Environmental Risk Factors for Multiple Sclerosis. Part I: The Role of Infection

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Although genetic susceptibility explains the clustering of multiple sclerosis (MS) cases within families and the sharp decline in risk with increasing genetic distance, it cannot fully explain the geographic variations in MS frequency and the changes in risk that occur with migration. Epidemiological data provide some support for the "hygiene hypothesis," but with the additional proviso for a key role of Epstein–Barr virus (EBV) in determining MS risk. We show that whereas EBV stands out as the only infectious agent that can explain many of the key features of MS epidemiology, by itself the link between EBV and MS cannot explain the decline in risk among migrants from high to low MS prevalence areas. This decline implies that either EBV strains in low-risk areas have less propensity to cause MS, or that other infectious or noninfectious factors modify the host response to EBV or otherwise contribute to determine MS risk. The role of infectious factors is discussed here; in a companion article, we will examine the possible role of noninfectious factors and provide evidence that high levels of vitamin D may have a protective role, particularly during adolescence. The primary purpose of these reviews is to identify clues to the causes of MS and to evaluate the possibility of primary prevention.

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Geography and Migration

Multiple sclerosis (MS) is a relatively common disease in Europe, the United States, Canada, New Zealand, and parts of Australia. Incidence is low in childhood, increases rapidly after age 18, reaches a peak between 25 and 35 years (about 2 years earlier in women than men), and then slowly declines, becoming rare at age 50 and older.[1–3] Risk is greater in women than in men; the female-to-male ratios are between 1.5 and 2.5 in most populations, with a trend toward greater values in the most recent studies.[4] In high-risk populations, the lifetime risk for MS is about 1 in 200 for women and somewhat less for men.[1,5] MS is rare in Asia, and in all continents, it is rare in the tropics and subtropics. Within regions of temperate climate, MS incidence and prevalence increase with latitude, both north and south of the equator.[6]

Genetic predisposition most likely contributes to geographic variations in MS incidence,[2,7] but it cannot explain the remarkable differences in risk among people of common ancestry who migrate to areas of high or low MS prevalence.[8] Caution is warranted in interpreting these differences, because migrants to a certain region may differ in socioeconomic status, health, and genetic constitution from nonmigrants or other migrants. Furthermore, differential access to health care and uncertainty about the denominator, which may not include illegal immigrants, are sources of potential bias in studies of international migration.[8] Nevertheless, some of these concerns do not apply to studies of migration within the United States, and an overall critical review of the data shows a rather compelling pattern. 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.[8] A difference, however, has been noted according to the direction of migration. MS risk declines among individuals migrating from high- to low-risk areas,[9–14] but does not consistently increase with migration in the opposite direction. An example is the lack of increase in MS risk with migration to the United Kingdom among natives of the Caribbean and Asia,[15–17] whose genetic susceptibility is proven by the high rates of MS among their UK-born children.[18,19] The interpretation of these data has been questioned on the grounds that most immigrants from low-risk areas had been in the United Kingdom for less than 5 years, which may not have been sufficient time for the effect of migration to become manifest.[20] This objection notwithstanding, the data on migration from

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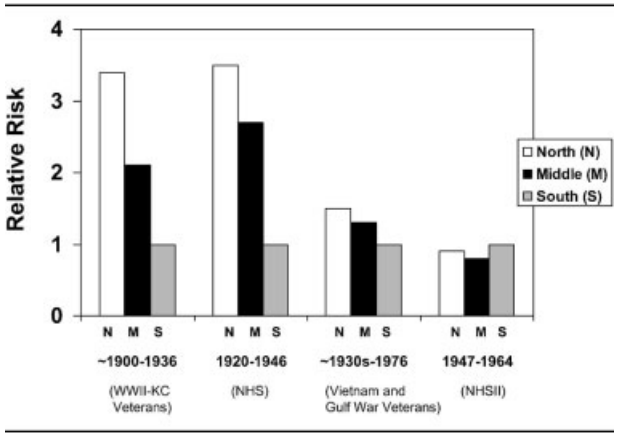

Fig 1. Relative risk for multiple sclerosis (MS) by latitude at birth in different birth cohorts of US white women. Birth cohort dates for veterans are approximate. Data for veterans are from Wallin and coworkers[21] and those from the two nurses study from Hernán and colleagues.[5] Among white male veterans, the north:south relative risk declined from 2.5 (WWII and Korea) to 2.0 (Vietnam and later), whereas no change was reported in black men (relative risk 1.9 in both groups).

low- to high-risk areas support the hypothesis that individuals born in low-risk areas appear to benefit from some long-lasting protection that is not, however, transmitted to their children.

Attenuation of the Latitude Gradient

The latitude gradient, which for many decades has been the most salient feature of MS epidemiology, is disappearing. The most striking data are from white women in the United States; in a cohort of female nurses born before 1946, we found that MS risk was three times greater among those born in the north (42° N and above) than those born in the south (37° N and below), whereas there was no latitude difference in a similar cohort of nurses born after 1946 (Fig 1).[5] A marked attenuation of the latitude gradient among white women within the United States has been independently confirmed in a study among US veterans[21] (see Fig 1). In the same study, a less prominent attenuation of the latitude gradient was seen among white men, whereas no change was seen in black men (the number of black women was insufficient for analysis). Because MS incidence in the north of the United States appears to have been constant over the past several decades,[3] the disappearing latitude gradient most likely reflects an increased incidence in the south. Although a greater degree of underrecognition of MS in the southern states as compared with the northern states in the earlier cohorts cannot be completely excluded, it is highly unlikely that it accounted for a threefold difference among individuals of similar socioeconomic status[5] and access to health care.[21] In Europe, there is also evidence of an increase in MS incidence in some southern regions. Increased recognition in some countries is difficult to exclude, but a true increase appears likely in selected areas that were intensively and repeatedly surveyed.[22]

Overall, these studies implicate environmental factors in childhood, and possibly during adult life, as strong determinants of MS risk, but do not identify the nature of these factors. Both infectious and noninfectious putative factors deserve consideration. We discuss infectious factors here, whereas noninfectious factors are discussed in Part II of our review.

Infection as Possible Explanation of Geographic Variations and Migrant Data

Most research on infectious causes of MS is based on clinical and pathological observations or experiments in animal models, as reviewed elsewhere.[23] Here, however, we follow a complementary approach: we start by examining to what extent an infectious agent could provide a plausible explanation for the *epidemiology* of MS.

In this context, two general hypotheses were originally proposed. A common aspect to both is that a widespread microbe, rather than a rare pathogen, causes MS. The first has been called the *poliomyelitis hypothesis,* which postulates that there is a virus that increases the risk for MS if acquired in late childhood or adulthood, but is less harmful and confers protective immunity if acquired in infancy.[24] The second is the *prevalence hypothesis,* championed by Kurtzke[25] and colleagues and partly based on his investigation of the Faroe Islands epidemic (see later), which postulates that MS is caused by a pathogen that is more common in regions of high MS prevalence. According to Kurtzke,[25] this pathogen is widespread and in most individuals causes an asymptomatic persistent infection; only rarely, and years after the primary infection, does this agent cause neurological symptoms (ie, MS).

The poliomyelitis hypothesis appears to have gained more favor than the prevalence hypothesis, but it has evolved into a more general "hygiene hypothesis," an idea initially proposed 30 years ago from observations on sanitation and MS incidence in Israel.[26] According to the hygiene hypothesis, exposure to several infectious agents early in life is protective against MS, as in the poliomyelitis model, but there is not a specific agent responsible[27–29]; rather, MS is an autoimmune reaction that is triggered in susceptible individuals in response to infection by multiple microorganisms, with risk increasing with age at infection.[28,29] This hypothesis is in many ways attractive, because it could explain the latitude gradient and the apparent protection from MS of individuals born in low-risk areas who migrate to high-risk areas, as well as other features of MS epidemiology, such as the greater incidence rates among

Table 1. **Odds Ratios of Multiple Sclerosis in Epstein–Barr Virus Seronegative versus Seropositive Subjects**

Study	Cases, N		Control Subjects, N		OR of MS for Seronegativity	Exact 95% CI*
	+	−	+	−		
1. Sumaya and colleagues, 1980[37]	155	2	76	5	0.2	0.02–1.24
2. Bray and colleagues, 1983[38]	309	4	363	43	0.11	0.03–0.31
3. Larsen and colleagues, 1985[39]	93	0	78	15	0	0–0.05
4. Sumaya and colleagues, 1985[40]	104	0	99	5	0	0–1.07
5. Shirodaria and colleagues, 1987[41]	26	0	24	2	0	0–5.29
6. Ferrante and colleagues, 1987[42]	29	1	31	11	0.1	0–0.76
7. Munch and colleagues, 1997[43]	137	1	124	14	0.06	0–0.44
8. Myhr and colleagues, 1998[44]	144	0	162	8	0	0–0.67
9. Wagner and colleagues, 2000[45]	107	0	153	10	0	0–0.66
10. Ascherio and colleagues, 2001[46]	143	1	269	18	0.1	0–0.68
11. Haahr and colleagues, 2004[47]	153	0	50	3	0	0–0.82
12. Sundström and colleagues, 2004[48]	234	0	693	9	0	0–1.5
13. Ponsonby and colleagues, 2005[34]	136	0	252	9	0	0–0.96
Total	1770	9	2374	152	$OR_{MH} = 0.06$	0.03–0.13[a]

[a]Cornfield confidence interval; $p < 0.000000001$.

*Calculated as described in: Mehta CR, Patel NR, Gary R. J Am Stat Assoc 1985;78:969–973.

OR = odds ratio; MS = multiple sclerosis; CI = confidence interval.

individuals of higher education and income,[30–32] the trend toward a later age at infection with childhood viruses in MS cases than control subjects,[33] the lower risk for MS among individuals exposed to infant siblings in early life,[34] and possibly the attenuation of the latitude gradient within the United States, which could be ascribed to improved hygienic conditions, and thus lower incidence of childhood infections in the south.

Beyond the Hygiene Hypothesis: The Epstein–Barr Virus Paradox

Two limitations of the hygiene hypothesis of MS should be considered. One is that, considering the multiplicity of infectious agents and the diversity of host immune responses that they generate, it appears unlikely that all microbes or parasites are equally involved in predisposing to MS. In fact, observations in animal models of autoimmune diseases suggest that some infections enhance whereas others may contribute to prevent autoimmune diseases.[27,35] Thus, one is left with the task of identifying which infections are important and which are not. Without this further step, the hypothesis is lacking and insufficient to formulate new preventive or therapeutic measures. The second, and most critical, limitation is what could be called the Epstein–Barr virus (EBV) paradox; that is, the extremely low risk for MS among individuals who are seronegative for EBV. Having escaped infection in childhood, these individuals will, on average, have experienced a more "hygienic" upbringing than their EBV-positive coetaneous peers, a concept supported by the positive correlation between age at infection with EBV and socioeconomic status.[36] Thus, according to the hygiene hypothesis, these individuals should have a high MS risk; in contrast, their MS risk is many folds less than that of their EBV-positive peers (Table 1),[34,37–49] a finding recently confirmed in studies of pediatric MS.[50,51] The fact that EBV infection is associated with a dramatic increase in MS risk has been obfuscated for many years by the fact that EBV infects more than 95% of the adult population. This high rate of infection resulted in a low power of individual studies attempting to establish an association and, perhaps most importantly, to the illogical conclusion that a virus infecting almost everyone cannot cause a relatively rare disease such as MS. The fallacy of this conclusion should be clear from many examples, including that of poliovirus itself, which in the prevaccine years infected virtually all children in endemic countries, but caused clinical poliomyelitis only in a few.[52] Furthermore, the risk for MS is significantly increased among individuals with a history of infectious mononucleosis, a common manifestation of EBV infection in adolescence or adulthood,[53] as compared with individuals without such a history,[54] a finding that suggests that later age at infection with EBV further increases the odds of development of MS.

A summary plot of the relative risk for MS as a function of EBV infection and infectious mononucleosis is shown in Figure 2. Using individuals infected with EBV in early childhood as the reference, the risk is about 10-fold *less* among EBV-negative individuals,

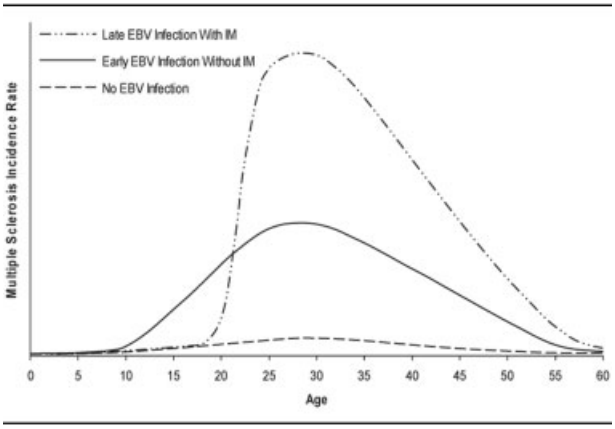

Fig 2. Schematic representation of multiple sclerosis incidence according to Epstein–Barr virus infection. Reprinted with permission from Thacker and colleagues.[54]

and about 2- to 3-fold *greater* among those infected with EBV later in life (as inferred from history of mononucleosis); thus, there is at least a 20-fold increase in risk among individuals with a history of mononucleosis compared with those who are EBV-negative, despite their sharing a similar "high hygiene" childhood environment. Although based on multiple snapshots at different ages and in different populations rather than on the ideal longitudinal follow-up of a large cohort of children recruited at birth, the findings summarized in Figure 2 are robust, because EBV serology has high positive and negative predictive values, and a single test can determine with high accuracy whether the subject has ever been infected.[55] As with all herpes viruses, EBV establishes a lifelong infection providing continuous stimulation to the immune system, and antibody titers to diagnostic EBV antigens in healthy subjects tend to remain constant over time. Furthermore, it is extremely unlikely that these data reflect an increase in EBV infection after the onset of MS because there is a conspicuous absence of recent EBV infection among individuals with MS[56] and because the association between mononucleosis and increased MS risk has been confirmed in longitudinal studies.[54,57]

The above data support in part the hygiene hypothesis, but with the additional caveat that what determines the increased MS risk for children brought up in an hygienic environment is that they are infected with EBV late in life; if EBV infection could be prevented, their MS risk would remain minimal. Before accepting this hypothesis, which we will call the "EBV variant" of the hygiene hypothesis, however, it is important to examine the specificity of the association of EBV with MS, and to explore alternative explanations for the EBV paradox. An obvious question is whether evidence analogous to that presented earlier for EBV is available for other infectious agents. Unfortunately, in many cases, there are no data assessing the association of interest, either because there is no specific laboratory test to determine the presence of infection, or because if such a test exists it has not been applied in an appropriately designed investigation. Furthermore, the potential role in MS of truly ubiquitous viruses such as human herpes virus (HHV6) cannot be addressed in this manner, because everybody is infected in childhood. Nevertheless, existing seroepidemiological data do not support strong associations with at least some selected viruses, including herpes simplex virus 1,[42,56] varicella zoster,[38,44,48,58] measles,[38,41,42,48,58–63] mumps,[38,60,62] or rubella.[41,58,60,62] Data are less clear for herpes simplex virus 2, which was more prevalent among MS cases in two studies based on concomitant control subjects (Table 2) and in a third study based on historical control subjects,[64] although in the latter, results were incompletely adjusted for age, which is a strong confounder. Nevertheless, EBV clearly stands out for the strength and consistency of its association with MS (see Tables 1 and 2). Another possible explanation of the EBV paradox is that susceptibility to EBV infection and MS could share common genetic determinants. Two scenarios can be postulated. One is that those individuals who are still EBV-seronegative in adulthood are genetically resistant to both MS and EBV infection. Evidence against this explanation comes from two studies on pediatric MS, one from Canada[50] and one from Germany.[51] In those studies, 28 to 54% of the control children were EBV-negative (in contrast with 1–17% of the children with MS); because in the same populations the seronegativity to EBV among healthy adults is less than 10%, genetic resistance to EBV infection is an improbable explanation for the low rates of infection among control children. The other hypothetical scenario is that of a genotype that increases both the risk for MS and the susceptibility to EBV infection in childhood. Such a genotype could explain the increased MS risk among seropositive children, but would be uncommon among individuals with history of mononucleosis (infected with EBV late in life) and could not explain their increased MS risk.[54] Although more complex explanations that involve multiple genes with different specificities cannot be excluded, it appears that the EBV-hygiene hypothesis is the most plausible explanation for the data presented so far.

Anti–Epstein–Barr Virus Antibodies and Risk for Multiple Sclerosis

Increased antibody titers to EBV[39,41] and other viruses[41,48,58,60] have been reported in individuals with MS, but these elevations could simply reflect the immune dysregulation in MS and their causative significance is uncertain.[29] An important contribution to understanding the relation between EBV and MS has thus come from longitudinal studies of antibodies in serum of healthy individuals who later developed MS.

Table 2. **Odds Ratios of Multiple Sclerosis and Exact 95% Confidence Intervals for *Seronegativity* to Selected Viruses**

Virus	Cases, N		Control Subjects, N		OR (95% CI)	Laboratory Methods
	+	−	+	−		
HSV						
Haire and colleagues, 1973[58]	56	1	48	9	0.10 (0–0.74)	Immunofluorescence
Bray and colleagues, 1983[38]	145	86	234	124	1.1 (0.78–1.6)	Complement fixation
Myhr and colleagues, 1998[44]	117	27	148	22	1.6 (0.80–3.0)	ELISA
Sundström and colleagues, 2004[48]	189	45	567	135	1.0 (0.67–1.5)	ELISA
HSV1						
Ferrante and colleagues, 1987[42]	25	5	32	10	0.64 (0.15–2.4)	ELISA
Wandinger and colleagues, 2000[56]	84	24	127	36	1.0 (0.53–1.9)	ELISA and Western blots
HSV2						
Ferrante and colleagues, 1987[42]	19	11	12	30	0.23 (0.08–0.70)	ELISA
Wandinger and colleagues, 2000[56]	21	87	17	146	0.48 (0.23–1.0)	ELISA and Western blots
VZV						
Haire and colleagues, 1973[58]	48	7	45	10	0.66 (0.19–2.1)	Immunofluorescence
Bray and colleagues, 1983[38]	102	102	110	142	0.77 (0.53–1.1)	Complement fixation
Myhr and colleagues, 1998[44]	141	3	167	3	1.2 (0.16–9.0)	ELISA
Sundström and colleagues, 2004[48]	225	9	682	20	1.4 (0.54–3.2)	ELISA
CMV						
Bray and colleagues, 1983[38]	110	123	169	128	1.5 (1.0–2.1)	Complement fixation
Myhr and colleagues, 1998[44]	91	117	53	53	1.3 (0.78–2.1)	ELISA
Wandinger and colleagues, 2000[56]	39	69	64	99	1.1 (0.67–2.0)	ELISA
Ascherio and colleagues, 2001[46]	72	72	161	127	1.3 (0.83–1.9)	Immune adherence hemagglutination
Measles						
Clarke and colleagues, 1965[59]	26	0	49	3	0 (0–4.9)	Hemagglutination inhibition
Panelius and colleagues, 1971[60]	135	2	135	2	1.0 (0.07–14)	Hemagglutination inhibition
Haire and colleagues, 1973[58]	57	0	56	1	0 (0–39)	Immunofluorescence
Poskanzer and colleagues, 1980[61]	59	22	119	27	1.6 (0.82–3.3)	Hemagglutination inhibition and ELISA
Leinikki and colleagues, 1982[62]	17	1	88	0	Undefined	ELISA
Bray and colleagues, 1983[38]	61	59	83	256	0.31 (0.20–0.50)	NA
Krakowka and colleagues, 1983[63]	17	3	15	5	0.53 (0.07–3.3)	NA
Ferrante and colleagues, 1987[42]	29	1	40	2	0.69 (0.01–14)	ELISA

Table 2. Continued

Shirodaria and colleagues, 1987[41]	26	0	26	0	Undefined	Immunofluorescence
Sundström and colleagues, 2004[48]	234	0	698	4	0 (0–4.6)	ELISA
Mumps						
Panelius and colleagues, 1971[60]	134	1	136	1	1.0 (0.01–80)	Hemagglutination inhibition
Leinikki and colleagues, 1982[62]	14	4	86	2	12 (1.5–142)	ELISA
Bray and colleagues, 1983[38]	76	157	83	216	0.79 (0.54–1.2)	Complement fixation
Rubella						
Panelius and colleagues, 1971[60]	131	3	132	4	0.76 (0.11–4.6)	Hemagglutination inhibition
Haire and colleagues, 1973[58]	52	5	54	3	1.7 (0.32–11)	Immunofluorescence
Leinikki and colleagues, 1982[62]	18	0	88	0	Undefined	ELISA
Shirodaria and colleagues, 1987[41]	25	1	25	1	1.0 (0.01–81)	Hemagglutination inhibition

Including only studies based on concomitant (rather than historical) control subjects, and reporting results adjusted for age and sex. Odds ratio (OR) and 95% confidence intervals (CIs) not provided in original articles were calculated by the authors of this study.

ELISA = enzyme-linked immunosorbent assay; HSV = herpes simplex virus; VZV = varicella zoster virus; CMV = cytomegalovirus; NA = not available.

Several indirect immunofluorescence assays are commonly used to characterize the antibody response to EBV. These assays measure antibody responses to the viral capsid antigen (VCA) and early antigen, which are expressed in the lytic cycle, and to the EB nuclear antigen (EBNA) expressed in latently infected cells.[55] EBNA is a complex of six distinct proteins, one of which (EBNA 1) is primarily recognized in the conventional anti-EBNA complex assay. Specific assays for antibodies to EBNA 1 and 2 are also frequently used for clinical and research purposes. The antibody response to EBV infection in naive individuals has been well characterized in individuals with mononucleosis. By the onset of symptoms, most patients have high titers of IgM antibodies to VCA and increasing IgG titers to VCA and early antigen. Although anti–early antigen often becomes undetectable after convalescence, the anti-VCA IgG titers persist at stable levels indefinitely.[55] Reactivity to EBNA 1 and 2 appear at distinct times. The IgG response to EBNA 2 appears in the acute phase of the disease and declines during convalescence, whereas anti-EBNA 1 IgG antibodies become detectable only during convalescence and remain stable throughout life.[55] Anti-EBNA 1 and anti-EBNA 2 IgG also behave differently during immunosuppression: anti-EBNA 2 titers increase, whereas anti-EBNA1 titers decrease, leading to an inversion of the anti-EBNA 1/anti-EBNA 2 ratio, which in healthy individuals is greater than 1.[65] Furthermore, titers of anti-EBNA 1 antibodies are positively correlated with the number of cytotoxic lymphocytes to EBV infected cells, and thus could be a marker of strong cellular immunity to EBV.[66]

In diseases caused by EBV, such as Burkitt's lymphoma[67] and nasopharyngeal carcinoma,[55] and in EBV-related Hodgkin's disease,[68] antibodies to EBV have been found to be increased several years before diagnosis. In Burkitt's lymphoma, there are prediagnostic elevations of anti-VCA but not anti-EBNA antibodies,[67] whereas elevation of both anti-VCA and anti-EBNA IgG has been associated with risk for Hodgkin's disease[68] and nasopharyngeal carcinoma,[69] but in the latter, the strongest predictors of risk are IgA antibodies to VCA.[70] In MS, an increase of anti-EBV antibodies, but not of antibodies to other herpes viruses, becomes significant 5 or more years before the onset of symptoms. The pattern of these elevations differs from that observed in Burkitt's lymphoma or nasopharyngeal carcinoma; the most consistent finding in MS is an increase of anti-EBNA antibodies, driven by a marked increase of anti- EBNA 1 antibodies and a less prominent increase in anti-EBNA 2, and only a marginal or absent increase of anti-VCA.[46,48,71,72] The increase of titers to EBNA complex and EBNA 1 suggests a more severe primary infection or reactivation of infection accompanied by a vigorous cellular immune response.[66,73,74] The correlation of cellular immunity to EBV with MS is supported by the recent finding that T cells specific to EBNA 1 are present at signifi-

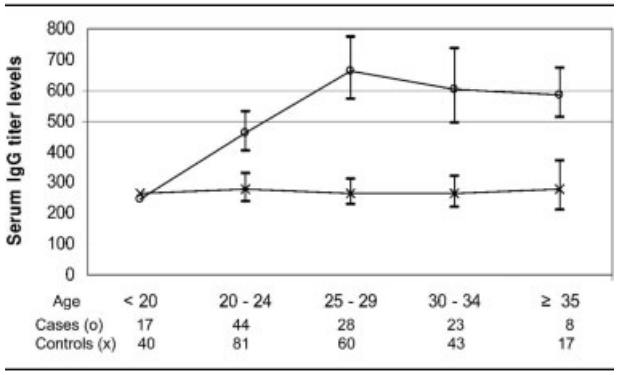

Fig 3. Epstein–Barr nuclear antigen (EBNA) IgG titer levels by age at blood collection: cases and control subjects. Reprinted with permission from JAMA (2005;293:2496–2500). Copyright © 2005, American Medical Association. All rights reserved.[71]

cantly greater frequency and have a broader specificity in MS patients than in control subjects.[75]

An intriguing finding of the investigation of prediagnostic anti-EBV antibodies in MS is the strong interaction with age (Fig 3). In blood samples collected before the age of 20 years, the mean anti-EBNA antibody titers of EBV-positive individuals who later develop MS are identical to those of EBV-positive age- and sex-matched control subjects. Whereas anti-EBNA titers in healthy subjects remain remarkably constant over decades, titers in individuals who later developed MS increase at some point between the ages of 20 and 30 years and then plateau. This divergence between individuals who will develop MS and those who remain healthy is remarkable: a fourfold increase in anti-EBNA titers between a serum sample collected before the age of 20 and a subsequent sample predicts a 15-fold greater MS risk. Furthermore, this antibody increase appears unrelated to the age at onset of MS symptoms or the age at MS diagnosis.[71] The causes for this age-related increase in antibody titers remains unknown; it could be due either to infection with a separate microorganism that alters the immune response to EBV,[76] or to infection with a strain of EBV different from that originally carried by the host. Coinfection with multiple EBV strains is common even in healthy individuals,[77] but its relation to antibody titers has not been investigated. Alternatively, the increase in anti-EBNA titers could be a marker of the developing autoimmune reaction, which ultimately leads to MS. Whatever its cause, the interaction between age and anti-EBNA titers indirectly supports the concept of an age of vulnerability for the acquisition of MS.[25] As we discuss in Part II, some evidence also exists that age modifies the effects of ultraviolet light exposure and vitamin D on MS risk.

Beyond Epstein–Barr Virus: What Remains Unexplained

Many, but not all, features of MS epidemiology appear consistent with a role of EBV. Particularly striking are the similarities between the epidemiology of MS and mononucleosis, which were noted more than two decades ago.[36] Both diseases affect mostly young adults, follow a similar latitude gradient, are rare among populations where children are infected with EBV at an early age,[6,78] occur at an earlier age in women than in men, are more frequent in individuals with high socioeconomic status, are less frequent in blacks[79–81] and Asians[82] than in whites, and occur rarely among Eskimos.[83,84]

Also, the low rates of MS among individuals born in low-risk areas could be explained by the protective effect of early acquisition of EBV infection. However, the reduction in MS risk among migrants from high- to low-risk areas, which, as discussed earlier, is a well-established feature of MS epidemiology, is not easily explained by differences in age at EBV infection. EBV infection is common in the first years of life, when young children exchange saliva by sharing toys, utensils and food, and then again during adolescence when the exchange of saliva becomes more direct, but less common between 5 and 12 years of age.[85] A reduction in MS risk among migrants from a high- to a low-risk area would thus only be expected for migration at an early age, probably younger than 5 years, which could result in earlier acquisition of EBV, and thus a lower risk for infectious mononucleosis and MS. A marked reduction in risk, however, has been reported for migration up to the age of 15[8] and even for migration in adulthood.[11] This finding suggests that other factors may be at play.

Several factors may explain the reduction in MS risk among migrants from high- to low-risk areas, in the context of the EBV variant of the hygiene hypothesis. First, the effect of EBV infection on MS risk may be modified by other environmental factors, which could be of a toxic, nutritional, or infectious nature. For example, the effect could be modulated by the vitamin D status of the host, which depends on sunlight exposure and thus strongly correlates with latitude,[86] or by coinfection with a microbe that increases (and is more common in areas at high risk for MS) or decreases (and is more common in low-risk areas) MS risk. The possible protective effect of vitamin D is examined more closely in Part II of this review. A candidate microbe that could explain the migration data remains to be discovered, but there is at least one well-described example of a clinically relevant microbe-microbe interaction involving EBV: EBV-specific T-cell responses are inhibited by acute malaria, an effect that has been invoked to explain the overlapping geographic distribution of EBV-induced Burkitt's lymphoma and falcipa-

rum malaria.[87] Also, HHV6 has been reported to transactivate EBV in vitro[88] and has been related to MS risk,[89,90] but because of its ubiquitous prevalence and uniform early age at infection, it cannot explain the decrease in risk with migration to low-risk areas.

Second, EBV itself may vary across regions, and some EBV strains may be more likely to increase MS risk than others. EBV occurs in two types, 1 and 2, and within each type there are multiple strains identified by mutations in the latent membrane protein 1 and other genes,[91] and EBV isolates from the same geographic area tend to be closer to each other than to those from different areas.[91]

It is noteworthy that known EBV-related diseases have a distinctive geographic distribution. Thus, Burkitt's lymphoma is common in equatorial Africa, with a distribution that overlaps that of endemic malaria,[55] whereas nasopharyngeal carcinoma is common only in certain areas of South East Asia and in Eskimos.[92] Whether these differences are due to variations in EBV itself or in other factors modifying the outcome of infection remains unknown.[92] The possibility that there is an MS-related EBV strain has so far been little investigated, but some supportive evidence has been reported.[93]

Important clues may also come from the investigation of MS epidemics. Of the several reported, the most convincing, albeit not uncontroversial, occurred in the Faroe Islands. The Faroes are a group of islands in the North Atlantic Ocean that have been a semi-independent unit of the Kingdom of Denmark since 1948.[94] According to investigations by Kurtzke and others, MS was virtually absent among indigenous Faroese until the islands were occupied by the British troops in 1940. After the occupation, 25 cases were identified with onset between 1943 and 1960, most among residents of parishes where the troops were located.[94] A detailed analysis of the time course of the epidemic[25] lead Kurtzke to postulate that there is a widespread transmissible agent that causes an asymptomatic persistent infection or "primary MS affection"; rarely, and years after the primary infection, this agent would cause neurological symptoms (MS). Overall, the data support the possibility that an environmental factor introduced by the British troops caused an increase in MS incidence. Critics have pointed out that the epidemic could be an epidemic of recognition coinciding with the arrival to the Faroes of the first resident neurologist.[95–97] Although this criticism cannot be dismissed, it should be noted that MS has a chronic course and relatively low mortality; therefore, some MS cases with onset before 1942, if they existed, should have been alive and recognized in 1957, when a resident neurologist first arrived. In fact, Kurtzke did identify two Faroese with MS onset before the British troop occupation, but he excluded them from his analyses because they had spent 3 or more years in Denmark before the onset of symptoms. This exclusion was criticized as arbitrary,[96] but in the context of Kurtzke's hypothesis of an infectious cause of MS successfully introduced among the Faroese by the British troops appears legitimate, and could, in fact, be invoked as evidence that if they existed, cases of MS with onset before 1942 among Faroese would have been identified. Because some doubts remain about the Faroe epidemic, it would be unwise to use it as a pillar of the entire theory of MS causation, but equally unwise to disregard its implications. In the context of our discussion of factors that explain the reduction in risk among migrants from high- to low-risk areas, the Faroe epidemics clearly and strongly support the occurrence of a factor that is prevalent in areas of high MS risk, and rare or absent in areas of low risk, most likely an infectious agent. Although there are no data to prove it, Faroese were almost certainly infected with EBV before 1940. It is possible, however, that the British troops introduced a new, MS-related EBV strain. Because of these considerations, we have maintained the hypothesis of an MS-related EBV strain high in our research agenda.

If there is a specific infectious agent that is more common in areas of high MS frequency, be it an MS-related EBV strain or an unrelated microbe, then some degree of space-time clustering of cases might be expected. Results of several investigations have not been fully consistent. No convincing evidence of clustering was found in a few early studies,[98–101] but an investigation in Norway supported space-time clustering during adolescence with a peak at age 18 among individuals in the same community who developed MS,[102] whereas a study in Sardinia reported clustering in early childhood.[103] The limited and inconsistent evidence for clustering could be explained if this putative infectious agent were ubiquitous in areas of high MS risk, or difficult to transmit among siblings. Clustering at the time of disease onset may not occur because of the restricted age of susceptibility, the hypothetically long asymptomatic period (at least 6 years according to Kurtzke[104]), and the fact that individuals with MS may no longer be infectious. Furthermore, even for diseases known to be caused by viruses, and most notably infectious mononucleosis itself, evidence of clustering is often absent.[105] In the case of infectious mononucleosis, lack of clustering is probably explained by the high prevalence of protective immunity in the population and the low probability of transmission within families in the age groups at risk.

Epstein–Barr Virus and Multiple Sclerosis Pathology

A major obstacle to the broad acceptance of a role of EBV in MS cause has been the putative absence of

EBV itself from MS lesions.[106,107] There are, however, multiple mechanisms by which EBV infection could increase the risk for MS without directly infecting the central nervous system.[108] The leading hypothesis is that the immune response to EBV infection in genetically susceptible individuals cross-reacts with myelin antigens. This response could include both cross-reacting T lymphocytes[109–111] and antibodies.[112] The discovery that MS patients have an increased frequency and broadened specificity of CD4+ T cells recognizing EBNA 1[75] and the identification of two EBV peptides, one of which from EBNA-1, as targets of the immune response in the cerebrospinal fluid of MS patients[113] have recently provided strong support to this molecular mimicry theory. Other proposed hypotheses include the activation of superantigens,[114] an increased expression of α B-crystallin,[115] or infection of autoreactive B lymphocytes.[116] Considering the pathological heterogeneity of MS lesions,[117] it is also possible that there are variations across patients in the predominant mechanisms. The observation that MS tends to occur several years after mononucleosis[57] may also be important, because it suggests that EBV acts as a priming factor or initiator of the pathological process, and that other events may be needed to trigger disease expression.[118] Further clues on how EBV may increase MS risk may also come from the investigation of other autoimmune diseases, particularly systemic lupus erythematosus,[119] which, like MS, appears to be strongly related to EBV infection.

Role of *Chlamydia pneumoniae* and Human Herpes Virus 6

As mentioned earlier, the fact that EBV is the only infectious agent for which there is compelling evidence of an increased MS risk among those individuals who are infected as compared with the uninfected does not in itself exclude a possible causative role of other microbes. Among the candidates that have received most intensive investigation in the past few years are *C. pneumoniae*[120–122] and HHV 6.[89,90,123] Enthusiasm for *C. pneumoniae* appears to be fading after lack of confirmation of the original findings of *C. pneumoniae* DNA in the cerebrospinal fluid of MS patients.[120,124,125] In contrast, a role for HHV 6, a neurotropic virus, is still strongly proposed.[126] Because HHV 6 infects virtually all children by the age of 2 years,[127] it has not been possible to compare the MS risk for infected versus noninfected individuals, as it was done with striking results for EBV. For the same reason, although HHV 6 could be an important causative factor in MS, it contributes little to explain the migrant data or other aspects of MS epidemiology. Evidence of an involvement of HHV 6 in MS includes pathological data showing the presence of the virus in MS lesions postmortem,[128–130] its neurotropic nature,[131] immunological and molecular studies showing an increase in viral DNA or other markers of HHV 6 infection in blood cells, serum, or cerebrospinal fluid of MS patients,[131,132] and some evidence of greater frequency of reactivation in MS patients as compared with healthy control subjects.[89,133] Furthermore, results of some studies suggest that the HHV 6A variant may be more likely to be related to MS, although data supporting this hypothesis remain inconclusive.[134,135] Most importantly, although the results are intriguing, none of these studies can exclude the possibility that the changes observed are merely a consequence of MS, rather than its cause.

Summary

Among the infectious agents that have been proposed to be associated with MS, only EBV stands out as a consistent and strong risk factor, although an important or even critical role of other known or unknown microbes cannot be excluded. As compared with individuals not infected, MS risk is about 10 times greater among individuals who experienced an undiagnosed EBV infection in childhood, and at least 20-fold greater among individuals who developed mononucleosis. Nevertheless, some features of MS epidemiology are not explained by EBV and strongly imply either an MS-related EBV strain or a key role of other infectious or noninfectious agents.

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