Analysis of specific pathogens as possible triggers of Multiple Sclerosis

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I. Overview

Multiple sclerosis (MS) is believed, based on familial concordance data, to be a multifactorial disorder, requiring the presence of both genetic and environmental factors to initiate the mechanisms that lead to demyelination and neural damage. Although the environmental factors that trigger MS are still being determined, infectious agents such as bacteria and viruses have long been speculated to play a role in the etiology of MS. Scientists have investigated many different pathogens as candidate triggers of MS, using a variety of experimental techniques that have evolved over time as knowledge about infectious agents and disease has grown.

This document surveys a number of pathogens that have been evaluated for a role in MS, summarizing the results of the experiments that have been conducted to identify possible associations. It does not provide an exhaustive analysis for each pathogen; detailed reviews already exist for a number of them. Instead, it attempts to give an overview of the types of investigations that have been conducted across the spectrum of infectious agents explored in MS, and provide a sense of what is known and not known regarding the involvement of these potential infectious triggers of MS.

Each pathogen discussed in this document is presented in a standardized template. The templates contain a brief description of the pathogen being evaluated and a summary of the available experimental evidence, primarily obtained from case-control or epidemiological studies. The evidence is organized into these topics:

- Is the presence of the actual pathogen different in subjects with MS compared with non-MS subjects?
- Does the immune response to the pathogen differ between MS subjects and non-MS subjects?
- Does the age of infection or another characteristic of infection differ significantly between MS subjects and non-MS subjects?
- Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
- If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

At the end of each template, an opinion is given as to the strength of the evidence associating the pathogen with the risk of MS.

For most of the pathogens listed, few conclusions can be drawn about their possible involvement in MS. A common finding is that positive results provided by one or more studies are contradicted by another study or group of studies. There are several possible reasons for this situation, including:

- Differences and/or deficiencies in the experimental methods and techniques used (see Moore and Wolfson[1] for an analysis of how poor methodological choices or incomplete descriptions of methods limit reliance on results)
- Differences in choices of cases and control subjects and differences in recruitment methods used
- Small sample sizes resulting in a lack of statistical power
- Inherent difficulties in detecting or isolating certain pathogens (such as *Chlamydia pneumoniae*), which may prevent replication of results from lab to lab
- Non-specific findings implicating multiple pathogens (none, one, or some of which may be true susceptibility factors)
- Lengthy time spans between the age of initial infection, the age of MS onset, and the age at which tissues or data are collected for analysis; the infection-to-analysis span can be especially long for studies examining MS autopsy brain tissue
- The possibility that MS may be a etiologically heterogeneous disease and therefore evidence for any given infectious trigger may be subtle or modest, or not even present in a given population under study
- The possibility that differences detected between MS and non-MS subjects may be artifacts of the disease process (for instance, the presence of pathogens in the central nervous system in MS subjects may be an artifact of blood-brain barrier leakage)

Indeed, because of time lapses, disease heterogeneity, and other factors, it is probably difficult to conclusively *exclude* the involvement of any particular pathogen of interest in any type or form of MS. Still, we believe there is value in presenting the available data as it will help to highlight areas where additional research could more conclusively indicate whether infections trigger MS and if so, which pathogens are involved.

Note: Accompanying this document is a spreadsheet (continually being expanded) that lists relevant studies for each pathogen, including characteristics such as technique used, number of subjects and controls, and results. A copy of this spreadsheet can be downloaded at www.acceleratedcure.org/downloads/phase2-infectiousagents-studies.xls.

II. Viruses

Pathogen: Adenovirus
Description:
Adenoviruses are common human pathogens that can cause respiratory infections, conjunctivitis, and occasionally gastroenteritis. These viruses most frequently infect children although outbreaks in adult groups are also possible. Adenoviruses persist in human tonsils and are capable of integrating their genome into the host DNA, making them candidate vectors for gene therapy.
Is presence of actual pathogen different in MS subjects compared with non-MS subjects?
Evidence is limited – one study[2] found adenovirus-like particles in brain tissue of 1 of 20 MS subjects but these were not correlated with demyelinating lesions; another study[3] found no adenovirus DNA in MS brain tissue.
Does immune response to pathogen differ between MS subjects and non-MS subjects?
The few studies found that compared adenovirus antibodies in MS subjects and controls (e.g., Appel *et al*[4]) did not find a significant difference between the two groups.
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
Not assessed
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
Not assessed
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
Evidence is limited – one study[5] found a correlation between upper respiratory infections (URIs) associated with increased cerebrospinal fluid (CSF) adenovirus titers and subsequent MS relapses.
Conclusion:
Only a few studies were found that explore a causal role for this virus in MS. The correlation found between adenovirus titers, URIs and subsequent relapses could indicate a possible interaction between the presence and activity of adenoviruses and MS disease mechanisms.

Pathogen: Canine distemper virus (CDV)
Description:
CDV is a virus that infects dogs and other animals, including wildlife, and can result in encephalomyelitis. It is closely related to the measles virus. Humans can be infected with CDV asymptomatically, but the measles vaccine protects humans against CDV infection. A vaccine against CDV was introduced in the 1960's and is now widely available for pet dogs.
Is presence of actual pathogen different in MS subjects compared with non-MS subjects?
Evidence so far is unsupportive. One tissue staining study showed features characteristic of CDV[6] in MS plaques; however, subsequent studies[7-9] showed no evidence of CDV-specific genes or antigens in MS brain samples.
Does immune response to pathogen differ between MS subjects and non-MS subjects?
Evidence is inconclusive: • A few studies found an increase in anti-CDV antibodies in MS serum and/or CSF compared with controls, but increases in measles antibodies were also seen (e.g., Cook *et al*[10]). Other similar studies found no significant difference in antibody levels between MS and control subjects (e.g., Kurtzke *et al*[11]), and another study showed no IgM response to CDV in MS CSF.[12] • No difference was seen between MS subjects and controls in lymphocyte production upon exposure to CDV.[13]
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
Some epidemiological studies have shown connections between CDV outbreaks, dog ownership, etc. and MS; however, others have found no such association. A review of CDV epidemiological studies[14] concludes that weaknesses in these studies have prevented demonstration of a relationship, if any exists.
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
As mentioned, immunization for dogs is now routine in many countries which has significantly reduced the incidence of CDV. However, no corresponding reduction in the risk of MS has been demonstrated.
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
CDV is known to cause demyelination in animals, and one study showed a loss of beta-adrenergic receptors on astrocytes in subjects with MS as well as CDV-infected dogs which may indicate involvement of a common pathogenic factor.[15]
Conclusion:
There is no strong evidence at this time to support a role for CDV as a cause of MS. If CDV were an important trigger of MS, it would be expected that control of this virus would produce an eventual reduction in the incidence of MS, but such a reduction has not yet been documented.

Pathogen: Coronavirus
Description:
Human coronaviruses (HCV) are responsible for approximately 20 percent of colds in humans. Incidence of coronavirus infection peaks in the winter. Immunity does not persist and individuals can be periodically reinfected. There are two main serotypes of HCV, OC43 and 229E. While human coronaviruses typically infect respiratory epithelial cells, they have been found in brain tissue as well, and the coronavirus murine hepatitis virus (MHV) is known to cause a chronic demyelinating disease similar to MS in mice.
Is presence of actual pathogen different in MS subjects compared with non-MS subjects?
Studies of the presence of coronavirus in brain tissue have provided mixed results. Initial reports of coronavirus or particles resembling coronavirus in some MS brains[16, 17] motivated several subsequent efforts to analyze the presence of coronavirus in brain tissue. Some (e.g., Stewart *et al*[18] and Arbour *et al*[19]) detected coronavirus RNA (either OC43 or 229E) more frequently in brain tissue of MS subjects compared with controls, but others such as Dessau *et al*[20] found no statistical difference between the two groups.
Does immune response to pathogen differ between MS subjects and non-MS subjects?
Evidence so far is unsupportive. Several studies have reported finding no difference in serum antibody titers between MS and controls. Results are mixed from the few studies that examined whether CSF titers to coronavirus are elevated in MS.[21-23]
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
Not assessed
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
Not assessed
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
One study found that MS subjects had more HCV 229E/MBP cross-reactive T cells than did controls;[24] this may suggest molecular mimicry which could contribute to the development of MS, or may instead be a result of greater access to myelin antigens in MS. Another study demonstrated that HCV infection of human astrocytes can spur an increase in the production of inflammatory molecules which may be damaging to brain tissue.[25] Also, as mentioned above, the mouse coronavirus MHV does cause a form of demyelination similar to MS.
Conclusion:
At this time no strong evidence has emerged to support a causal role for coronavirus in MS. Once the role of MHV in demyelination is better understood, it may be possible to compare it with HCV to determine which of MHV's pathogenic factors are also present in HCV.

Pathogen: Cytomegalovirus

Description:

Human cytomegalovirus (CMV or HCMV) is a member of the herpes family and is a common pathogen worldwide. It causes three types of clinical syndromes: (1) congenital infection can cause hepatosplenomegaly, retinitis, rash, and CNS involvement, although 90% of infected infants are asymptomatic; (2) primary infections in older children and adults can cause mononucleosis, although 90% of primary infections at this age are asymptomatic, and (3) primary or reactivated infections in immunocompromised individuals (e.g., people with AIDS) can develop into a life-threatening systemic infection involving the CNS, lungs, GI tract, liver and retinas. Transmission occurs via contact with infected secretions such as saliva or breast milk. HCMV can persist in and be chronically excreted by a variety of tissues, including salivary glands and blood cells.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

Evidence so far is unsupportive:

- Three studies found no HCMV DNA or antigen in MS brain tissue,[3, 26, 27] while another found similar percentages of MS and control subjects (neurological and non-neurological) positive for HCMV DNA in brain tissue.[28]
- Of those studies searching for HCMV DNA in other tissues, one found it present in the PBMCs of MS subjects but not healthy controls.[29] However, another found no difference in HCMV DNA prevalence in PBMCs of MS subjects and controls,[30] while others failed to detect any HCMV DNA in CSF or serum samples from MS subjects or controls.[30-32]

Does immune response to pathogen differ between MS subjects and non-MS subjects?

Again, the available evidence is unsupportive:

- Most studies of anti-HCMV antibodies using serum or CSF have found similar levels or prevalence in MS and control subjects (e.g., Alvarez-Lafuente *et al*,[30] Ascherio *et al*,[33] and Alotaibi *et al*[34]).
- Similarly, other types of immunological studies have found either no HCMV-specific differences or similar findings in MS and control subjects (e.g., Haahr *et al*[35], Hollsberg *et al*[36] and Lunemann *et al*[37]).
- Scotet *et al*[38] detected CMV- and/or EBV-reactive T cells in lesions from a variety of different inflammatory and/or autoimmune diseases, suggesting an involvement of antiherpes immune factors in inflammation that may be not specific to MS.
- Interestingly, one study (Zivadinov, *et al*[39]) reported that more favorable clinical and MRI outcomes in MS subjects were associated with HCMV antibody positivity and higher titers.

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

Not assessed

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

Not assessed

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

One study[40] that investigated viral activity during acute MS attacks reported that HCMV DNA was found in the blood of some MS subjects on the first day of the acute attack

and during follow-up. However, other viruses were detected as well.
Conclusion:
Although its persistence and potential for CNS involvement make it a plausible candidate for an MS trigger, at this time little evidence exists to support a specific role for HCMV in causing MS.

Pathogen: Epstein-Barr virus

Description:

Epstein-Barr virus (EBV) is a ubiquitous member of the herpes family; 95% of the world's population is thought to have been infected with this virus. Primary EBV infection can be asymptomatic or can manifest as infectious mononucleosis (IM), especially in adolescents and young adults. (Primary infection in children is often mild or asymptomatic.) Development of IM during primary infection is associated with large expansions of T cells in the blood. Why IM develops in some people but not others is not known but it may be due to host factors (such as genetics) and/or characteristics of the infection. EBV is trophic for B cells and is thought to cause lymphoproliferation in immunocompromised individuals. EBV persists in B cells and epithelial cells as a latent infection that periodically reactivates, and is primarily exchanged through transmission of saliva.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

Several studies have looked into this question and provide a range of findings:

- Brain tissue: Six studies have been conducted to investigate the presence of EBV DNA in brain tissue. Of these, Hilton *et al*[41] and Virtanen *et al*[27] found no evidence of EBV RNA in MS plaques, Sanders *et al*[28] reported that EBV DNA was not more prevalent in MS samples than controls, and Challoner *et al*[3] and Morre *et al*[42] detected no EBV DNA in MS brain. However, a recent study by Serafini *et al*[43] found EBV-infected B cells in 21 of 22 MS brain samples studied (no infection was found in samples from subjects with other neurological diseases). EBV-infected cells were found both in active lesions and in ectopic B cell follicles located in cerebral meningeal tissue.
- CSF: Neither Martin *et al*[31] nor Morre *et al*[42] detected EBV in the CSF of any MS subjects. Serafini *et al*[43] detected low copy numbers of EBV DNA in the CSF of only 2 of 16 MS cases, Mancuso *et al*[44] found similar prevalence of EBV DNA in MS subjects and controls; and Alvarez-Lafuente *et al*[32] found EBV DNA in the CSF of only 1 of 48 MS subjects.
- Blood/serum: Several analyses of EBV DNA have been conducted using the blood and/or serum of MS subjects and controls, with mixed results:
    - EBV was found in none of the subjects or controls in Martin *et al*[31], Villoslada *et al*[45], or Riverol *et al*[46] (serum).
    - EBV was found in all subjects and controls in Hay and Tenser[47] (PBLs)
    - EBV was found in equivalent percentages of subjects and controls by Ferrante *et al*[29] (included both acute and stable MS), Alvarez-Lafuente *et al*[30], Lunemann *et al*[37], and Sotelo *et al*[48] (PBMCs); and Alvarez-Lafuente *et al* [49](serum and PBMCs).
    - A prospective study[50], using samples drawn before onset or diagnosis, found that the presence of EBV in plasma (but not the viral load) was associated with an increased risk of MS.
    - The presence of EBV DNA was associated with MS exacerbations by Ferrante *et al*[40] (which detected EBV DNA in the PBMCs of 42.8% of MS subjects on day 1 of attack) and Wandinger *et al*[51] (which detected DNA in the serum of 72% of MS subjects with exacerbations vs. 0% of stable subjects). However, another study failed to find increased anti-EA-D IgG (a sign of viral reactivation) in MS subjects during relapse as compared with baseline.[52]

- Level of disease activity has also been associated with the presence of EBV. Fraser *et al*[53] established spontaneous B lymphocyte transformation from 8 of 10 active MS subjects but only 3 of 18 stable MS subjects and only 4 of 20 healthy controls; all transformed cells carried EBV antigens. Hollsberg *et al*[54] also found that the presence of EBV DNA in MS samples was associated with disease activity.

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Does immune response to pathogen differ between MS subjects and non-MS subjects?

A variety of studies concerning the immunological response to EBV have been conducted with respect to MS; most have found some type of association with MS although not all of these results have been consistently verified:

- Of those studies examining antibody titers or seropositivity to EBV, a preponderance found either higher titers or a higher prevalence of seropositivity in MS or optic neuritis subjects vs. controls (although in some studies, these effects were found for other pathogens as well). Notably, Alotaibi *et al*[34] and Banwell *et al*[55] showed significantly higher seropositivity in MS pediatric subjects than in matched controls (83% vs. 42% and 86% vs. 64%, respectively).
- In addition, five studies[33, 56-59] associated higher titers against EBV with the subsequent development of MS. The presence of higher antibody titers to EBNA-1 and EBNA complex prior to the development of MS may indicate a severe or recent primary infection or reinfection that stimulates a vigorous cellular immune response[58].
    - Note however that Sundstrom *et al*[57] suggests that higher pre-onset titers may be due to the development of proteasomal autoantibodies that can occur during MS, which may block EBV's ability to hide from immune system and make it more immunogenic.
- Haahr *et al*[60] found equal antibody evidence of EBV reactivation in MS subjects vs. controls, but reactivation was found more frequently in recently diagnosed MS subjects than those of longer disease duration.
- Two separate studies[61, 62] investigated the interaction between the HLA DR15 gene and anti-EBNA1 titers in influencing MS risk; each found significantly increased MS risk in DR15-positive individuals with high antibody titers.
- Rand *et al*[63] found an oligoclonal banding pattern specific for the EBV antigen EBNA-1 in 5 of 15 MS subjects but 0 of 12 controls; Cepok *et al*[64] also found that oligoclonal bands from MS subjects specifically bound EBNA-1 and BRRF2, another EBV protein. Serafini *et al*[43] found EBV-specific OCBs in seven of 16 MS subjects studied; when present, OCBs were generally few in number and faint.
- Three studies[65-67] identified a failure of T cells from MS subjects to control EBV infection of cells, which may be related to an increased CD4+/CD8+ ratio as opposed to overall numbers of CD8+ cells or T-cell mediated cytotoxicity.
- On the other hand, Lunemann *et al*[37] determined that EBNA1-reactive CD4+ cells from MS subjects were more numerous, produced more IFN-gamma, and recognized a broader range of EBNA1 antigens than cells from controls. A follow-up study demonstrated that EBNA-1-reactive CD4+ cells from MS subjects were more likely to also react to myelin antigens than to other autoantigens not associated with MS.[68]
- Hollsberg *et al*[36] found a higher frequency of CD8+ cells responding to two EBV antigens, EBNA-3A and latent membrane protein 2 (but not to other EBV antigens and not to two CMV epitopes) in MS subjects vs. healthy controls.

Cepok *et al*[64] found greater CD8+ T cell responses to latent EBV proteins in MS subjects than in non-MS neurological controls. Similarly, Jilek *et al*[69] described an increased frequency of IFN-gamma secreting EBV-specific CD8+ cells in subjects with a clinically isolated syndrome; this T-cell response tended to decline over the course of a year. However, no differences in the prevalence or levels of EBV-reactive cytotoxic lymphocytes were found between MS cases and controls in a study by Gronen, *et al*[70]. In addition, as mentioned in the HCMV template above, Scotet *et al*[88] detected CMV and/or EBV-reactive CD8+ T cells in lesions from a variety of different inflammatory and/or autoimmune diseases, suggesting an involvement of antiherpes immune factors in inflammation that may be not specific to MS.

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

Numerous sources indicate that MS is associated with a history of infectious mononucleosis,[60, 71-80] and that primary (initial) infection is absent in MS subjects, presumably because of previous exposure.[51, 81, 82]

One study, Munch *et al*[81], investigated EBV subtypes, finding all 8 members of an MS cluster in Denmark to harbor the same EBV subtype. However, another study did not find any particular strain of EBV to be associated with MS.[83]

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

The effect of anti-herpes treatment on MS disease activity was examined by Bech *et al*,[84] who found that valacyclovir appeared to reduce new active lesion formation in a subset of subjects who had active lesions at baseline. Another trial of valacyclovir found evidence of clinical efficacy in more severely affected subjects but detected no effect on imaging evaluation[85]. Administration of valacyclovir in MS subjects appeared in one study to reduce EBV expression in saliva but not in plasma[54].

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

Several different types of evidence have been produced to explain how EBV could cause or trigger MS:

- Multiple reports of immune cross-reactivity to EBV and self-antigens have been published:
    - van Sechel *et al*[86] showed that EBV-infected B cells produce alpha beta crystallin which is an immunodominant antigen of CNS myelin in MS subjects, and Rand *et al*[87] found that oligoclonal bands (OCBs) in some MS subjects and neurological controls reacted to epitopes that are found both in EBNA and alpha beta crystallin. Unlike other stress proteins, alpha beta crystallin is not expressed in lymph nodes so it may evade tolerance mechanisms.
    - Esposito *et al*[88] demonstrated antibody cross-reactivity between human transaldolase epitope (271-285) and an EBV as well as an HSV-1 peptide.
    - Vaughan *et al*[89] detected in MS subjects cross-reactive antibodies to EBNA (Epstein-Barr nuclear antigen) and lymphocyte and neuroglial proteins.
    - Lang *et al*[90] found T-cell receptor (TCR) contact surface similarities between DRB5*0101-EBV (DNA polymerase peptide) and DRB1*1501-MBP(85-99).
- Several studies have explored possible connections between EBV and

retroviruses that may relate to MS. Haahr *et al*,[91] Munch *et al*,[92] Christensen *et al*,[93] and Christensen *et al*[94] established B cell lines from MS subjects that produced retrovirus-like particles as well as EBV; they also detected reverse transcriptase activity via PCR.

- Pender[95] suggested that EBV-infection of autoreactive B cells lodging in the central nervous system may promote the survival of activated autoreactive T cells which then effect an inflammatory response leading to tissue damage. Subsequently, a study described EBV-infected B cells in the brain and meningeal tissue of MS subjects and also detected the presence of activated CD8+ T cells and evidence of cytotoxic activity in association with the infected cells.[43]

Conclusion:

Numerous types of evidence exist to suggest a possible role for EBV in causing MS, including several findings that suggest possible etiological mechanisms. It is possible that some evidence (e.g., increased antibody titers in MS cases vs. controls) results from MS disease processes rather than reflects a cause of MS. However, the existence of multiple types of evidence supporting a causal role, particularly the increased seroprevalence of EBV in pediatric MS cases[34] and the detection of EBV-infected B cells in lesions and meningeal tissues,[43] is striking and makes EBV a pathogen of particular interest in MS. Questions worth exploring further include why mononucleosis appears to increase the risk of MS, what is the full significance of EBV-infected B cells in the central nervous system, and whether interactions between EBV and other elements such as endogenous retroviruses play a key role in MS.

Pathogen: Herpes simplex virus

Description:

Herpes simplex virus (HSV) 1 and 2 are closely related neurotropic viruses whose primary symptoms are oral lesions (HSV-1) and genital lesions (HSV-2). Other manifestations of HSV infection include lesions at other skin sites, meningitis, encephalitis, and disseminated disease. HSV is transmitted by oral and sexual contact. Infection is established through replication of the virus in epithelial cells and movement up the peripheral sensory nerve to the dorsal ganglia, where it can further replicate, move back down the sensory nerves to form a new lesion, or assume a state of latency.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

- Several studies have searched for the presence of HSV in brain tissue, with varying results:
  - HSV antigen or DNA has been found in brain or plaque tissue of MS subjects and/or other subjects by Warren *et al*[96] (HSV was isolated from the trigeminal ganglia), Martin *et al*[97] (HSV-2 but not HSV-1 was found in 3 of 31 MS tissue samples, restricted to glial cell nuclei in and around demyelinating lesions), and Nicoll *et al*[98] (HSV-1 but not HSV-2 DNA was found in 1 of 77 plaques from 23 MS subjects).
  - Another study found no HSV DNA in MS brain tissue.[26]
  - In comparison with controls, results are slightly positive: see Challoner *et al*[3] (HSV-1 was found in the brain tissue of 2 of 25 MS vs. 2 of 42 controls) and Sanders *et al*[99] (HSV was more often detected in MS brain tissue than OND or NND control tissue and was also more prevalent in active vs. inactive plaques).
- Mixed results have also been found in studies of other tissues:
  - One study[31] failed to detect HSV or other herpesvirus DNA in the serum or CSF of MS subjects; similarly, another study failed to detect herpes simplex virus DNA in the PBMCs of MS subjects (in relapse or remission) or controls[48].
  - Other studies examining PBMCs[30] and CSF[32, 44] found HSV DNA in similar levels in MS subjects and controls.
  - HSV-1 or -2 DNA was found in the PBMCs of a subset of MS subjects at the beginning of relapses and during a follow-up period, but EBV and HTLV-1 were found more frequently.[40]
  - Similarly, HSV-1 mRNA and DNA were found in the PBMCs of acute MS subjects but not stable MS subjects or controls.[29]

Does immune response to pathogen differ between MS subjects and non-MS subjects?

Evidence to date does not show significant differences between MS and non-MS subjects:

- Antibody evidence so far is unsupportive. Most antibody studies of serum or CSF have reported similar titers or rates of positivity in MS and control subjects, although a few found higher titers or positivity rates in MS subjects, one using tear samples.[100] One study of pediatric MS subjects[34] showed lower positivity rates for MS subjects vs. controls.
- In studies of T cell immunity to HSV, the majority showed no differences in lymphocyte response between MS and controls (e.g., Chou *et al*[101]), while a few showed lower responses to HSV by MS subjects (e.g., de Silva and

McFarland[102]). However, Brudek, *et al.* demonstrated increased IFN-gamma production in PBMCs from MS subjects vs. controls after stimulation with HSV-1 antigens, both alone and in combination with HERV antigens. (IL-2 and IL-10 production were unchanged.)[103]

- Two studies[104, 105] found serum or CSF immune complexes containing HSV antibodies and/or antigen in MS subjects (however, antibodies or antigens for other viruses were also detected).

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

Not assessed

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

The effect of anti-herpes treatment on MS disease activity was examined by Lycke *et al*,[106] who found that acyclovir reduced the number of exacerbations in RRMS subjects. Also, Bech *et al*[84] found that valacyclovir appeared to reduce new active lesion formation in a subset of subjects who had active lesions at baseline.

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

A few relationships have been proposed involving retroviral activation or molecular mimicry:

- Brudek *et al*[107] found a synergistic effect of HSV-1 (and HHV-6A) with HERV-H on cellular immune responses in both MS and control subjects, and in a subsequent study[108] showed that HSV-1 and other viruses induced higher reverse transcriptase activity in peripheral lymphocytes of MS subjects compared with controls
- In addition, Perron *et al*[109] detected increased activation of LM7 retrovirus in cells infected with HSV-1.
- Cortese *et al*[110] and Esposito *et al*[88] found antibodies cross-reactive for HSV-1 and brain proteins in MS sera and CSF.

Conclusion:

The results of case/control studies do not strongly support a role for HSV in causing MS. However, antiviral treatment studies showing a reduction in MS lesion formation or relapses may suggest a role for HSV or other herpesviruses in stimulating disease activity.

Pathogen: Human herpesvirus 6

Description:

Human herpesvirus 6 (HHV-6) is a common virus that infects most people in early childhood, most likely through salivary transmission. It is tropic for CD4 T cells but also has been shown to inhabit CNS tissue, and like many of the viruses described in this document has been detected in the CNS tissue and/or CSF of subjects with neurological conditions such as encephalitis. Two strains have been identified: HHV-6A and HHV-6B. The B strain is associated with the childhood disease exanthem subitum, also known as roseola; both strains have been found to be neurotropic. Like many other viruses, HHV-6 can be reactivated; its reactivation has been associated with several diseases.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

Numerous studies have explored the question of whether HHV-6 is more prevalent in MS compared with non-MS subjects, or in plaque tissue vs. non-plaque tissue; other studies have focused on the presence of the two strains of HHV-6.

- HHV-6 has been detected in both MS brain tissue and control tissue, with some studies reporting that the virus is more prevalent in MS samples.[28, 111, 112]
- Some studies indicate a higher frequency or greater activity of HHV-6 in MS plaque tissue compared with normal appearing white matter (NAWM) or tissue from controls. For example, Challoner *et al*[β] detected HHV-6 gene expression in the oligodendrocytes of MS subjects, particularly around plaques, but not in those of OND or NND controls. Goodman *et al*[113] found HHV-6 DNA to be prevalent in the oligodendrocytes, lymphocytes, and microglia from biopsied acute MS lesions. Virtanen *et al*[27] detected the presence of HHV-6 variant B antigen in 67% of MS lesional samples vs. 30% of normal control brain samples; HHV-6 antigen was located most often in oligodendrocytes, and frequently co-located with tissue plasminogen activator (tPA) protein in MS samples. A recent study of HHV-6 gene expression[114] found higher levels of mRNA in MS brain samples (lesional samples, and to a lesser extent, NAWM) compared with normal control samples. On the other hand, Tuke *et al*[115] found similar viral loads in lesion and non-lesion MS brain tissue samples, and Mameli *et al*[116] could not detect HHV-6 replication in MS brain samples.
- Studies of HHV-6 presence or activity in other tissues (blood, serum, PBMCs, and CSF) have provided mixed results; some studies detected no HHV-6 in MS or controls (e.g., Villoslada *et al*[45], Gutierrez *et al*[117], and Riverol *et al*[46]), others found similar frequencies of the virus in both MS and control samples (e.g., Ferrante *et al*[29]), and others found a higher prevalence in MS compared with controls (e.g., Tejada-Simon *et al*[118] and Alvarez-Lafuente *et al*[49]).
- In comparisons of strain A vs. strain B, some studies report detecting mostly HHV-6A in serum, urine or PBMCs, while others found mostly HHV-6B in serum or brain tissue. One study[119] found only the B strain in PBMCs and only the A strain in cerebrospinal fluid or cells, whereas other studies found only variant A in PBMCs and serum of RRMS subjects with active HHV-6 replication[49], in serum from RRMS and SPMS subjects[120], and in CSF from RRMS subjects.[32] In addition, HHV-6 DNA from the CSF of two natalizumab-treated MS subjects was subtyped as HHV-6A.[121] Studies comparing the immune response to each strain (lymphoproliferative response, antibody analysis) in MS and control subjects offer mixed results (e.g., Ongradi *et al*[122] and Soldan *et al*[123]).

- A case report of a subject who developed Balo's concentric sclerosis describes a concurrent primary HHV-6 infection of the CNS which may have been related or may have been a coincidence[124].

Does immune response to pathogen differ between MS subjects and non-MS subjects?

- Antibody evidence from serum and CSF analysis has been mixed, although more studies (e.g., Villoslada *et al*[45], Caselli *et al*[125], and Virtanen *et al*[126]) found higher titers or positivity in MS subjects vs. controls than found no differences between the groups. Associations with MS have been found for both IgG and IgM antibodies, as well as antibodies to various proteins (early and late antigens, U94/rep).
- One study found a higher lymphoproliferation response to HHV-6 in MS cases than controls (to 6A only);[123] another found a similar response in both groups.[127]
- Incubation of PBMCs with HHV-6 antigens did not result in an increased production of IFN-gamma in MS cases compared with controls, although an increased response was seen when combined HHV-6 and HERV antigens were used.[103]
- One study showed a higher frequency in MS cases vs. controls of T cells cross-reactive to MBP and HHV-6 epitopes.[128] However, another study found that cross-reactive T cell lines were more readily generated in MS samples following MBP stimulation than HHV-6 stimulation and that the percentage of cross-reactive cell lines following HHV-6 stimulation was roughly equivalent in MS subjects and controls.[129]

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

Not assessed

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

Although the MS disease-modifying drug interferon-beta does not specifically target HHV-6, IFN-b has been found to reduce HHV-6 viral replication and perhaps exerts a beneficial effect in MS by this mechanism.[130-132] Although it is unclear whether to what extent the antiviral drug valacyclovir can inhibit HHV-6[54], two clinical trials of valacyclovir have been performed, using doses which may have some efficacy against HHV-6. Results from these trials indicate a possible clinical or MRI effect in subjects with severe or highly active MS.[84, 85]

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

Several observations or hypotheses have been proposed and investigated to explain how HHV-6 might trigger MS:

- Infection of T cells with HHV-6 may be indirectly toxic to oligodendrocytes.[133]
- HHV-6 and endogenous retroviruses may interact in a way that contributes to MS. HHV-6 and other herpesviruses have been shown to induce higher reverse transcriptase activity in blood cells of MS subjects compared with controls[108], and the combination of HHV-6 and HERV antigens has a synergistic effect on stimulating cellular immune responses.[103, 107]
- There is evidence, although mixed, for molecular mimicry based on increased T-cell reactivity (see above).
- Elevated levels of soluble CD46, the receptor for HHV-6, have been found in the sera of MS cases compared with healthy and OND controls, particularly in MS cases positive for HHV-6 DNA in serum (but note that elevated soluble CD46 levels were also detected in the sera of subjects with other inflammatory

diseases compared with healthy controls).[134]

- As with other viruses such as EBV, reactivation may be associated with disease activity/exacerbations and progression.[48, 49, 120, 132, 135-138]
- Infection with HHV-6 has been shown *in vitro* to induce morphological changes in and inhibit proliferation of glial precursor cells[139]; this effect may impair remyelination processes in MS or other diseases.
- Chronic infection of astrocytes with HHV-6 impairs their glutamate uptake, which may lead to glutamate levels in the CNS that are harmful to oligodendrocytes.[140]
- HHV-6 encodes the protein U24, which shares a 7 amino acid stretch in common with an MBP phosphorylation site; MBP in MS subjects has lower levels of phosphorylation which may be a result of phospho-U24 confounding of signaling pathways.[141]

Conclusion:

It is possible that HHV-6 is involved in triggering MS. There is some evidence that HHV-6 is preferentially found in plaque tissue of MS subjects but the significance of this is unknown. Some studies have also identified differences in the antibody response to HHV-6 between MS subjects and controls. Many theories exist concerning how this virus may participate in the pathogenesis of MS (molecular mimicry, impairment of glutamate uptake in astrocytes, interaction with HERVs, infection of T cells, etc.).

Pathogen: Human immunodeficiency virus (HIV)
Description:
Human immunodeficiency virus (HIV), also referred to in older texts as HTLV-III, is the human retrovirus that causes AIDS (acquired immune deficiency syndrome). HIV infects CD4 T cells and macrophages, and AIDS is characterized by depletion of CD4 T cells which leads to opportunistic infections and tumors as well as dementia and other neurological abnormalities. HIV is chiefly transmitted through sexual contact, through contact with blood (e.g., transfusion of contaminated blood), and perinatally.
Is presence of actual pathogen different in MS subjects compared with non-MS subjects?
Searches for HIV genetic sequences in MS CNS tissue and blood have produced negative results (e.g., Hauser *et al*[142] and Rozenberg *et al*[143]).
Does immune response to pathogen differ between MS subjects and non-MS subjects?
Several antibody analyses have been conducted using MS blood samples. Most found no reactivity to HIV/HTLV-III in MS subjects, although Ferrante *et al*[40] did find a clear or weak antibody response to HIV in 10% of MS subjects, and Perron *et al*[144] did find increased reactivity to reverse transcriptase fragments in MS cases compared with controls.
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
Not assessed
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
Not assessed
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
Not assessed
Conclusion:
No strong evidence exists for an association between MS and HIV. However, there is evidence for possible increased reactivity to retroviral components (see also the discussions of HTLV-1 and endogenous retroviruses).

Pathogen: Human T-cell leukemia (or T-lymphotropic) virus (HTLV)
Description:
There are two known strains of HTLV: HTLV-1 and HTLV-2. HTLV-1 is an exogenous human retrovirus that is prevalent to varying degrees in different parts of the world and is particularly common in southwestern Japan and the Caribbean. Most HTLV-1 infected individuals have an asymptomatic infection, but a small percentage develop an associated disease such as adult T-cell leukemia/lymphoma. Approximately 0.25% of infected people develop HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a demyelinating disease that resembles MS in many aspects. Transmission of the virus typically occurs through sexual contact, contact with contaminated blood, or breastfeeding. HTLV-2 is a closely related virus that has not yet been conclusively associated with any human disease.
Is presence of actual pathogen different in MS subjects compared with non-MS subjects?
HTLV-1 DNA has been detected in tissues of MS subjects by some studies but not by others: • Although a few early studies associated MS with the presence of HTLV DNA sequences such as pol, env, and p24,[145, 146] a large number of follow-on studies failed to find various HTLV DNA sequences in a variety of tissues (PBMCs, brain, CSF, etc.) in MS subjects (e.g., Watanabe *et al*,[147] Oksenberg *et al*,[148] and Ehrlich *et al*[149]). • One study found increased detection of HTLV-1 tax-rex DNA (detected in 35.7% of cases) in MS PBMCs on day 10 of an acute attack; other viruses were also detected.[40]
Does immune response to pathogen differ between MS subjects and non-MS subjects?
Evidence is mixed: • Approximately 40 studies have assessed the presence of HTLV-1 antibodies in MS subjects. Most found no evidence of seropositivity in MS subjects (e.g., French *et al*,[150] Merelli *et al*[151]), a few found equal rates in MS subjects vs. various types of controls (e.g., Kuroda *et al*,[152] Brookes *et al*[153]), and a handful found higher seropositivity in MS subjects vs. controls (e.g., Odum *et al*,[154] Ferrante *et al*[155]). It has been pointed out that some cases of seroreactivity to HTLV antigens in MS subjects could be due to cross-reactivity with autoantibodies to endogenous retroviruses.[156] • No evidence has been produced of lymphocyte proliferation indicating HTLV-1 infection,[157] of cytopathic effects,[158] or of seronegative HTLV-1 infection.[159] One study did find that cells from MS subjects but not controls reacted to anti-p19 and anti-p24 antibodies.[160]
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
Not assessed
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
Not assessed
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
Not assessed
Conclusion:
At this time the evidence does not indicate a causal role of HTLV-1 in MS. However,

the existence of a similar demyelinating disease (HAM/TSP) that is thought to be caused or triggered by HTLV-1 suggests that the involvement of retroviruses should continue to be studied in MS.

Pathogen: Human endogenous retroviruses (HERVs)

Description:

HERVs are retrovirus-like elements that make up as much as 8% of the human genome and are found in both coding and non-coding regions. Most are unable to replicate because of genetic defects, but many HERVs are capable of encoding retroviral proteins. Expression of HERV RNA may be increased at sites of inflammation; HERVs may also be transactivated by herpesviruses[108]. Several different HERVs have been investigated in MS. One, multiple sclerosis-related virus (MSRV, previously called LM7), is particularly noteworthy because it was first isolated from cells from people with MS. Following are brief research summaries for each of the individual HERVs investigated for involvement in MS.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

- HRES-1: One study found that expression levels of HRES-1 in brain tissue did not differ between MS and non-MS subjects.[161] A few studies of HRES-1 genotypes have identified allelic differences between European MS and non-MS subjects, but no differences have been found in Chinese populations.[162-165]
- ERV3: In studies of ERV3 transcription, most identified no differences between MS and control groups, although one study[161] showed that healthy controls had a higher frequency of an ERV3/zinc finger composite transcript. No allelic associations have been found for ERV3 and MS.[166, 167]
- RGH/HERV-H/RTVL-H: Three studies have reported an association with MS for the expression of RGH sequence or splice variants in plasma;[168-170] however, another study using brain tissue found no HERV-H upregulation in MS.[171] A study of CSF in MS and other neurological disease subjects found no samples positive for HERV-H.[32]
- HERV-K: One study reported that HERV-K expression was increased in brain tissue of subjects with MS or AIDS compared with healthy controls, as was expression of TNF-alpha;[172] however, two other studies[161, 171] found no increased expression of HERV-K in blood or brain tissue. Another study found equal expression of HML6 RNA in MS plaque versus control tissue.[173] With respect to HERV-K genotypes, an investigation of the HERV-K113 allele found that its presence was not significantly increased in MS subjects compared with their parents,[174] contradicting results from a previous, smaller study.[175] However, Tai, *et al*[176] did find a genetic association with MS for the K18.3 allele of HERV-K18 Env.
- MSRV/LM7/HERV-W/ERVWE1:
  - Numerous studies have found the expression of HERV-W RNA to be increased in the blood, brain, or CSF samples of MS or optic neuritis subjects compared with other disease controls or healthy controls (e.g., Perron *et al*[177], Menard *et al*,[178] Serra *et al*,[179] Nowak *et al*[180], Mameli *et al*[116]). However, increased expression of MSRV RNA has also been shown in unaffected close relatives of MS subjects[181], as well as in subjects with other diseases, particularly other inflammatory neurological diseases.[182] Also, Petzold, *et al*[183] detected no evidence of MSRV viremia in acute optic neuritis subjects and Alvarez-Lafuente, *et al*[32] found no MS or other neurological disease CSF samples to be positive for HERV-W sequences.
  - DNA levels of syncytin-1 (the Env protein of ERVWE-1) have been

detected at higher levels in MS vs. non-MS brain tissue.[184] In addition, a study comparing two members of the HERV-W family, MSRV and ERVWE-1, found ERVWE-1 but not MSRV DNA to be more abundant in MS brain tissue than control tissue; DNA levels for both ERVs were similar in MS and control PBMC samples. ERVWE-1 mRNA was also amplified in MS brain samples vs. control samples whereas no difference was seen for MSRV[185].

- Expression of MSRV/HERV-W has been associated with disease severity and/or duration of MS. For example, Dolei *et al*[182] found MSRV RNA in the CSF of 50% of MS subjects at onset, with the prevalence increasing with disease duration, and Mameli *et al* similarly found viral load to be directly related to disease duration.[186] Sotgiu, *et al*[187], associated MSRV RNA in the CSF with faster progression, more relapses, and greater risk of conversion to a secondary progressive course. The presence of MSRV RNA in blood and/or CSF of acute optic neuritis subjects was associated in another study (Sotgiu *et al*[188]) with conversion to MS.
- Perron *et al*[189], using immunohistochemical methods, found HERV-W GAG and ENV expressed in both MS and normal brain, although the cells expressing these proteins differed between the two states. However, Mameli *et al* found MSRV/HERV-W immunoreactivity within MS plaques (generally only active ones) but not within normal appearing white matter, perilesional areas, or tissue from normal controls.[116]
- Regarding genomic differences, one study of MSRV *pol* sequence copy number found a greater number of sequences in the chromatin of MS subjects compared with controls.[190]

Does immune response to pathogen differ between MS subjects and non-MS subjects?

- HRES-1: Elevated antibody titers were found in MS subjects compared with healthy controls, but titers were also elevated in other disease states such as Sjogren's.[153]
- RGH/HERV-H/RTVL-H: Christensen *et al*[170] found increased antibody responses to HERV-H/RGH-2 in the serum and CSF of MS subjects compared with healthy controls and patients with other diseases. A follow-up study found that MS subjects, particularly those with active disease, had increased seroreactivity to HERV-H antigens compared with unaffected relatives; degree of reactivity (high or low) to HERV-H was often found to be similar among family members.[191]
- MSRV: Stimulation of PBMCs by the surface protein ENV-SU was shown to induce a stronger production of cytokines IFN-gamma, IL-6 and IL-12p40 in MS subjects compared with controls.[192] However, Ruprecht *et al* found serum antibodies against HERV-W Env (Syncytin-1) in only one of 50 MS subjects and no subjects positive for antibodies against MSRV matrix, Gag and Env proteins; similarly, no cytotoxic T-lymphocyte responses were seen to various MSRV/HERV-W-derived peptides.[193]

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

Not assessed

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

Although interferon beta is not specifically targeted at inhibiting HERV activity, a small eleven-person trial did find that initiation of IFN-b therapy reduced MSRV RNA blood levels in all subjects to below detection levels. These levels were maintained for a

year in ten of the subjects; MSRV levels rebounded in one subject, whose EDSS score also increased.[186]

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

- RGH/HERV-H/RTVL-H: Combinations of HERV and herpesvirus (such as HHV-6A, HSV-1, and VZV) antigens have been shown to exert a synergistic effect on stimulating cellular immune responses in both MS subjects and controls.[103, 107]
- LM7/MSRV/HERV-W: Antony *et al*[171] showed that the HERV-W encoded protein syncytin is upregulated in glial cells in acute MS lesions, and that syncytin is cytotoxic to oligodendrocytes through the release of redox reactants. Previously, Menard *et al*[178] had reported that monocyte/macrophage culture supernatants were toxic to astrocytes and oligodendrocytes, and that this gliotoxicity was correlated with the reverse transcriptase activity in these cultures. In addition, Perron *et al*[194] demonstrated that MSRV envelope protein can induce an abnormal T-lymphocyte response and may act as a superantigen. This hypothesis was further explored in a transfer of MSRV-particles into SCID mice[195] and in a study of cytokine production in response to MSRV ENV-SU exposure.[192] The latter study showed greater pro-inflammatory cytokine production in MS vs. control samples, which was correlated with disease severity.

Conclusion:

No conclusion can yet be drawn about the involvement of endogenous retroviruses in MS. The increase in MSRV expression activity in MS subjects, combined with its production of proteins that may be harmful to oligodendrocytes or act as superantigens, suggest a role for this virus. However, it should be noted that increased MSRV expression activity is not specific to MS but has also been seen in other diseases. Interestingly, two studies have identified familial patterns in seroreactivity to ERV antigens[191] and expression of ERV sequences[181]; whether and how this might relate to familial patterns of MS is not known.

Pathogen: JC virus

Description:

JC virus (JCV) is a relatively common virus that is found worldwide, with prevalence varying according to geographic region. In some areas the majority of the adults studied have been found to be seropositive for JCV. Normally the virus establishes a latent, asymptomatic infection in the kidneys (and possibly other sites in the body), which is kept under control by the immune system. However, if the immune system is suppressed, JCV may travel to and/or become activated in the brain, where it can cause a demyelinating disease called progressive multifocal leukoencephalopathy (PML). Notably, PML has been diagnosed in a small number of MS patients receiving the MS drug Tysabri, causing a temporary withdrawal of the drug from the market and the development of programs for monitoring those receiving the drug.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

The available evidence does not demonstrate an increased prevalence of JCV in MS compared with non-MS subjects:

- None of the searches for JCV DNA or antigens in MS brain tissue samples have produced a positive result.[3, 196-198]
- Of studies analyzing the presence of JCV DNA in CSF, one detected JCV in 9% of MS subjects studied but none of the controls[199], another detected it in 5% of early MS subjects but none of the controls,[200] while another found similar prevalence of JCV DNA in the CSF of MS subjects compared with controls[44]. Four studies, including a study of 1,869 natalizumab recipients, failed to detect JCV DNA in MS CSF samples.[201-204]
- Several studies (e.g., Agostini *et al*[201]) have analyzed the excretion of JCV in urine in MS subjects and controls, as a marker of JCV activation, but none report an association with MS.
- The presence of JCV DNA in blood or serum has also been found not to differ between MS subjects and controls,[40, 199, 204, 205] and does not appear to be increased by interferon beta treatment.[205, 206]

Does immune response to pathogen differ between MS subjects and non-MS subjects?

One study assessed the synthesis of intrathecal antibodies to JCV in subjects with PML and other neurological diseases; oligoclonal antibodies to JCV were found in the CSF of one subject with MS, but these may have been part of a polyspecific reaction.[207] Du Pasquier, et al analyzed cytotoxic lymphocyte response to JCV and found it to be stronger in MS subjects than in normal controls.[208]

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

Not assessed

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

Not assessed

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

Not assessed

Conclusion:

Although the similarities between PML and MS might suggest a causal role for JCV in MS, there is currently no strong evidence to support this idea.

Pathogen: Measles

Description:

The measles virus is a paramyxovirus that, in an unvaccinated person, can cause a disease involving rash, fever, runny nose, conjunctivitis and other complications. The disease is marked by a viremia that spreads to the lymphatic system, skin, respiratory system, intestines and urinary tract. The virus may also spread to the brain where it occasionally results in encephalitis. It can persist in the brain and at a later time reactivate to cause an inflammatory, demyelinating disease called subacute sclerosing panencephalitis (SSPE). Measles RNA can be detected in the brain tissue of people with SSPE. Note that an effective vaccine to measles became available in the US in 1963; since the implementation of public health vaccination programs, the incidence of measles in the US and other countries has been dramatically reduced.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

Mixed results have been provided by studies investigating the presence of measles antigens or RNA/DNA sequences in MS brain; some found the virus to be present in certain MS samples (e.g., Rastogi *et al*[209]) while others failed to detect it (e.g., Dowling *et al*[210]). The most recent study of this type was Geeraedts *et al* [9] which did find evidence of a conserved morbillivirus protein in MS brain samples, but no evidence of specific measles proteins or mRNA using immunostaining and RT-PCR. Very few studies seem to have compared the presence of measles in MS vs. control brain. A small number of studies have searched for the presence of measles in other tissues such as the jejunum or peripheral blood lymphocytes (e.g., Woyciechowska *et al*,[211] Gupta *et al*,[212] Brankin *et al*[213]), but again their results are mixed.

Does immune response to pathogen differ between MS subjects and non-MS subjects?

This question has been analyzed extensively, particularly in studies of anti-measles antibodies which often report finding increased titers and/or prevalence in MS subjects compared with controls.

- Approximately 50 studies (e.g., Fewster *et al*[214]) have analyzed measles antibody titers in MS subjects vs. various types of controls (OND subjects, healthy controls, siblings and other family members, for example). Most of these studies have analyzed serum but occasionally have tested CSF or other substances such as tears. The majority of these studies found higher titers in MS subjects. Subjects with SSPE were often also tested and likewise found to have higher titers.
- Several studies also found an increased prevalence of anti-measles antibodies in subjects with MS vs. controls. Evidence of intrathecal production of measles antibodies has been repeatedly found in MS subjects (e.g., Rostrom *et al*,[215] Schadlich *et al*,[216] Reiber *et al*[217]). One study found measles antibodies in brain tissue from MS subjects, along with antibodies to other viruses;[218] however, another study found no measles antibodies in MS brain tissue.[219]
- Many other studies have searched for measles-related immunological differences between MS subjects and controls (based on lymphocyte transformation, interferon response, lymphocyte-mediated cytotoxicity, and other assays). Although a few studies appeared to indicate a deficient response to measles virus (e.g., Neighbour *et al*,[220] Jacobson *et al*[221]), others reported similar responses in cases and controls (e.g., Tovell *et al*,[13] Santoli *et al*[222]).
- Some studies (e.g., Walker *et al*,[223] Salonen *et al*,[224] and Makela[225]) searched for correlations between immune responses and HLA status such as DR2 status. It

is possible that the prevalence of the DR2 allele in MS may underlie some of the immunological responses to measles since class II genes have been found to influence responses to measles antigens (for instance, see Ovsyannikova *et al*[226]).
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
Approximately 17 studies have analyzed age of infection or other features of prior measles infections in people with MS vs. controls. Several showed that people with MS tended to have been infected later in life than controls (e.g., Haile *et al*,[227] Sullivan *et al*,[228] and Bachmann and Kesselring[229]); however, other studies showed that infection at earlier ages, such as before age 7, is associated with MS (e.g., Casetta *et al*[230]). Still others found no significant correlation between risk of MS and age of measles infection (e.g., Bager *et al*[231]).
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
The dramatic reduction in measles cases starting in the 1960's due to vaccination may appear to rule out measles infection as a cause of MS (as is postulated in Svenningsson *et al*[232]). However, since the vaccine is live attenuated virus, there is a possibility that it could persist dormant somewhere in the body and later cause disease. It is also possible that physiological changes induced by the vaccine, such as a humoral or cellular immune response, could contribute to the etiology of MS.
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
Several investigators have looked into the possibility of immune cell cross-reactivity or molecular mimicry triggered by measles infection. Matossian-Rogers *et al*[233] found T cell lines cross-reactive to MBP and measles virus in healthy controls, but in Richert *et al*[234] and Pette *et al*,[235] no measles-specific T cell lines from MS subjects or healthy controls showed any reactivity to MBP. Also, in Jingwu *et al*[236] and in Rubio and Cuesta,[237] no antibody cross-reactivity was found between MBP and measles virus.
Conclusion:
Higher prevalence and titers of anti-measles antibodies have been found in MS subjects vs. controls; however, similar findings have also been reported for other pathogens. Due to vaccination programs, measles infection has nearly been eliminated in many countries where MS prevalence rates are the highest (for example, the US). This would suggest that, were the measles virus an important trigger of MS, incidence rates should have begun to decline in these countries unless the vaccine itself can also trigger MS.

Pathogen: Mumps

Description:

The mumps virus is a paramyxovirus that is found throughout the world and transmitted through close person-to-person contact. Mumps epidemics can occur where vaccination is not practiced. Distribution of the virus from the respiratory system through the bloodstream results in systemic infection involving target organs such as the salivary glands, testes, ovaries, and the pancreas. The central nervous system can also be affected through the development of mumps meningitis, or more rarely, mumps encephalitis. Approximately one-third of mumps infections are subclinical. A vaccine for mumps was licensed in the US in 1967; since then the incidence of reported clinical mumps infection in this country has declined by 99%.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

Only one study in this category was identified;[238] it failed to find mumps RNA in MS brain tissue.

Does immune response to pathogen differ between MS subjects and non-MS subjects?

Evidence is mixed:

- A few studies of antibody titers showed elevated mumps titers in MS serum or CSF samples compared with those taken from controls (e.g., Berr *et al*[239]). Likewise, a few analyses of MS CSF samples found evidence of intrathecal antibody production, generally in a minority of MS subjects (e.g., Sindic *et al*[240]).
- Studies involving leukocyte migration, lymphocyte blast transformation, rosette formation, T-cell reactivity and/or interferon production have produced mixed results, with some but not all indicating a reduced immune response in the case of MS subjects (e.g., Tovell *et al*,[13] Ilonen *et al*[241]).
- A few studies (e.g., Salonen *et al*[224]) found immune response to be correlated either with the presence of exacerbations or with genetic (Dw2) status.

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

- Of the seven epidemiological studies that assessed the age of mumps infection in MS cases vs. controls, five (e.g., Hays[242]) found an older age of infection in MS cases, one found infection by age 7 to be associated with MS,[243] and one found no correlation between MS and age of mumps infection.[231]
- Most studies of past mumps infection have found MS subjects no more likely to have had mumps than controls (e.g., Bansil *et al*[244]).

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

As stated above, mumps vaccination has been practiced since the late 1960's but this has not had a demonstrated effect on MS incidence.

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

Not assessed

Conclusion:

There is no strong evidence to support involvement of the mumps virus in the development of MS. Later age of mumps infection was associated with MS in multiple studies, but this was often also found true for other types of infections in these same studies. Similarly, the immunological differences between MS and control subjects found concerning the mumps virus have also been reported for other pathogens.

Pathogen: Parainfluenza and related paramyxoviruses (6/94 virus, Sendai virus)

Description:

In addition to measles and mumps, other paramyxoviruses have been investigated for a role in MS. These include strains of the human parainfluenza virus (which cause primarily respiratory system symptoms), animal viruses such as Sendai, simian virus 5, Newcastle disease virus, and canine distemper virus (described separately in this document), and the 6/94 virus which was originally isolated from MS brain tissue.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

Evidence concerning the presence of these paramyxoviruses in MS is limited:

- A few groups reported seeing filament-like inclusions in MS brain tissue that were suggestive of paramyxovirus (e.g., Tanaka *et al*[245]); however, other investigators identified them as normally occurring cellular products, such as byproducts of degeneration or postmortem autolysis.[246,247]
- The 6/94 virus was originally discovered through analysis of MS brain tissue samples,[248] but little subsequent research appears to have been performed to determine its prevalence in MS or control brains.

Does immune response to pathogen differ between MS subjects and non-MS subjects?

- Prevalence of antibodies to these viruses has not been determined to be significantly increased in MS subjects vs. controls in either serum or CSF. A few studies of antibody titers to parainfluenza-1 or 6/94 found levels to be higher in MS subjects (e.g., Cernescu *et al*[249]), but others found no such increase (e.g., Whitaker *et al*[250]). One study determined SV5 antibodies to be present in a large percentage of MS CSF samples and that SV5 antigen was able to absorb oligoclonal bands;[251] however, subsequent studies were either not able to replicate OCB absorbance or found it to be a phenomenon not specific to MS.[252, 253]
- A few studies (e.g., Ciongoli *et al*[254]) found a decreased response to parainfluenza or 6/94 virus in MS subjects vs. controls using leukocyte migration or lymphoblast transformation studies. Leukocyte migration and interferon production studies using the Sendai virus did not reveal any difference between MS cases and controls.[13, 255-257] Also, mixed results were reported by two studies of interferon alpha production using Newcastle disease virus.[258, 259]

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

Not assessed

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

Not assessed

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

Not assessed

Conclusion:

The available evidence does not strongly support the involvement of these paramyxoviruses in MS. Follow-up investigations of indications connecting one of these viruses with MS have generally failed to replicate the findings or have shown them not to be specific to the particular virus or to MS.

Pathogen: Rubella (German measles)
Description:
Rubella is a togavirus that causes German measles, a disease characterized by rash and lymphadenopathy. Rubella infection can be transmitted transplacentally and can cause birth defects in a developing fetus. The incidence of rubella infection in the US has declined by approximately 99% since the introduction of the rubella vaccine in 1969.
Is presence of actual pathogen different in MS subjects compared with non-MS subjects?
Two studies that searched for rubella genomic sequences in MS brain tissue failed to detect them.[7, 238]
Does immune response to pathogen differ between MS subjects and non-MS subjects?
• Several studies found evidence for intrathecal production of rubella antibodies, but often detected the production of antibodies to other viruses as well (e.g., Reiber *et al*,[217] Rostasy *et al*[260]). • Two studies found no impairment of rubella-induced inhibition of leukocyte migration in MS subjects;[255, 261] however, two other studies found a decreased response in MS subjects using lymphocyte transformation[241] and interferon alpha production[224] assays. • One additional study[104] found immune complexes containing rubella antibodies in around one-quarter of MS cases (other viruses were also found in these complexes).
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
A few studies found earlier development of German measles to be associated with MS (e.g., Casetta *et al*[230]), others found later infection to be associated with MS (e.g., Compston *et al*[262]), and yet another found no association with age of infection.[231]
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
As with other viruses such as measles and mumps, widespread vaccination has nearly eliminated the incidence of rubella in several countries without a corresponding reduction in risk of MS
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
Not assessed
Conclusion:
The available evidence does not strongly suggest a role for rubella in causing MS. Immune abnormalities involving rubella, such as intrathecal synthesis of anti-rubella antibodies, have also been detected for other viruses and may be a nonspecific feature of the disease.

Pathogen: Vaccinia
Description:
The vaccinia virus is a poxvirus that is used to vaccinate people against smallpox. Serious complications from vaccination are rare but can include CNS effects, most notably encephalitis. Routine smallpox vaccination was ended in the US in 1972, but the vaccinia virus is still used to prepare recombinant vaccines for other diseases.
Is presence of actual pathogen different in MS subjects compared with non-MS subjects?
Not assessed
Does immune response to pathogen differ between MS subjects and non-MS subjects?
• There is evidence of an abnormal antibody response to vaccinia in people with MS, demonstrated both by higher titers in CSF and serum compared to different types of controls,[263] and by a higher prevalence of CSF antibodies and signs of intrathecal production in MS subjects (e.g., Cremer *et al*[264]). • Regarding cellular immunity, one study showed a weaker lymphocyte transformation response in MS cases vs. healthy controls[265] but two other studies found no impairment in migration inhibition or lymphocyte proliferation.[266, 267]
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
Not assessed
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
Not assessed
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
Not assessed
Conclusion:
Although vaccinia antibodies have been detected more frequently and at higher levels in MS cases than controls, this is also true of other viruses. The fact that routine vaccinia vaccination was discontinued in 1972 but nonvaccinated people continue to be diagnosed with MS also points away from a significant causal role for this virus in MS.

Pathogen: Varicella zoster

Description:

Varicella zoster virus (VZV) is the cause of chickenpox and historically has been encountered by most susceptible individuals in childhood, although a vaccine has recently been introduced to prevent infection. In unvaccinated people, the initial infection develops and resolves over a period of a few weeks. The course of the infection includes initial replication in the oropharynx, dissemination throughout the body by viremia, and development of a characteristic rash. VZV may subsequently persist in the dorsal root ganglia. If reactivated, VZV can cause herpes zoster, also known as shingles; this mainly occurs in older adults.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

- A handful of studies have looked for VZV genetic sequences in brain tissue or blood; most found either no evidence for viral DNA or similar prevalence rates in MS cases and controls (e.g., Challoner *et al*[β], Martin *et al*[β1]).
- An analysis of CSF detected VZV DNA in 31.6% of MS subjects (43.5% of RRMS subjects) compared with 10.7% of subjects with other neurological diseases.[44] However, another study of CSF samples taken from MS subjects at their first demyelinating event and from neurological controls found no samples positive for VZV DNA.[32]
- One study[268] found the presence of VZV DNA in blood cells only in MS subjects experiencing a relapse, but not in MS subjects in remission nor in controls. A follow-up study reported similar results, with VZV DNA detected in PBMCs of 95% of MS subjects in relapse, 17% of MS subjects in remission, and no controls.[48] Further study detected VZV DNA and viral particles identical to VZV in the CSF of MS subjects during relapse, which decreased and/or disappeared during remission.[269]

Does immune response to pathogen differ between MS subjects and non-MS subjects?

- Results have been varied on the subject of whether serum titers or seropositivity to VZV is increased in people with MS vs. controls (e.g., Sundstrom *et al*,[57] Myhr *et al*[β70]). However, the prevalence of CSF antibodies may be higher in MS subjects,[271] and multiple studies have found evidence of intrathecal production of antibodies to VZV, as well as to other viruses such as rubella and measles (e.g., Reiber *et al*,[217] Sindic *et al*[β40], Bednarova *et al*[β72]).
- One study of cellular immunity[35] found a lower interferon production response to VZV by lymphocytes of MS subjects compared with controls. However, Brudek *et al* detected an increased production of IFN-gamma in PBMCs of MS subjects compared with controls when stimulated with VZV antigens or VZV combined with HERV antigens.[103]

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

One study describes a north-south diminishing gradient of varicella infection that resembles that which has been found for MS.[273] Another study identified six MS subjects out of 82 who had varicella or zoster concurrent with the development or progression of MS.[274]

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

The effect of anti-herpes treatment on MS disease activity was examined by Bech *et al*,[84] who found that valacyclovir appeared to reduce new active lesion formation in a

subset of subjects who had active lesions at baseline. Another study[275] found improvement in 28% of MS subjects who were administered an attenuated varicella virus vaccine, but this was a short-term uncontrolled trial.
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
A recent study (Brudek *et al*[108]) determined that VZV antigens were capable of inducing reverse transcriptase expression from lymphocytes and that this effect was sustained for longer (> 6 days) than could be achieved with other viral antigens.
Conclusion:
A few associations between MS and VZV have been reported, such as a notably increased prevalence of DNA in MS relapses and presence of VZV antibodies in MS CSF samples. However, at this time, no conclusions can be drawn about any potential role of VZV in the etiology of MS.

III. Bacteria

Pathogen: Borrelia burgdorferi
Description:
Borrelia burgdorferi is a Gram-negative spirochete that causes Lyme disease, a disease that can affect multiple organ systems including the central nervous system. In fact, the neurological symptoms of chronic Lyme disease can closely resemble those of MS, which creates challenges in the differential diagnosis of these diseases. Diagnosis is also complicated by the possibility of false positives and false negatives in detecting antibody responses to B. burgdorferi which is the standard diagnostic approach for Lyme disease. In the US, B. burgdorferi is transmitted by the bite of hard ticks such as the deer tick in the northern and midwestern US and the western black-legged tick in western states. Lyme disease is endemic to those regions inhabited by these ticks, notably wooded areas in the northeast US, the Great Lakes region, and the Pacific Northwest.
Is presence of actual pathogen different in MS subjects compared with non-MS subjects?
Not assessed
Does immune response to pathogen differ between MS subjects and non-MS subjects?
Evidence is inconclusive – several studies have measured the prevalence of antibodies to B. burgdorferi in MS and control subjects, but the results have varied greatly. In some studies, antibodies were found at low frequencies in MS subjects and/or less often than in controls (e.g., Schmutzhard *et al*[276]). In other studies, antibodies were found in a high percentage of MS subjects (for instance, 38.5% according to one study[277]) and more frequently than in controls. Differences in antibody positivity rates between studies may be influenced by overall Lyme prevalence in the geographic regions where these studies were performed and/or differences in lab methodologies.
Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?
Not assessed
Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?
Not assessed
If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?
Some studies have found evidence for cross-reactivity between borrelia proteins and neural antigens such as MBP (e.g., Weigelt *et al*[278]).
Conclusion:
Because it is endemic only to certain geographic locations, B. burgdorferi is unlikely to be an MS trigger in all cases. However, assuming that MS is an etiologically heterogeneous disease, in individuals who are predisposed to MS, it is plausible that B. burgdorferi infection may initiate certain pathogenic events leading to MS (see Karussis *et al*[279] which discusses an apparent case of concomitant Lyme disease and MS).

Pathogen: Chlamydia pneumoniae

Description:

Chlamydia pneumoniae (Cpn) is a common obligate intracellular bacteria that causes respiratory tract infections and is transmitted by respiratory secretions. It has been implicated as a risk factor in other diseases, most notably heart disease, but also other neurological disorders such as Alzheimer's disease.

Is presence of actual pathogen different in MS subjects compared with non-MS subjects?

Some positive findings have been reported, but overall the evidence is inconclusive:

- Four studies have searched for the presence of Cpn DNA in MS brain tissue; three reportedly failed to detect any DNA[280-282] but the fourth detected MOMP and 16s RNA DNA in 5 of 8 MS subjects and 3 of 18 OND controls[283]. This last study also performed immunohistochemical staining of brain tissue, and found evidence of staining with three anti-chlamydial antibodies in 7 of 20 MS subjects vs. 0 of 17 OND subjects. (Staining was confined to ependymal surfaces and periventricular regions.)
- Over 20 studies have analyzed the presence of Cpn DNA in MS CSF, with or without an OND control group. Several failed to detect DNA in any of the samples (e.g., Saiz *et al*,[284] Derfuss *et al*[285], Budak *et al*[286], Lindsey and Patel[287]); however, several others were able to detect it in at least some samples. Of those studies that detected DNA and compared its prevalence in MS and controls, some found it to be more common or present only in MS subjects (e.g., Sotgiu *et al*,[288] Hao *et al*[289]), while the rest found it to be equally prevalent in MS subjects and controls (e.g., Contini *et al*[290]). These discrepancies in CSF PCR studies may be due to a number of factors such as variations in the techniques used; currently no standardized process exists (see Yamamoto[291] for a discussion).
- One study using electron microscopy detected structures resembling chlamydial bodies in the CSF of 11/20 MS subjects and 2/12 OND controls[283].
- Studies that have analyzed blood or serum samples have provided mixed results; a recent analysis of PBMCs from 112 MS subjects using real-time PCR detected Cpn DNA in only two subjects, and in these subjects the viral loads were low[292].

Does immune response to pathogen differ between MS subjects and non-MS subjects?

- Several studies have analyzed the prevalence of antibodies to Cpn in MS subjects and controls, usually OND controls, but occasionally healthy controls. In general, these studies have found serum positivity to be equivalent in MS subjects and controls, but results from studies analyzing CSF positivity or intrathecal synthesis were mixed, with some showing higher frequencies in MS subjects (e.g., Krametter *et al*[293]) and others showing similar frequencies in MS and OND control groups (e.g., Sotgiu *et al*[288]). A study that found greater evidence of active Cpn infection in MS cases than controls (see Parratt *et al* below) found serum Cpn-specific IgA to be more prevalent in healthy controls, indicating that IgA is not a reliable indicator of current infection.[294]
- Antibody titers in CSF have generally been found to be higher in MS subjects vs. controls, usually OND controls (e.g., Sriram *et al*[295]), but serum titers have often been found to be similar in MS subjects and OND or healthy controls (e.g., Villoslada *et al*[45], Krametter *et al*[296]).
- One prospective study using serum samples from two cohorts (US Army and Kaiser Permanente) found no association in either cohort between Cpn

seropositivity and risk of MS, but did report an association between serum IgG anti-Cpn levels and MS risk in the Kaiser Permanente cohort[297].

- Serum Cpn-specific immune complexes, a sign of active infection, were found by Parratt *et al* in 24% of MS subjects compared with 16% of other neurological disease controls and 15% of healthy controls (4.7% when residents of a certain town were excluded). The presence of Cpn-specific immune complexes was not associated with MS disease duration, and is therefore probably not simply a function of disease burden. 30%-54% of the subjects who were positive for immune complexes were negative for IgG and IgA antibodies to Cpn.[294]
- Yao *et al*[298] found oligoclonal bands from MS subjects to be partially or completely adsorbed by Cpn antigens but not by a variety of other antigens. However, Derfuss *et al*[285] reported a lack of reactivity of oligoclonal bands to Cpn elementary or reticulate bodies.

Does age of infection or other characteristic of infection differ significantly between MS subjects and non-MS subjects?

Not assessed

Have any therapies aimed at controlling or eliminating the pathogen been found to alter the risk or clinical features of MS?

Sriram *et al*[299] presents a case report of a man with MS who was positive for Cpn DNA and antibodies in his CSF and who experienced improvement of his MS symptoms following anti-chlamydial treatment. A small (8 subject, 14 month) follow-on study of azithromycin and rifampin showed no significant treatment effect on lesion volume or number, but reported a significant increase in brain atrophy in the placebo group[300]. However, a separate study (Woessner et al[301]) found no significant therapeutic effect of a different regimen consisting of three six-week cycles of roxithromycin.

An analysis of medical records of MS cases in the three years prior to MS onset and matched controls did not find a protective effect for antibiotics active against Cpn, but did detect a lower risk for subjects treated with penicillin[302].

If any significant differences appear to exist for any of the above, have cause-effect relationships been established to rule out other types of connections?

- MacIntyre *et al*[303] suggests that Cpn infection of human brain microvascular endothelial cells may alter the permeability of the blood-brain barrier.
- Lenz *et al*[304] reports on potential mimicry between an MBP peptide and a Cpn peptide that can induce EAE in rats.
- Four studies found Cpn DNA positivity in MS to be associated with MRI and/or clinical activity[289, 290, 305, 306]; another found Cpn infections to be associated with risk of MS exacerbation.[307] Whether these findings derive from a causal role of Cpn in the pathogenesis of MS has yet to be determined.

Conclusion:

The available evidence concerning Cpn and MS is quite mixed. Although some studies have linked the presence of Cpn DNA or antibodies in CSF to MS, not all have shown such a connection. Furthermore, the one prospective study conducted[297] to analyze Cpn antibodies prior to MS onset showed only a possible association between IgG levels and risk of MS. However, the presence of chlamydial bodies in the CSF of MS subjects and the higher prevalence of anti-Cpn immune complexes in MS subjects support the possible involvement of this bacterium in MS. Investigations of anti-chlamydial treatments in MS have been limited and have evaluated different treatment regimens.

IV. Other pathogens investigated in MS

In addition to the pathogens presented in the templates above, a number of other pathogens have been evaluated for an etiological role in MS but to a lesser degree. For each of the pathogens listed below, only a few studies (often only one or two) could be found that evaluated experimental evidence for its involvement in MS. Interestingly, a few of these infectious agents (such as hepatitis B virus and helminths) have been proposed to have a protective effect against MS, perhaps by mediating a shift in the T cell repertoire towards a Th2 profile.

Our infectious agents studies spreadsheet (which can be downloaded at www.acceleratedcure.org/downloads/phase2-infectiousagents-studies.xls) contains a listing of the studies found to date for each of these pathogens.

Viruses:

- Acute human encephalomyelitis virus
- Adult T-cell leukemia (ATL) virus
- BK virus
- Borna disease virus
- Bovine leukemia virus
- Caprine arthritis encephalitis virus
- Enteroviruses
- Hepatitis B
- Hepatitis C
- Hepatitis G
- Human herpesvirus 1
- Human herpesvirus 2
- Human herpesvirus 7
- Human herpesvirus 8
- Human herpesvirus 7
- Human herpesvirus 8
- Human papilloma virus
- Human parvovirus B19
- Inoue-Melnick virus
- Influenza virus
- Lipovnik virus
- Maedi-Visna virus
- Marek's disease virus
- Parvovirus B19
- Poliovirus
- Respiratory syncytial virus
- Simian immunodeficiency virus
- Simian virus 40

- Spumavirus/spumaretrovirus
- Torque Teno virus
- Vesicular stomatitis virus

Bacteria:
- Acinetobacter
- Bacillus Calmette-Guerin
- Bifidobacterium
- Bordetella pertussis
- Clostridium tetani
- Corynebacterium diphtheriae
- Enterococcus
- Escherichia coli
- Helicobacter pylori
- Hemophilus influenzae
- Microsporidia
- Mycobacteria bovis
- Mycobacteria leprae
- Mycobacteria tuberculosis
- Mycoplasma pneumoniae
- Parachlamydia-like organisms
- Pseudomonas aeruginosa
- Staphylococcus/S. aureus
- Streptococcus

Fungi:
- Candida albicans

Protozoa:
- Plasmodium falciparum
- Toxoplasma gondii

Helminths:
- Toxoplasma gondii
- Trichuris trichiura

V. Conclusion

The information presented in the templates above show that although many different infectious agents have been studied, some quite extensively, for a potential role in MS, currently no specific pathogen can be conclusively labeled as an MS trigger. There are many challenges inherent in identifying the triggers of a disease which may take many years to manifest itself and which may require

the contributions of multiple etiological factors. Assuming that MS is a multifactorial and heterogeneous disease, it is likely that the evidence implicating infectious triggers of MS will not be clear cut, will be opposed by conflicting findings, and will require careful interpretation. It is also possible that identifying and confirming infections triggers of MS will require the use of new investigative techniques and expanding the scope of research to pathogens not previously evaluated in MS.

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