**SPECIAL REPORT**

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The ‘hygiene hypothesis’ and the development of multiple sclerosis

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**Practice Points**

- There is some support for a role of a hygienic environment in early life in the development of multiple sclerosis (MS) but the evidence is not conclusive.
- Population-level studies of regional MS prevalence and studies of migration patterns are consistent with the hygiene hypothesis but are limited by methodological issues.
- Over time, childhood infection rates have declined and both MS and asthma incidence appear to have increased.
- The contribution of a hygienic early-life environment appears likely to differ for MS compared with asthma.
- Late infection with Epstein–Barr virus has been prospectively associated with MS and must be incorporated into any model where the hygiene hypothesis is implicated in the development of MS.
- One possibility is that inadequate microbial exposure in early life contributes to a dysregulated host immune response to Epstein–Barr virus.
- Two randomized controlled trials are currently underway to examine the potential role for helminthic infection in biasing the human immune response away from the immune profile associated with MS.

**SUMMARY**

We review evidence linking inadequate microbial exposure in early life to the development of multiple sclerosis (MS). There is some supportive, but not conclusive evidence for a role of a hygienic environment in early life and MS. Population-level studies of MS are consistent with the hygiene hypothesis but are limited by methodological issues. Late infection with Epstein–Barr virus (EBV) has been prospectively associated with MS and must be incorporated into any model where the hygiene hypothesis is implicated in the development of MS. One possibility is that inadequate microbial exposure in early life contributes to a dysregulated host immune response to EBV. Two areas of particular interest include the potential role for helminthic infection in biasing the human immune response away from the immune profile associated with MS and also the role of microbial exposure in training the development of the EBV-specific immune response.

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The hygiene hypothesis (HH) was proposed in 1989 to explain the inverse association between larger family size and risk of hay fever [1]. The hypothesis was that having a greater number of siblings may decrease the risk of allergic disease because siblings are a source of infection. A high load of minor infections in early life may assist the infant immune system in developing correctly, away from an allergic disease profile [1]. These original findings of variation in hay fever and allergic disease by family size were objectively confirmed in the following decade of atopic disease research [2].

**Exposure measurement**

- **How do we determine the early life hygiene environment?**

In the ongoing investigations of all studies based on the HH, a significant issue is the accuracy and scope of exposure measurement. Early-life infections are difficult to measure accurately and precisely and even more difficult to recall. For this reason, a range of proxy markers have been used to indicate a higher probability of early-life infection exposure: sibling number, day-care attendance and high residential density. Biomarkers of past infection have also been examined; for example, host IgG seropositivity to hepatitis A virus, *Helicobacter pylori*, Herpes simplex virus 1 (HSV1), Cytomegalovirus (CMV) and tuberculin sensitivity, have all been associated with a reduced risk of atopic disease [3]. However, such findings should be interpreted in the context that these measures reflect not only infectious exposures but also the resultant host response [4].

More recently, it has become clear that noninfectious microbiological exposures may also be of importance, with several studies indicating that living on a farm, pet exposure, a probiotic diet and even the bacterial contamination of drinking water are associated with reduced risk of allergic disease [5,6]. Thus, we can conceptualize the relevant exposures as extending beyond infection alone, to include innocuous microbial exposure as well. Furthermore, microbial exposures cannot be examined in isolation, but must be untangled from possible confounding factors such as socioeconomic status or child nutrition.

The HH is relevant to autoimmunity as well as allergic disease. Both allergic and autoimmune diseases have increased in incidence over time from the 1950s to early 2000 [7], a period of documented decline in major childhood infections such as whooping cough [7]. The prevalence of innocuous infections has also declined. For example, in The Netherlands, the prevalence of HSV1 IgG seropositivity among 20–30-year-olds declined from 81 to 59% between 1977 and 1984 [8] and, in the UK, a corresponding decline in HSV1 IgG seropositivity was noted from 1986 to 1995 [8]. Furthermore, both allergic and autoimmune conditions coexist at the population level [9] and are more common in developed countries where the infectious and microbial load on children is lower [7]. For example, these immune disorders are rare in Africa and over 90% of children in Eritrea or traditional Africa are HSV1 IgG seropositive by 5 years of age compared with less than a third of children in Germany and the UK [8]. Over the past two decades, these epidemiological and other findings have led to a broadening of the HH to be considered as a possible contributing factor to the development of other immune-mediated disorders such as Type 1 diabetes [10], Crohn’s disease [11] and MS [12–15].

Currently, it is the broadest form of the evolved HH that is the subject of much investigation. That is, that modern hygiene-related factors isolate the human population from microorganisms, pathogens, commensals or innocuous environmental microbes. Exposure to these agents is crucial to the development of an optimal immune response and the prevention of various allergic, autoimmune and inflammatory diseases. This concept is part of what is sometimes termed ‘Darwinian’ medicine [16]. In addition, it is evident that the timing of early life microbial exposure, relative to a putative antigenic stimulus or an age window of developmental vulnerability, as well as the sequence of antigenic stimulation may be important [17].

**Multiple sclerosis & the hygiene hypothesis**

Multiple sclerosis (MS) is a disease with environmental and genetic determinants. While twin studies support a genetic contribution to MS susceptibility, a modest concordance rate in monozygotic twins of less than 30% [18–21] and a similar concordance rate among dizygotic twin pairs (4.7%) and non-twin siblings (5.1%) [21] suggest that environmental factors are also important. However, heritability estimates vary widely with large confidence intervals [22], reflecting relatively small sample sizes in the published studies.

The concept that MS may result from an early life environment characterized by altered microbial exposure was first formulated in the
early 1960s. In 1963, the descriptive epidemiological features of paralytic poliomyelitis and MS were compared and found to be similar, leading to the suggestion that widespread exposure to an infectious organism may be of etiological importance in MS [18]. In 1966, a study from Israel reported that people with MS were more likely to have spent their childhood in high sanitary conditions [19]. Modeled on the findings of age-dependent poliomyelitis infection, the disease causation model proposed that early infection with a widespread agent was protective, but that late infection with the same agent was deleterious [19]. This is sometimes also referred to as the poliomyelitis HH [20]. We will now consider both the evidence indicating that early life microbial exposure may alter MS risk and the possible underlying mechanisms involved.

**Animal models**

In relation to animal studies, more severe demyelination occurs in immune-deficient anti-myelin basic protein T-cell receptor transgenic rats when kept in germ-free conditions [21], whereas immunization or infection of mice with the helminth *Schistosoma mansoni* reduces the severity of experimental autoimmune encephalomyelitis [22–24].

**Population-level human studies**

Multiple sclerosis has a striking geographical distribution, being rare in the Tropics (close to the Equator) and increasing in incidence and prevalence with increasing latitude [25] with some exceptions (e.g., Sicily is approximately the same latitude as Malta but has a 10-fold lower MS prevalence) [26]. There is an inverse association between the prevalence at the population level of *Trichurus trichiura*, a common intestinal helminthic infection and MS [27]. Using a critical threshold of *Trichurus trichiura* prevalence cut-off (of 10%) there is a strong inverse correlation \( r = 0.53; \ p < 0.001 \) between the prevalence of *Trichurus trichiura* and MS prevalence [27]. In addition, in regions where MS is rare, a higher proportion of children show positive titers to many viral diseases early in life, suggesting MS could be an age-dependent response to a common viral infection [28].

The natural history of MS epidemic outbreaks on the isolated Faroe Islands, following the arrival of British troops during the Second World War, was thought to possibly indicate that MS may partly result from an introduced pathogen from the troops who had arrived from a region of higher MS incidence [29].

Studies of immigrant populations moving from areas of high MS prevalence to areas of low prevalence, and vice versa, aim to tease out the relative importance of genetic susceptibility and environmental exposures. Furthermore, by studying whether there is any difference in effect depending on the age at migration, the timing of any important environmental exposures can also be further elucidated. Nevertheless, there are many possible sources of error with migration studies (reviewed in [26]). Migrants can differ in their age, health and lifestyle compared with compatriots who do not migrate [26]. There may be difficulties in accurately determining both the numerator and the denominator in both the country of origin and the destination country. Many studies fail to standardize the incidence rates or prevalence by age or sex, so that the findings are not truly comparable across different regions. However, in a systematic review of the migrant studies in MS, two consistent patterns emerged [26]. Migrants moving from a region of high MS risk to one of lower risk have a lower than expected prevalence of disease, particularly when migration occurs before the age of 15 [26,30]. By contrast, migrants from an area of lower risk to one of higher risk tend to retain the lower MS risk of their country of origin with no clear age at migration effect [26]. Recent studies of migration patterns, within the USA [31], of Iranians to Sweden [32] and Canada [33], and migration from Africa and Asia to Israel [34], from Asia to the UK [35] and from the UK to Australia [36], have largely confirmed these findings.

Perhaps the strongest evidence comes from the studies of migration and reverse migration between the French West Indies, where MS was rare prior to the 1960s but recurrent neuromyelitis optica (RNMO) was occasionally seen, and France [36]. Not only did returning migrants have higher prevalence of MS, but only a small proportion was RNMO [36]. Return migration initially favored the island of Martinique, compared with its neighbor, Guadeloupe. On Martinique, MS prevalence was higher, MS was eight-times more common than RNMO and there was a prevalence peak in the 35–44 year age group followed by a fall in prevalence in the 45–54 age groups [36]. In Guadeloupe, MS was only twice as common as RNMO and the prevalence peak was in the 25–34 age group. These findings
are consistent with recent emergence of MS in Martinique but even more recent emergence in Guadeloupe [36]. In addition, age-corrected statistics show that the greatest MS risk was in those who were in France under the age of 15 [36].

In another recent study, based in Toronto, patients with pediatric-onset MS were more likely to report Caribbean, Asian or Middle-Eastern ancestry and less likely to report European ancestry, than those with adult-onset MS [37]. Two possible explanations were proposed: firstly, individuals raised in a region of high MS prevalence, but whose ancestors originate from regions in which MS is rare, have an earlier age of MS onset. Alternatively, the place of residence during childhood determines lifetime MS risk [37]. Nevertheless, these are ecological studies and, thus, migrant bias, incomplete ascertainment and confounding by individual-level factors cannot be ruled out as possible explanations for these findings.

**Individual-level human studies**

In contrast to asthma, an association between birth order and MS has not been consistently found [38–43]. However, greater exposure to younger siblings has been associated with reduced MS risk [44,45]. Likewise, in Canadian studies, although an overall pattern was not found, higher birth order in larger families was associated with reduced MS risk, again consistent with a beneficial effect of exposure to young children [46].

The association between child care and MS risk has been little studied to date. Furthermore, in a disease of predominantly adult-onset, the accurate ascertainment of antibiotic administration in the first years of life is problematic. With regard to studies examining animal or farm exposure, little evidence exists to support a protective role of these factors for MS risk. Early reports that human exposure to dogs was associated with higher MS risk [47,48], an association postulated at the time to possibly reflect exposure to canine distemper virus [49], were neither consistent [50] nor confirmed by later work [51].

Case-control studies have predominantly, but not consistently, found that MS cases acquire childhood infection at later ages [28,52].

**Epstein–Barr virus infection & MS**

In particular, the risk of MS is increased two- to three-fold among individuals with a history of infectious mononucleosis, a disease linked to late infection with Epstein–Barr virus (EBV) in adolescence or adulthood, compared with those without this disease history [53–55]. Similarly, a Danish registry study reported a risk ratio of 2.81 for developing MS after infectious mononucleosis [56] and a prospective Swedish registry study reported a relative risk of 3.7 for subsequent MS among patients with infectious mononucleosis [57]. Consistent with these findings, a history of tonsillectomy has also been associated with MS (adjusted odds ration: 1.25; 95% CI: 1.11–1.40) [58], with recurrent tonsillitis separately linked to recurrent EBV infection and reactivation [59].

A striking and consistent feature of MS is that cases are more likely to have had past EBV infection than controls [33,44]. There is a significant difference between the seroprevalence of EBV in MS patients compared with controls (99.7 vs 90.2%; p < 0001) [60]. The pooled odds ratio (based on 13 studies) for EBV seronegativity and MS in a recent meta-analysis was 0.06 (95% CI: 0.03–0.13) [14]. The fact that MS is extremely rare among EBV-negative individuals challenges a simple interpretation of the HH; that is, that being in a high hygiene early-life environment increases future MS risk [61]. Rather, it indicates that some combination of an adverse EBV infection and the HH may be important, where inadequate early-life microbial exposures are either associated with an adverse characteristic of EBV infection, such as a later age of infection, and/or an altered adverse host response.

With regard to EBV serology, the most consistent pattern is a marked increase in anti-EBV nuclear antigen (EBNA) antibodies, and a more modest increase in antiviral capsid antigen (VCA) IgG. A second virus, human herpesvirus-6 (HHV6), which is the causative agent in roseola exanthum subitum – a common febrile illness in children – has also been inconsistently associated with MS. This neurotrophic virus has been detected among MS lesions post mortem [62] and also in cerebrospinal fluid [63,64] but the significance of these findings is uncertain.

**Helminth infection & MS**

In a small cohort study, during a 4–6-year follow-up, parasite-infected MS patients (n = 12) had lower rates of clinical and MRI-measured progression compared with uninfected patients [65]. In addition, IL-10 and TGF-β production were increased and CD26+CD4+Foxp3+ T cells were induced, indicating that regulatory cells induced
The ‘hygiene hypothesis’ & the development of multiple sclerosis

The contribution of the hygiene hypothesis to both asthma & MS

One approach to investigating the HH in MS is to compare the overlap between MS and asthma. A meta-analysis of three markers of exposure to infection (latitudinal gradient, infectious agent contacts [e.g., sibling number] and history of infection), concluded that 40% of asthma could be attributed to variation in these markers [70]. An earlier review of 53 studies also concluded that sibling-related factors could account for at least 30% of all cases of asthma, eczema and hay fever [71]. Thus, the contribution of the HH in asthma appears substantial. A review of the literature examining the association between MS and asthma (Table 1) demonstrates that no clear strong association between MS and asthma exists. This suggests that the same HH-related factors do not appear to be acting in the same way and to the same extent for MS and asthma.

Mechanisms

The core concept is the notion that the microbial environment interfaces with the innate immune system and also modulates adaptive immune responses, particularly when such interactions occur in early life [72].

■ Innate immunity

Innate immunity appears important for the beneficial effects of HH-related factors in asthma but has been little studied in relation to HH-related factors and MS. For asthma, for example, maternal farm exposure during pregnancy results in protection from allergy among preschool offspring and is associated with an upregulation of innate immunity genes such as TLR2 and TLR4 [73]. Substantial amounts of microbial antigens, ranging from plant antigens to parasites, may assist the correct development of a balanced immune system [74]. Within the T-cell adaptive immune responses, a balancing square model has been proposed whereby Th1, Th2, Th17 and Treg cells are in balance [74]. MS, long thought to reflect Th1 overactivity [75], is now considered to involve upregulation of Th17 cells and a defect in thymic natural Treg cells [74]. The reduction in Treg activity associated with MS is now well-known [76,77], but the role of early life microbes on the development of different T-cell types is still poorly understood [67].

■ Specificity of the T-cell antigenic response

There is emerging evidence in MS that a lack of specificity of the T-cell response may be important. MS patients provide antibodies primarily to herpes simplex 1 (HSV1) virus but not significantly bound by healthy EBV seropositive controls. Furthermore, these antibodies cross react with myelin basic protein. Animal studies demonstrate that mice immunized with EBNA 206 also develop antibodies to myelin basic protein and MS symptoms [78]. Other studies have also demonstrated that T cells in MS patients have an altered specificity against EBNA-1 compared with controls [79–81]. The specificity of such responses may depend not only on initial but also repeated EBV or similar herpes virus exposure. Seronegativity to herpes simplex 1 (HSV1) virus has been over-represented among pediatric or adult MS cases at onset [17,45] but not in a multinational pediatric study examining this issue [43]. However, there is molecular evidence that HSV1 may be associated with MS exacerbations [82,83]. These herpes viruses now require examination in tandem not in isolation.

■ Immune priming

Immune priming, particularly in early life may be important for precise, well-targeted host responses to herpes viruses.

Re-exposure to active viral infection is known to boost established immunity with rising IgG titers in seropositive individuals [84]. In addition, repeated antigen stimulation induces affinity maturation of the B cell [85], and influences T-cell phenotype [86] and T-cell receptor diversity [87]. It has also been postulated that microbial exposure may influence the regulation of human endogenous retroviruses, encoded within the human genome [15,16].
Epstein–Barr virus, vitamin D & the hygiene hypothesis

A recent model, based on the epidemiological features of MS natural history, incorporated both genetic susceptibility and environmental risk factors, with three sequential factors implicated in the environmental risk – one operating near birth, one in childhood and one later [88]. The candidate environmental factors proposed were vitamin D deficiency and EBV infection. It is possible that suboptimal microbial exposure in early life is not acting alone but involves an important interplay with these two factors. For example, inadequate exposure to herpes virus or other microbes in early life could result in an aberrant host response to EBV, resulting in not just an elevated EBNA titer but a difference in the quality of the targeted host response to EBV. Secondly, inadequate vitamin D status in early life can hamper the innate immune response [89] and adaptive immune response, with RCT evidence demonstrating an improved child response to viral infection with higher vitamin D administration [90]. This interplay between vitamin D and early life microbial exposure and associated host responses is also plausible.

Conclusion & future perspective

Future work is needed to comprehensively examine the HH in MS. A key target would be to focus on the broader T-cell specificity against EBNA-1 and understand what determines this in terms of genetic risk, such as human leukocyte antigen-DRB1*1501-DQB1*0602 (HLA-DR15) genotype or environmental HH-related factors, such as sibship structure, or late infectious mononucleosis infection. As MS is a disease characterized by a variable disease course, immunomodulatory trials based on HH-related factors, such as helminthic infection, will be worthwhile.

An improved understanding of gene–environment interactions in relation to the HH will also be important. For asthma and other immune diseases, this work has improved our understanding of HH-related mechanisms [91,92]. Polymorphisms in the genes for TLR4, TLR2 and NOD2 have been demonstrated to interact with the farm environment, modulating the asthma and allergy protective effect. Furthermore, a significant interaction between genetic variation in CD14 and unprocessed cow’s milk consumption has been observed [93–95]. These findings suggest that a protective effect of various farm exposures is modified by an individual’s genetic make-up.
It is likely that gene–environment interactions related to the HH also exist for MS. Studies with adequate statistical power are required to assess this. The risk of developing MS has been associated with the human leukocyte antigen-(DRB1*1501 (HLA-DR15) genotype, low infant sibling exposure, and high EBNA IgG levels [96]. In a population-based, case-control study (Tasmania, Australia), we reported that the combined effect of HLA-DR15 positivity and low infant sibling exposure on MS (OR: 7.88; 95% CI: 3.43–18.11) was 3.9-fold greater than expected if each risk factor was present but operating independently (test for interaction; p = 0.019). This suggests that genetic immune mechanisms involving HLA can be modified by HH-related factors, such as infant siblings, in early life [96].

In conclusion, there is some supportive, but not conclusive, evidence for a role of a hygienic environment in early life and the development of MS. It appears that the role of such an environment is likely to differ for MS compared with asthma. Late EBV infection has been prospectively associated with MS and must be incorporated into any model of the HH in MS. One possibility is that inadequate microbial exposure in early life contributes to a dysregulated host immune response to EBV. Two areas of particular interest are the potential role of helminthic infection, to bias the human immune response away from the immune profile associated with MS and also the role of early-life exposure to other herpes viruses that share many features with EBV, in training the development of the EBV immune response. The outcome of the helminth immunomodulatory trials in MS are awaited with great interest. Future work should also include an examination of the specificity of adaptive immune responses to EBV and seek to better understand the potential role of inadequate microbial exposure in determining this response.

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Bibliography

Papers of special note have been highlighted as:


Summarizes the epidemiological evidence that has emerged during the 1990s relating to family size and infections to atopy and allergic disease and discusses the research agenda.


7 Discusses epidemiologic and experimental studies concerning the hygiene hypothesis in relation to asthma, allergic diseases and autoimmune disorders.


This review of the epidemiological evidence argues for a causal role of Epstein–Barr virus (EBV) and multiple sclerosis (MS). It proposes a EBV variant hygiene hypothesis that also incorporates a possible causal role for EBV and accounts for the low risk of MS among EBV-zero negative individuals.
Discusses similarities in the distribution of both MS and poliomyelitis and proposes that MS may represent the occasional neurological manifestation of a widespread enteric infection.

This case–control study in Israel reported that MS cases were more likely to have lived in a childhood with a high level of sanitation.


Demonstrated that in a small case–control study, pediatric MS cases had a broadened recognition of EPNA1-specific IgG than controls. This would be consistent with an upregulated and qualitatively distinct immune recognition of EBNA1 in children with MS.
This population-based case–control study reported that the combined effect of human leukocyte antigen-DR15 positivity and low infant sibling exposure on MS risk was 3.9-fold greater than that expected. This suggests that immune mechanisms involving human leukocyte antigen class II molecules are susceptible to modulation in early life.


■ Websites

201 Immunoregulation by Controlled Parasite Exposure in Multiple Sclerosis (WIRMS-1) http://clinicaltrialsfeeds.org/clinical-trials/show/NCT00630383

202 Helminth-induced Immunomodulation Therapy (HINT) in Relapsing-remitting Multiple Sclerosis http://clinicaltrials.gov/ct2/show/ NCT00645749