking of vitamin supplementation that included vitamin D was associated with an approximate 40% reduction in risk for developing multiple sclerosis, but the amounts taken were insufficient to cause much change in vitamin D levels. Small uncontrolled studies suggest that vitamin D supplementation may decrease multiple sclerosis relapses (for review, see the report by Brown [24]). A prospective, nested case–control study conducted in more than 7 million military personnel in the USA, for whom serum samples were stored, found that lower risk for multiple sclerosis was associated with high serum 25-hydroxyvitamin D levels [25]. In white people the risk for developing multiple sclerosis decreased significantly with increasing levels of 25-hydroxyvitamin D (odds ratio for a 50 nmol/l increase in 25-hydroxyvitamin D: 0.59, 95% confidence interval 0.36–0.97; $P = 0.02$). Using the lowest quintile as the reference, the odds ratios for each subsequent quintile were 0.57, 0.57, 0.74 and 0.38; the odds ratio for the highest quintile was significantly different from 1.00 (odds ratio 0.38, 95% confidence interval 0.19–0.75; $P = 0.006$). Surprisingly, in black and Hispanic people no significant associations between serum vitamin D levels and risk for multiple sclerosis were found.

A pooled analysis of data from Canada, the UK, Denmark and Sweden, including more than 42 000 patients, found that significantly fewer people with multiple sclerosis are born in November and significantly more are born in May [26]. This observation that month of birth and risk for multiple sclerosis are associated implies an interaction with the environment that may act during gestation or shortly after birth. An hypothesis proposed was that this month of birth effect is linked to the vitamin D status of the mother.

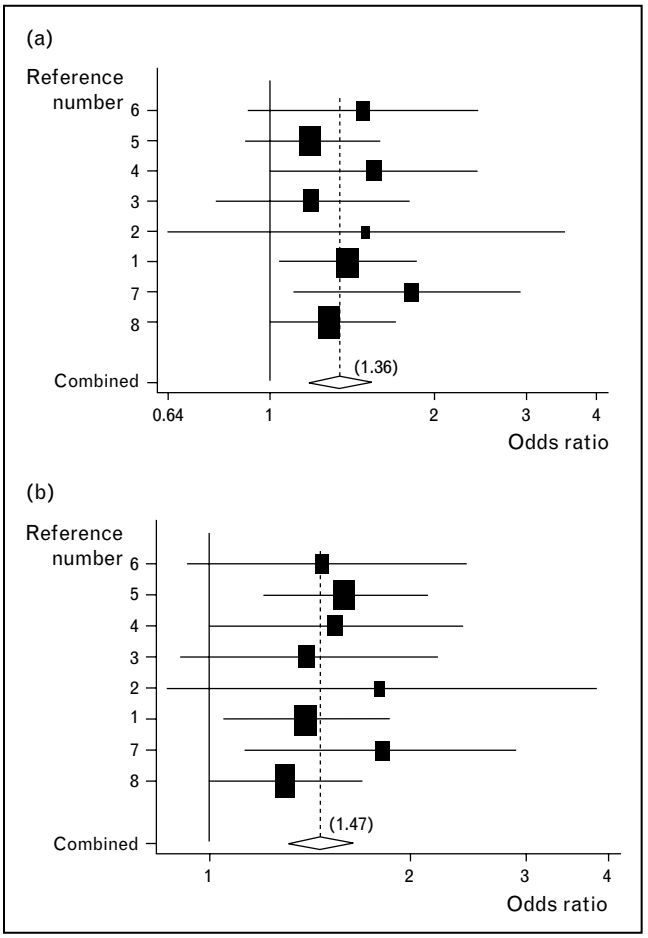
1,25-Dihydroxyvitamin D inhibits experimental allergic encephalomyelitis (EAE) [24]. A recent study [27] confirmed previous observations that vitamin D_3 and 1,25-dihydroxyvitamin D inhibit myelin oligodendrocyte glycoprotein induced EAE, but they were unable to prevent it in mice lacking interleukin-10 or its receptor. In another study [28], treatment of mice with 1,25 dihydroxyvitamin D_3 intraperitoneally ameliorated EAE, and inhibited interleukin-12 production and antigen-specific T-helper-1 responses. In-vitro treatment of activated T cells with 1,25 dihydroxyvitamin D_3 inhibited interleukin-12 induced tyrosine phosphorylation of several tyrosine kinases and transcription factors (Janus kinase-2, tyrosine kinase-2, and signal transducer and activator of transcription-3 and -4) in association with reduced T-cell proliferation. These studies add to previous observations that vitamin D is immunomodulatory and has complex immunological effects on T cells. Population-based prevention studies and adequately powered phase II and III clinical studies are now

required to define further the role of vitamin D metabolism in multiple sclerosis.

Smoking and other environmental factors

A recent review of the literature related other environmental factors (e.g. smoking, alcohol consumption, recreational drugs, use of the oral contraceptive pill and diet) to multiple sclerosis risk [29]. Only smoking before onset of multiple sclerosis emerged as a significant, albeit moderate, risk factor for the subsequent development of multiple sclerosis. In the retrospective meta-analysis, ever versus never smoking status from four studies yielded a pooled relative risk estimate for developing multiple sclerosis of 1.51 (95% confidence interval 1.24–1.83; $P < 0.0001$). Prospective analyses were also conducted using conservative and a nonconservative approaches to represent the likely exposure to cigarettes. For the conservative approach, three categories were incorporated, namely ever, previous smoker and smoking 1–14 cigarettes per day; this gave a pooled relative risk estimate of 1.25 (95% confidence interval 1.05–1.49; $P < 0.01$). In the less conservative analysis, three groups were also considered: ever, current and more than 15 cigarettes per day. This yielded a pooled relative risk of 1.45 (95% confidence interval 1.22–1.72; $P < 0.0001$). The pooled analysis of all eight studies (retrospective and prospective), using the most conservative comparisons, yielded an odds ratio of 1.36 (95% confidence interval 1.19–1.54; Fig. 1a). A combined analysis using less conservative comparisons gave an odds ratio of 1.47 (95% confidence interval 1.29–1.67) (Fig. 1b). This meta-analysis is supported by the findings of another, more recent case–control study [30]. It remains to be seen whether smoking explains some of the increase in the female : male multiple sclerosis sex ratio reported from Canada [19••].

Figure 1 Smoking and multiple sclerosis: a meta-analysis of smoking habit and relative risk for developing multiple sclerosis



(a) Retrospective and prospective smoking studies combined using a conservative analysis. (b) Retrospective and prospective studies combined, using less conservative analysis. The reference numbers given are those from Hawkes [29]. Modified with permission from [29].

Transmissible agents

Reports implicating specific transmissible agents as possible causes of multiple sclerosis continue to appear regularly [10,31]. Leading candidates include Epstein–Barr virus (EBV), human herpesvirus (HHV) type 6, multiple sclerosis-associated human endogenous retrovirus (HERV) and *Chlamydia pneumoniae* [12•,32].

Human endogenous retroviruses and human herpesvirus-6

HERV and HHV-6 have been hypothesized to play a role in autoimmune diseases both as putative susceptibility genes and as pathogenic viruses [33,34]. The sequences of both HERV-H and HERV-W have been isolated from samples of plasma, serum and cerebrospinal fluid from patients with multiple sclerosis. HERV and herpesviruses are known to have complex interactions [35], which raises the possibility of a two-hit hypothesis (i.e. multiple sclerosis may be caused by two viruses interacting with one another). In neuronal and brain endothelial cell cultures, HERV-W gag and env proteins are induced by herpes simplex virus-1 [36]. HERV-W gag and env protein expression occur in multiple sclerosis lesions, and an accumulation of gag antigen was also seen in axonal structures in demyelinated white matter and in endothelial cells of multiple sclerosis lesions from acute or actively demyelinating cases [37]. The latter was not seen in any of the control individuals. In comparison, expression of env proteins were seen in microglia in normal brain, and in macrophages in early multiple sclerosis lesions. The reported differential expression of these proteins in patients with multiple sclerosis is unexplained and may simply reflect differential regulation of inherited HERV-W copies rather than expression of 'infectious' multiple sclerosis associated retrovirus copies.

In another pathological study [38] all brains were shown to express HERV-W env and pol genes. Accumulation of HERV-W specific RNA was greater in multiple sclerosis brains than in control brains ($P = 0.014$ versus healthy control individuals; $P = 0.006$ versus pathological control individuals). No HERV-W env protein was detected in control brains, but it was shown to be upregulated within multiple sclerosis plaques and correlated with active demyelination and inflammation. In peripheral blood, all patients with multiple sclerosis expressed HERV-W env at higher copy numbers than did control individuals ($P = 0.00003$). No HHV-6 specific RNA was detected in brains of patients with multiple sclerosis, and no differences were found in peripheral blood between patients with multiple sclerosis and control individuals. The association between HERV-W, HHV-6 and multiple sclerosis is based mainly on pathological studies; different research strategies will be required to prove causation (see section entitled 'Causation', below).

Epstein–Barr virus

The epidemiological data associating EBV infection with multiple sclerosis remains strong. A consistent finding across eight case–control studies is the finding that almost all patients with multiple sclerosis (>99%) are infected with EBV, as compared with only about 90% of control individuals (see the meta-analysis by Ascherio and Munch [39]). A Turkish study [40] investigating the association between EBV exposure and multiple sclerosis in children found that 83% of children with multiple sclerosis had been infected with EBV at the time of diagnosis, as compared with only 42% of appropriate age-matched control individuals ($P < 0.001$). A similar European seroprevalence study conducted in 147 paediatric patients [41] reported that 99% of children with multiple sclerosis had detectable antibody against EBV virus capsid antigen, as compared with only 72% of age-matched control individuals ($P = 0.001$).

People with symptomatic EBV infection or infectious mononucleosis are at increased risk for developing multiple sclerosis compared with people who have not had infectious mononucleosis. A systematic review and meta-analysis of 14 case–control and cohort studies [42] reported a combined relative risk for multiple sclerosis after infectious mononucleosis of 2.3 (95% confidence interval 1.7–3.0; $P < 10^{-8}$). This risk was subsequently confirmed in a large Danish cohort study including more than 25 000 Danish patients with suspected infectious mononucleosis who were followed up for the occurrence of multiple sclerosis after the diagnosis of infectious mononucleosis or a negative Paul-Bunnell test [43]. The ratio of observed : expected multiple sclerosis cases was 2.27 (95% confidence interval 1.87–2.75). Interestingly, the risk for multiple sclerosis was persistently increased for more than 30 years after infectious mononucleosis, and it was uniformly distributed across all investigated strata of sex and age.

People with high titres of anti-EBV antibodies are at higher risk for developing multiple sclerosis than are those with low titres [44,45]. This is independent of antibody titres to cytomegalovirus, a related herpesvirus, suggesting that it is not a nonspecific phenomenon.

Another clue linking EBV infection to multiple sclerosis is the small cluster of cases of multiple sclerosis that occurred in a small Danish community called Fjelso [46]. All eight members of the cluster developed multiple sclerosis in close temporal association with an outbreak of EBV infection, and all members were harbouring the same subtype of the virus.

Hodgkin's lymphoma, which is aetiologically linked to EBV, occurs more frequently in families with multiple sclerosis and in patients with multiple sclerosis than in the general population [47]. Three independent groups have extracted peptides homologous with EBV from random peptide libraries using cerebrospinal fluid-derived multiple sclerosis immunoglobulin G and have shown intrathecal immunoglobulin G synthesis reactive to EBV protein [48–51]. Myelin basic protein-reactive T-cell clones derived from patients with multiple sclerosis cross-react with a nonhomologous EBV peptide presented by a different MHC class II molecule [52]. EBV-encoded nuclear antigen-1 specific $CD4^+$ T-cell responses from patients with multiple sclerosis preferentially recognize multiple epitopes within the central part of the carboxyl-terminal EBV-encoded nuclear antigen-1 domain, as compared with fewer more restricted epitopes in healthy EBV carriers [53]. These observations support EBV-induced molecular mimicry as a possible mechanism underlying autoimmunity in multiple sclerosis.

Individuals with multiple sclerosis are more likely to spontaneously produce immortal B cell lines, an EBV-dependent phenomenon, than are normal control individuals [54]. Multiple sclerosis patients exhibit a frequency of circulating EBV-specific cytotoxic T cells that is higher than healthy EBV carriers [51,55]. Whether these T cells are targeting EBV-infected cells or are part of an ongoing autoimmune response needs to be determined.

During relapse there is a suggestion that multiple sclerosis patients have evidence of active peripheral EBV replication as compared with patients with clinically stable disease [56]. Patients with multiple sclerosis and an antibody response to EBV early antigen were more likely to exhibit disease activity, as measured using gadolinium-enhanced magnetic resonance imaging [57].

Table 1 Hill's criteria for causation

Number	Criterion	General question
1	Consistency and unbiasedness of findings	Is there agreement among repeated observations in different places, at different times using different methodologies, by different researchers and under different circumstances?
2	Strength of association	What is the relative risk?
3	Temporal sequence	Does exposure precede the outcome variable?
4	Biological gradient	Is there evidence of a dose–response relationship?
5	Specificity	Is the outcome unique to the exposure?
6	Coherence with biological background and previous knowledge	Is the causal association compatible with present knowledge of the disease?
7	Biological plausibility	Does the causal relationship make biological sense?
8	Reasoning by analogy	Does the causal relationship conform to a previously described relationship?
9	Experimental evidence	Does controlled manipulation of the exposure variable change the outcome?

Hill's criteria are from [64].

EBV reactivation induces the expression in peripheral B cells of alpha-B-crystallin, a stress protein [58], which has been identified as one of the immunodominant antigens in central nervous system (CNS) myelin from patients with multiple sclerosis [59]. It has been proposed that EBV-induced peripheral alpha-B-crystallin expression may therefore drive a pathogenic autoimmune response in multiple sclerosis [58].

The association of EBV infection with multiple sclerosis may be causative, or EBV infection may simply be a ubiquitous epiphenomenon that is required at the onset of the disease. The observation that EBV has been linked to other putative autoimmune diseases [60,61], in addition to multiple sclerosis, suggests that it may be an important nonspecific trigger in the autoimmune cascade. One current hypothesis states that EBV randomly immortalizes B cells, which, as professional antigen-presenting cells, are capable of presenting autoantigens to autoreactive T cells [62]. These cells are resistant to apoptosis, and therefore they can perpetuate an aberrant autoimmune response [62]. An alternative hypothesis is that EBV operates indirectly by activating the pathogenic expression of endogenous retroviruses such as HERV-W, which may cause multiple sclerosis (see above) [34,63,64].

Causation

There are difficulties in applying Koch's postulates to infectious agents that cannot be cultured in the laboratory or infections that are specific to humans, and therefore most investigators adopt Sir Austin Bradford Hill's criteria for causation (Table 1) [65]. Bradford Hill's criteria were recently adapted in relation to infectious disease [66], and more recently they have been proposed as a framework for demonstrating causation in relationship to multiple sclerosis [12•]. Proving that an association between an environmental factor and multiple sclerosis is causal is complicated and may take years or even decades to achieve [12•]. There are several theories to explain how infection may cause multiple sclerosis, some of which are more plausible than others. One theory is that multiple sclerosis is due to an autoimmune reaction that is triggered by a monophasic infection, termed the 'hit-and-run molecular mimicry hypothesis'. A second possibility is that persistent peripheral infection drives an immune reaction that crossreacts with the CNS, termed the 'persistent infection molecular mimicry hypothesis'. Third, persistent infection of glial cells (e.g. oligodendrocytes) initiates focal inflammation within the CNS, termed 'direct infection hypothesis'. A fourth theory is that an infection deregulates the immune system and establishes an organ-specific autoimmune disease, termed the 'immune dysregulation hypothesis'. A final possibility is that multiple sclerosis is due to a dual infection, termed the 'the double infection hypothesis'. Much of the evidence favouring a role for EBV was adduced for measles a generation ago, however, and these lessons should not be forgotten [67].

Conclusion

Multiple sclerosis is a complex disease that is due to interactions between genes and environmental factors that lead to tissue injury by autoimmune mechanisms. Risk for developing multiple sclerosis is clearly determined by an interaction between HLA susceptibility and resistance genotypes in the HLA region. Family-based genetic epidemiological approaches have found no trace of nongenetic transmissibility. The environment influences multiple sclerosis risk at a population level. Important environmental factors include sunlight exposure, vitamin D status, smoking and infections. Of the infectious agents linked to multiple sclerosis, epidemiological evidence currently supports a role for EBV infection in the pathogenesis of multiple sclerosis.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 359–362).

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