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Epstein-Barr Virus and MS: Causality or Association?

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Introduction

The search for the aetiology of multiple sclerosis (MS) has led to many theories over the years. It has become clear, however, that MS is complex and multifactorial, involving many interlacing mechanisms, many of which appear to be playing a role in the causation and evolution of the disease. In the large part for epidemiological reasons, an infectious aetiology has always been a prime suspect in this search. Although many agents have been examined over the years, the Epstein-Barr virus (EBV) has re-emerged as a candidate pathogen, based to a large degree on sero-epidemiological evidence, and on the knowledge of the effects this virus is capable of producing in other situations. This commentary summarizes the strength of this evidence in the context of many of the other known factors present in MS, and suggests ways of extending the search for EBV or any other infectious agent in establishing causation.

In recent years, there has been an explosion of articles on the role of infectious agents in MS. These articles have documented the association of various viruses, especially EBV, with the tissues, clinical course and immune status of patients with MS. There have been many excellent reviews and comments on this subject,[1–3] including the article by Giovannoni in this journal. The object of this commentary is not to repeat the documentation, but rather to discuss the context in which these studies must be placed, to highlight those that show unique properties pointing toward causation, and suggest where future investigations might be directed.

While much progress has been made in the understanding, diagnosis and management of MS, after a century and a half of investigation, the basic cause remains elusive. Each new phase in general biomedical discovery has generated concurrent ideas as to the possible aetiology of MS. Thus, over the years, theories of causation have emerged, receded and at times re-emerged, as new ideas and especially new technologies have suggested promising directions. Vascular, toxic, infectious, genetic and immune hypotheses have been repeatedly

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invoked over the decades, with the advent of new evidence (and at times, even scientific fashion).

What has emerged in current thinking is that MS is a complex multifactorial disease, based on a genetic susceptibility, but requiring an environmental trigger,[4] and causing tissue damage through inflammatory/immune mechanisms. On the surface, this seems simple, and yet each of these statements raises fresh uncertainties. Firstly, is MS a disease or a symptom complex, caused by multiple aetiologies? The pathology has suggested to some that there is a heterogeneity in lesion types, sharing some common features, but with some variances possibly pointing to differing aetiopathogenetic mechanisms.[5] Although this has been contested by some authors, if true it makes looking for a uniform set of causes difficult. Secondly, widely varying environmental factors have been found to be associated with the disease, ranging from infectious agents to Vitamin D deficiency and smoking.[4] Even the autoimmune basis of the disease,[6] accepted as a given for decades, has now been questioned by some. In this respect it would seem that the debate revolves more around whether the immune pathogenesis is primary, or acts secondarily to some other trigger.[7] This assumes importance in the discussion of EBV, as the question arises whether any pathogenetic mechanism is based on a peripheral-originated immune reaction, or relies on immune destruction of EBV-containing B-cells resident in the CNS, by activated T-cells.[8]

Finally, there is widespread acceptance of the underlying genetic susceptibility. Convincing studies, based on studies of compatibility associations, complex families, adopted and natural siblings, twins, and maternal and paternal transmission, all point to a familial predisposition.[9,10] At this stage the only consistent genetic abnormality appears to be in the MHC HLA class 11 region, mainly HLA-DRB1.[4,11] Most recently, the genetic versus environmental contributions in MS were highlighted in a landmark *Nature* publication that evaluated the genomic epigenomic, and RNA sequences in purified CD4+ T-cells of monozygotic twins discordant for MS.[12] Remarkably, no reproducible differences were observed between siblings including one billion whole-genome sequences generated from one twin set. Therefore, by finding no evidence for genetic, epigenetic or transcriptome differences to explain MS disease discordance, this study strongly suggests the role of other (environmental?) factors in the MS disease process.

How to select from, or to tie genetic, environmental and immunological theories together, is the biggest challenge facing MS researchers today. Although much has been written about each of these areas, recent studies have again highlighted the role of infectious agents in causing the disease. In a sense this is ironic, because the infectious theory was first invoked in the late 19th Century, during the dawn of the great discoveries in bacteriology. Later, the striking observations on the incidence of MS in migrating communities led to a revival in the search for an infectious agent. These studies showed strong evidence for exposure to an environmental agent (infectious) by the age of about 15 years. Simple clinical studies almost two decades ago have also revealed a strong association between the onset of clinical infectious disease, especially respiratory, and MS attacks.[13,14] Traditional methods of isolating or demonstrating bacteria or viruses revealed a large number of putative agents, although none of these were consistently present enough to be convincing. These have included the measles virus,[15] parainfluenza virus, human herpesvirus type 6 (HHV-6), EBV,

canine distemper virus and retroviruses.[16] An updated list of potential viral pathogens that have been associated (and in some cases purported to be isolated directly from MS tissue) is shown in Table 1. Indeed, it appears that with every new virus discovery, there are reports that attempt to link this novel agent with chronic neurological diseases in which viruses have been thought to be involved, i.e. XMRV and chronic fatigue syndrome.[17] The relationship between MS and helminthic infection is complex, (see later), and may be immuno-protective.[18–20]

The advent of sophisticated molecular and immunological diagnostic methods has again revived a serious search for an infectious agent. Much has been learned from animal models of viral and helminthic neurological disease, especially Theilers murine encephalomyelitis virus, JHM virus and canine distemper virus.[21] These models have demonstrated the possible mechanisms by which an infectious agent could cause immune-mediated demyelination as in MS, including molecular mimicry, epitope spreading and bystander damage by activated lymphocytes secondary to tissue necrosis.[16,21]

In addition, the so-called hygiene theory of susceptibility to immune disease, suggests that a vigorous early immune response to infections is necessary to protect against the development of autoimmune disease. Improved sanitation interfering with this immune development has led to an increase in the incidence of immune-disease in both developed and developing countries. Specifically, it has been shown that patients with helminthic infections may have immunoregulatory molecules acting on dendritic and B-cells, to lower the incidence and severity of MS attacks.[18]

By this stage, there is a plethora of evidence associating MS with EBV. Almost 100% of patients with MS are seropositive for EBV, although the rate in the general public is about 90%. Patients with MS also have higher titres than controls. Other corroborative evidence shows that EBV DNA is higher in patients during relapses, that sera taken from patients prior to the onset of their disease, showed much higher titres than the normal population,[22] and that patients with MS are more likely to have had infectious mononucleosis. Many years ago, in patients with active MS, it was shown that circulating lymphocytes tended to transform spontaneously under EBV induction, and MS patients have a higher level of circulating EBV-specific cytotoxic T-cells than the general population. In Denmark, a cluster of MS cases following a community outbreak of infectious mononucleosis also supports this association. The serological differences between MS patients and the general population are even more striking in the paediatric population.[23]

There is also evidence that a higher number of antibodies in the cerebrospinal fluid (CSF) of MS patients recognize EBV antigen, than other viruses, and that there is a high level of intrathecal synthesis of these antibodies. In this regard, much has been discussed about the finding of extensive infection of MS patient B-cells by EBV, and the finding of lymphoid follicles in the meninges filled with EBV antigen[8] (see below). One of the hallmarks of MS, the oligoclonal banding (OCB) pattern in the CSF, has not been shown to be specific for most of the putative infectious agents, in contrast to their specificity in other known virological diseases. Indeed, Gilden has claimed that ‘if EBV or any other virus causes MS, it should be possible to demonstrate that MS OCBs contain antibody directed against the

suspected agent'.[24] However, recent evidence[25] based on protein arrays generated from cDNA libraries of human brain, have shown a high reactivity with MS CSF to EBV antigens BRRF2 and EBNA-1, some of which appears to be related to a small minority of oligoclonal IgG. There is also a suggestion that an increase in anti-EBV antibodies is associated with grey-matter atrophy in MS patients.[26]

One may therefore ask, with all this evidence, why there still is hesitancy in labelling EBV as the causative agent of MS? In fact, there are many arguments that show this strong association may be just that, and not a cause. The very high infection rates by EBV in the general population render the very slight increase in incidence in MS less convincing. A very real possibility exists that patients genetically predisposed to MS, may also be similarly more susceptible to EBV infection, or indeed other infections. The well-described phenomenon of MS attacks following respiratory infections may very well represent two parallel, but separate processes, with MS taking longer to manifest clinically. Although not the focus of this editorial, there is substantial evidence of the association between MS and other viruses; serological evidence of an association between MS and HHV-6 is well described. Although the incidence is lower than that of EBV, the occurence in the general population is similarly lower than that of EBV. Indeed, HHV-6 has been preferentially isolated from MS plaques by different investigators[27,28] using both *in situ* hybridization and polymerase chain reaction (PCR) on laser captured tissue. Interestingly, the relationship and interaction between HHV and human endogenous retroviruses (HERV) has suggested to some that both these two viruses, acting in concert, may have a pathogenetic role.[29] The same may hold true for EBV and HERV. It is interesting that the isolation from MS tissue of EBV-containing B-cells, especially the lymphoid follicle, reported by one group,[8] has not been substantiated by three other groups using more sensitive techniques.[30–32] It is far from clear whether the meningeal lymphoid follicles play a significant role, as they have been found by few other groups in any number. Far more work is needed to establish causality.

The concentration on viral aetiology should not preclude the search for bacterial and other infectious agents. In this regard there has been some interest in the role of *Chlamydophila pneumoniae* as a causative agent, but the evidence is still weak.[16] The long list of viral associations in MS (Table 1)[33] indeed suggests that there is no one specific agent that is associated with disease, or no 'MS virus'. Rather, multiple pathogens may act as environmental triggers in genetically susceptible individuals. It may not be merely coincidental that two of the viruses on this list that have received considerable attention for their association with MS are the double stranded RNA paramyxovirus, measles virus, and the DNA herpesvirus, HHV-6. Both these very divergent viruses use the same ubiquitous CD46 molecule as their virus receptor to bind and enter cells.[34] CD46 is a member of the family of regulators of complement activation (RCA) and has been shown to be the receptor for seven different pathogenic bacteria and viruses including HHV-6 variants A and B, the measles virus, group B adenoviruses, group A *streptococci*, *Neisseria gonorrhoeae* and *meningitis*, as well as *Helicobacter pylori.* Given the usage of CD46 as cellular receptor by multiple pathogenic agents and the frequent associations of MS with a wide range of infectious organisms may explain how multiple agents may be associated with the same disease by using a common pathway (receptor) to gain entry into a cell and dysregulate function.[35]

What then is needed to establish causality? The list of viral associations in MS (Table 1) could also suggest that, in fact, viruses may have nothing to do with causing this disease. Most of these associations are based on increased antibody responses in either the CSF and/or periphery and may be merely epiphenomenal of an aberrant or dysregulated global immune response. Proof of causation is much more complex than simply measuring antibody levels and is particularly challenging to establish links with ubiquitous human pathogens in which the majority of individuals have been exposed, e.g. HHVs such as EBV. Koch's postulates have been modified over the years to include the molecular detection of the suspected agent particularly in disease tissue.[36,37] More recently, Lipkin[38] has discussed these issues in the context of chronic disease when classical hallmarks of infection are absent or mechanisms of pathogenesis are indirect or subtle. He suggests that one 'may have to resort to a statistical assessment of the strength of epidemiological association based on the presence of the agent or its footprints (nucleic acid, antigen, and preferably, an immune response), and biological plausibility as indicated by analogy to diseases with other organisms where linkage is persuasive'. Has EBV (or any other agent) met these criteria? Given these comments, more substantial epidemiological proof of a causal relationship may be obtained with repeated documentation of clusters such as that seen in Denmark, or else the consistent and reproducible demonstration of pathogenetic mechanisms such as described in the paper by Serafini *et al.*[8] Another pathway to 'prove' causality in MS may be by well-controlled clinical trials when virus-specific antiviral therapies become available. This could preferably be done in patients in which evidence of the virus can be found (assuming that not all MS patients are associated with the same pathogen, i.e. there is no 'MS virus', see Table 1),[33] a condition which is critical in order to assess efficacy of the drug. Once met, the effect on MS disease, both clinically and radiologically, can be evaluated. It is of interest that one of the oldest and most established treatments in MS is beta-interferon therapy, initially envisioned as an antiviral compound administered intrathecally. The many mechanisms by which it has been shown to play a role in MS include the recent observations that beta-interferon therapy decreases the prevalence of HHV-6 in PBMC and sera.[39] Finally, it should never be forgotten that the search for, and discovery of new agents, viruses, bacteria, parasites and other, perhaps undiscovered, entities continues and must be considered in MS. The lessons learned from the Prion experience are relevant for any chronic disease. The advent of powerful new tools, such as phage-displayed random peptide libraries, single cell analysis of B-cell reactivity, and similar future advances will be critical in this quest.[16]

Any theory concerning causality in MS, especially with infectious disease, should also account for the strong association with other environmental factors such as Vitamin D and sunlight deficiency. Vitamin D has been shown to have a powerful immunomodulatory effect, and in addition, there is some evidence that it may actually interfere with virus replication or cause viral death through the production of antimicrobial peptides (AMPs).[40] Smoking has also been suggested to play a role. It is clear that genetics alone cannot explain MS and that infectious 'triggers' undoubtedly contribute to the aetiology of this disorder. Proving causation is much more challenging and will rely on molecular, immunological and radiologic signatures of these agents. Whether Epstein-Barr virus and/or any other pathogen

are involved in the pathogenesis of MS is still an open question, but in today's research environment the bar has certainly been raised.

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Table 1

Partial list of viruses and the year they were reported to be associated with MS (adapted from R Johnson, 1998)[33]

Virus	Years
Rabies	1946, 1964
Herpes simplex	1964
Scrapie agent	1965
MS-associated agent	1962
Parainfluenza virus 1	1972
Measles	1972
Simian virus 5	1978
Chimpanzee cytomegalovirus	1979
Coronavirus	1980
SIMON-like virus	1982
Tick borne encephalitus flavivirus	1982
HTLV-1	1986
LM7 (retrovirus) – MSRV	1989,1997
HSV-1	1989
MS1533 (retrovirus)	1994
HHV-6	1993,1995
Borna virus	1998
EBV	1998, 2003, 2007
Varicella zoster	2004
XMRV	2010?