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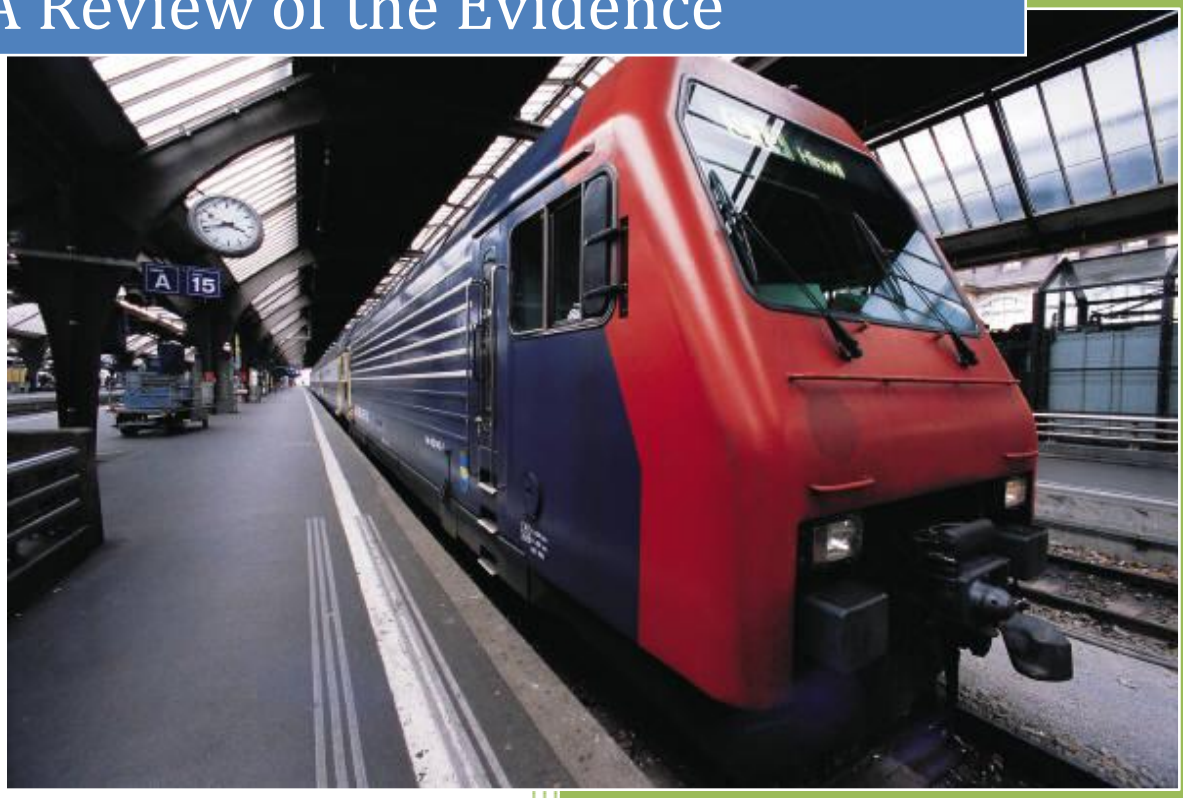
Epstein-Barr Virus and Multiple Sclerosis: A Review of the Evidence



STEVEN ROSNER

Epstein-Barr Virus and Relapsing Remitting Multiple Sclerosis (RRMS)

A Review of the Evidence

THIS IS A MAJOR CHANGE TO VERSION 1 (1/1/2019)

Steven Rosner

July 1, 2022

Version 4

VERSION 4: UPDATE INFORMATION

As you will see shortly, this review finds there is not the remotest evidence that the Epstein-Barr virus (EBV) is a major factor in contracting MS, and concludes there is no relationship that EBV precedes MS.

However, recent reviews of other "supposed causes," such as auto-immune, Sunlight/Vitamin D deficiency and Chronic Cerebral Spinal Venous Insufficiency (CCSVI), has convinced the author that Primary Progressive MS (PPMS), which affects 10-15 percent of the MS population, is a totally separate disease, and seriously confounds virtually every MS study—EBV or otherwise—and is likely the major reason (but far from only) that so little progress has been made in determining the cause of MS over the last 50 years.

Accordingly, opinions, assertions and conclusions within this review only relate to Relapsing Remitting Multiple Sclerosis (RRMS)—which affects 85 percent of those diagnosed with MS—using erroneous research standards.

As to the cause of Primary Progressive MS, the author has no strong opinions one way or another, but senses it is likely caused by a pathogen, a vitamin deficiency.

The paper has been modified, consistent with this new belief. If the author misses one or two references, the reader is advised they will be updated when he becomes aware of it. Needless to say, citations from studies that use PPMS in their text will not be modified.

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V4.0: July 1, 2022

DEDICATION

Audrey Rosner - Wife of 16 years
Afflicted with MS

When I married Audrey 16 years ago, she already had MS. So I did not know her when she went kayaking, did the triathlon, played fiddle in a band, and incredibly gifted, designed covers at McGraw Hill and other publishing houses. We met over the internet; she lived in Chicago, I, of all places, Des Moines, Iowa. At first sight she was coming down the escalator at the airport, looking just like you and me. Only when she got to the bottom and began walking did I see what the disease had done. In Chicago going to work every day, she needed a cane, sometimes two when it snowed, but wanting, I suppose, to make good impression, she eschewed it. It was somewhat of a risk, since she could have lost her balance, but fortunately we retrieved her luggage, I got my car, and we made it back to my apartment without incident. As for me, I resolved even before we met, not to stare, and to treat her as if she was perfectly fine.

16 years later, I did such a good job that I must admit most of the time I was totally oblivious to the struggle she goes through *every single day*; the difficulty walking; the numbness in her leg—sometimes her hand; the fatigue that prevents her from doing more around the house—often needing to go to bed by 7 PM—and especially, being unable to work at any sustained project, which she feels very guilty about. Then there are numerous trips to neurologists, ophthalmologists, and all sorts of other practitioners; some practicing alternative medicine, in the hope they can help her. . . . But despite everything, she just doesn't give up. Every day, she searches for an answer. . . .

Thank you, Audrey, for teaching me the most important lesson of all—compassion!

Mehrdad Golzad, MD, Neurologist

Elmhurst NY

Dr Golzad is an amazing neurologist, besides being brilliant, truly cares about his patients, even to the extent of researching alternate and less expensive ways for his patients to get their medicine. Having debilitating migraines for decades, he was the only doctor to figure out what might help. We tried several natural occurring substances, and after a bit of experimentation, one 400 mg magnesium tablet daily reduced my headaches by 80 percent.

Also, unlike most doctors, Dr. Golzad listens, takes into account what his patients say and asks his patients for feedback. When I wrote to him and mentioned a Yiddish expression, "“Don't ask the doctor—ask the patient,” He wrote back, "Dear Steve . . . . I totally love that last sentence: 'Don't ask the doctor—ask the patient.' As doctors, we always learn from our patients and this was obviously the case when you explained to me how you were using Toradol ampules for nasal administration. I already ordered a pack to have here and try on patients who come here with severe headaches. . . ."

To The Reader

This paper was written to review the theory that the Epstein-Barr virus is associated with contracting MS—some research papers going so far as stating it is a "cause." Before I started working on it, I believed a viral etiology was untenable, so the reader should be aware I am biased. Nonetheless, any bias is hopefully independent of the criticisms of the studies I have reviewed. They are either well-designed or not; conclusions valid based upon the data or not; bias non-existent or quite substantial—in short, whether the studies are good or poor science and can be relied upon. The author is more than happy to let each reader decide for her/himself.

I have no association with any organization, drug company, or medical establishment that might benefit by my conclusion. The study was not commissioned, but rather I took it upon myself to contribute what I can to the MS literature. While not part of the MS establishment or an "academic," with an M.S. in Experimental Psychology, as a writer (three published books since 2015), a systems designer, and author of complex reports in a number of arenas that I knew nothing about initially,[1] I treated this project like others I have taken on during my life. First learn the "language," in this case, the disease's demographics, the immune system, and especially, the basics of epidemiology, before reviewing the research to determine its validity. As it turned out, I deemed the latter two important enough to warrant a section each, which I hope can benefit the lay person as an introduction to that subject matter.

Unfortunately, given the amount of research time, effort, and money put in over the past 50 years relating to MS, the results, in terms of our knowledge of causation and a potential cure, are extremely disappointing! There are several reasons for this, a few which will be discussed in Part V. For now, MS is a problem that can—and must—be solved sooner than later. Every problem has a solution! However, I believe most research has been going down the wrong road for decades. We must start at the highest level possible—the demographics—reject any theory that cannot explain them, and not waste time and money in fruitless research of such theories.

This is written for the MS stakeholder, those with MS, their families, researchers, and medical doctors to challenge the way they think about the disease. This paper may be shared in part or in whole by anyone who might benefit from it. No permission is necessary! It can be downloaded at www.ebvms.com. The author welcomes comments, opinions, editorial mistakes, large or small, inconsistencies and links to new research. I will from time to time update my website after incorporating those I deem important. Please be respectful when you communicate; communication not respectful will be deleted immediately.

Although MS is not my main endeavor, I would nonetheless be happy to give feedback or discuss new research if one thinks I could be of service.

Any opinion expressed and conclusions reached herein are mine and mine alone*! *There is no implication that any of the individuals mentioned in the "Dedication," including my own wife, agree with my conclusions, and/or how I have expressed myself.

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[1] *God and Man: Love on the Rocks* (2018)—Award Finalist (Religion) in the Independent Author Network (IAN) 2018 contest.
God is Good (2016)
A Guide to the Psalms of David (2015)
Leading the Lambs to Slaughter: (Kings County Housing Court: Solutions for a Dysfunctional System) (2014)
Safety Issues, Inefficiencies & Inequities in the Taxi Industry in the City of Raleigh (2004)
*Anatomy of an outrage:*Iowa Federation of Families for Childrens Mental Health-loss of block grant funding (2004)
Critque of the Iowa Mental Health and Developmental Disabilities Commission's *MHDD System Redesign Report*-
An Alternative Approach (Submitted to the 80th Iowa General Assembly: 2004)
A Guide to Mental Health and Developmental Disability in Iowa (2003)
Co-author: Prototype *Fire Monitoring and Control System* software for high-rise office buildings in NYC (1978)

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Part V; Conclusion and Recommendations

Appendices

Introduction:

Multiple Sclerosis is a disease of the Central Nervous System in which the myelin sheath covering of nerves is compromised or destroyed, and which causes a variety of symptoms all over the body. It has been around for over 100 years. Yet to-date, there is no known cause—let alone a cure. Indeed, the number of theories over the years as to its cause easily number over two dozen. Some are fringe theories,[2] often in the form of a book, usually written by an individual who has MS and has supposedly "cured" him/herself.

By far, the most prevalent theory is that MS is an auto-immune (AI) disease, and the most common treatment is based upon that assumption, largely consisting of drugs curtailing or moderating the immune system so it less likely to attack the myelin of its own body. Arguably, the second most prevalent theory is that MS is caused by a virus—in opposition to the auto-immune theory. The viral theory actually preceded AI, and much research was done early on, but for reasons too detailed to go into, AI grabbed the spotlight and still holds it as of this writing.

Still, there is an enormous amount research going on related to a viral cause. While any number of known viruses have been proposed as the cause, by far, most research—literally hundreds of studies—relates to the Epstein-Barr Virus (EBV)—discovered around 1964.

This paper has five parts sub-divided into chapters. In Part I, Chapter 1 reviews the history, symptoms, and treatment of MS; Chapter 2 reviews what we know demographically about its prevalence, based upon gender, location, age, etc.; and Chapter 3 discusses the nature of a virus, in general, and EBV in particular.

Part II contains two "mini courses"—knowledge to make sense out of the EBV research relating to its proposed association to MS. Chapter 4 consists a fairly detailed description of our immune system, so that readers may be able to understand studies that are done at the cellular level, while Chapter 5 reviews "Observational" research used for studying various topics; otherwise known as *epidemiological* studies when it relates to diseases. This type of research can provide associations, correlations and clues for further directions, but by itself, can never prove X "causes" Y.

Part III evaluates four studies that are "pro" EBV-MS, each under a separate chapter (6-9) for the readers' convenience. Because of the author's belief, as stated earlier, that MS is not caused by a virus, he did not pick out random studies that could be easily critiqued, but chose the first three studies cited by an expert in the field, who also happens to have contracted MS. We refer to George Jelinek, MD,

[2] Fringe is used only in the sense that the theory is discounted by the medical establishment and little, if any, research has been done related to it.

and his book "Overcoming Multiple Sclerosis" in which he discuss a possible viral cause.[3] Knowledge of research techniques and the immune system of Part II will be critical for the reader to evaluate the studies, or better, evaluate the author's evaluation.

Part IV reviews several studies (Chapters 10-11) in opposition to the EBV-MS theory—that found no relationship between the two, as well pointing out some of the flaws in previous studies that claim a connection.

Finally, Part V reviews all the evidence, brings additional factors to bear, reviews the findings of the Tisch Research Center for MS in NYC, led by one of the foremost authorities of the disease, and makes suggestions for future research, and what changes of venue and/or direction might be helpful.

Several appendices list all the known human viruses, classification based upon the Baltimore System viral types, and a Chi Squared table to measure significant levels for one of the studies we review. An abbreviated glossary is added at the end, which we hope will be useful for lay readers.

It is hoped this paper will clarify some of the confusion in the MS universe, and provoke researchers and decision makers to rethink their approach to this debilitating disease, which seems to be increasing by leaps and bounds.[4]

[3] Jelinek, George, *Overcoming Multiple Sclerosis*, (Crow's Nest, Australia: Allen and Unwin, 2009) Page 259.
[4] For example, see: https://www.ncbi.nlm.nih.gov/pubmed/28087187 and https://www.ncbi.nlm.nih.gov/pubmed/27682228

PART I

Background

Chapter 1

Multiple Sclerosis: (MS)

Multiple Sclerosis (MS)

Multiple Sclerosis appears to be a disease of relatively recent origin (125+ years or so). Lack of an historical record, coupled with an almost endless array of symptoms has made it extremely difficult to diagnose. Even with today's modern technology, unlike cancer, pneumonia or tuberculosis, there is no *fool-proof* scientific method for doing so. Too often, MS is the "other" category. That is, if a person's symptoms cannot be explained by another disease, he/she is diagnosed with MS.

Symptoms[5]

We do know it is largely a disease of the Central Nervous System (CNS), but other than that, a person with MS can have almost any neurological symptom, with autonomic, visual, motor, and sensory problems being the most common. Specific symptoms are the presumed result of the locations of lesions within the nervous system, and may include loss of sensitivity or changes in sensation such as tingling, pins and needles, or numbness in extremities; muscle weakness, muscle spasms, and difficulty in moving; coordination and balance problems; speech or swallowing difficulties, visual problems (blurred vision, optic neuritis or double vision); fatigue, acute or chronic pain; and bladder and bowel difficulties; and difficultly sleeping. Foggy thinking and emotional problems, such as depression and mood swings, are fairly common. Uhthoff's phenomenon, which is a worsening of symptoms during high temperature, exercise, fever, or Lhermitte's sign, an electrical sensation that runs down the back when bending the neck, are common as well.

In 85 percent of cases, MS begins as an isolated symptom or group of symptoms. Of those, about 45 percent are motor or sensory problems, 20 percent are optic neuritis, and 10 percent are symptoms related to brainstem dysfunction.

The course of the disease seems to evolve in two main patterns: either as episodes of sudden worsening that last a few days to months (called relapses, exacerbations, or flare-ups) followed by improvement (85% of cases), or as a gradual worsening over time without periods of recovery (10–15% of cases).

Relapses are usually not predictable, occurring without warning. Some relapses, however, are preceded by several common triggers and they seem to occur more frequently during spring and summer. Infections, such as the common cold, influenza, or gastroenteritis increase the risk of relapse,

[5] Much of the following are excerpts from Wikipedia: https://en.wikipedia.org/wiki/Multiple_sclerosis

as does acute psychological stress, and even accidents. Women with MS who become pregnant experience fewer relapses—although pregnancy does not seem to improve long-term disability.

Pathophysiology

The three main characteristics of MS are formation of lesions (also called plaques) in the Central Nervous System, inflammation, and the destruction of neurons. These features interact in a complex and not fully understood manner to produce the breakdown of nerve tissue that turn into the signs and symptoms of the disease.

The name itself "Multiple Sclerosis," is totally descriptive, referring to the scars (*sclerae*–better known as plaques or lesions) that form in the nervous system. More specifically, MS usually involves the loss of *oligodendrocytes*, which are cells responsible for creating and maintaining a fatty layer—the *myelin sheath*—which protects nerves and is necessary to propagate the electrical signal of neurons. When the myelin is compromised, a neuron can no longer effectively conduct electrical signals. MS causes a thinning or complete loss of myelin and as the disease advances, even breaking down the axons and neurons themselves. Lesions that are most prominent affect the *white matter* in the optic nerve, brain stem, basal ganglia, and spinal cord, or white matter tracts close to the lateral ventricles,[6] whose function is to carry signals to grey matter, where the bodily functions are "controlled." The peripheral nervous system doesn't seem to be involved.[7] Often, a repair process, called remyelination, takes place in the early phases of the disease, but only partially.[8] Repeated attacks lead to successively less effective remyelination, until a scar-like plaque is built up around the damaged neurons.

History

In 1868, a famous French neurologist, Jean-Martin Charcot, known as the "father of neurology" first lectured on the symptoms of MS and gave it a name, Multiple Sclerosis or "many scars." He had a young woman patient with tremors, slurred speech and abnormal eye movements. After she died, an autopsy showed her brain had "plaques" similar to what we now know MS patients have.

Famous and respected, MS thus became a *neurological* disease. There is no diagnostic test that can unequivocally state the patient has MS. In fact, only during last decades of the 19th century was MS recognized as a diagnostic category. Myelin, which protects nerve cells, was discovered in 1878 by Dr. Louis Ranvier and could be seen under the microscope, and by the end of the century, careful

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[6] Compston A, Coles A (October 2008). "Multiple sclerosis." *Lancet*. 372 (9648): Pages 1502–17.
[7] Compston A, Coles A (April 2002). "Multiple sclerosis." *Lancet*. 359 (9313): Pages 1221–31.
[8] Chari,DM, (2007) "Remyelination in Multiple Sclerosis." *International Review of Neurobiology*. 79: Pages 589–620.

observation gave us a good deal of general knowledge—such as the disease is more common in women than men, that it is not directly inherited, and that it produces various, sundry and unrelated symptoms in those who have it.

In 1916, Dr. James Dawson at the University of Edinburgh performed detailed microscopic examinations of the brains of patients who had died with MS. Dawson described the inflammation around blood vessels and the damage to the myelin—but at the time, scientists had no idea of the reason for their destruction.

Over the years, theories for the cause of MS included "toxicity," and deficient circulation. The latter resulted in stimulating blood flow, including prescribing blood thinners and drugs to dilate blood vessels, but without real effect.

Finally, around 1950, steroids, such as Cortisone, were used to shorten relapses and reduce pain, but we now know long-term steroid effects are severe. It was in the 1960s, that scientists posited the immune system attacked the myelin coating of the nerves, and thus put MS in the category of an "auto-immune" disease; that is, the cells and proteins of the body's immune system, which normally defend it against infections, "abandon" their post, if you will, and enter into the brain and spinal cord destroying the myelin.

When in the late 1970s, Magnetic Resonance Imaging (MRI) was discovered, the brains of those with presumed MS could be seen, showing the damage to nerve cells, even when there were no outward clinical symptoms.

By the 1990s, drugs for MS "exploded" on the scene. First, Interferon[9] based drugs, an injectable drug used to treat cancer, was approved as a treatment for relapsing-remitting MS (RRMS) by the FDA. Virtually every year after, another drug claiming to benefit patients was approved. Most medications were developed to deflect the way the immune system attacked its own healthy tissues. Early drugs induced the immune system to attack what is injected, instead of the supposed attacking of healthy myelin. This despite the fact the specific triggering mechanism that modifies antibodies to attack its own healthy tissue was and remains unknown—assuming there even is one. Unfortunately, this treatment option leaves the body with a significant reduction of defenses against other invaders.

As of this writing, there still no known *cause*, let alone a *cure*, for multiple sclerosis. Treatments attempt to improve function after an attack, relieve symptoms, and prevent new attacks. Medications

[9] See Glossary

used to treat MS, while intermittently effective, can have serious side-effects and may be poorly tolerated. Physical therapy is said to be of help by some; as well as exercise, and dietary changes.[10]

Many people pursue alternative treatments, despite a "scientific" lack of evidence of benefit. The long-term outcome is difficult to predict, with better outcomes more often seen in women, those who develop the disease early in life, those with a relapsing course, and those who initially experienced few attacks.

Diagnosis

The major clinical measure of disability and severity is known as the Expanded Disability Status Scale (EDSS),[11] developed by a neurologist, John Kurtzke. It is widely used in clinical trials and in the assessment of people with MS. The EDSS scale ranges from 0 to 10 in 0.5 unit increments that represent higher levels of disability. Although the scale takes account of the disability associated with advanced MS, most people will not reach the higher scores. Nor is clinical data for a diagnosis sufficient if an individual has had separate episodes of neurological symptoms as described above.

For those who seek medical attention after only one attack, other testing is needed for the diagnosis. The most commonly used technological diagnostic tools are neuroimaging, analysis of cerebrospinal fluid and evoked potentials.[12] Magnetic resonance imaging (MRI) of the brain and spinal cord may show areas of demyelination, testing of cerebrospinal fluid obtained from a lumbar puncture can provide evidence of chronic inflammation in the central nervous system, while potential brain deficiencies can be examined using visual and sensory evoked potentials.

Despite the important information the above supply, there is still no single test that can provide a definitive diagnosis of the disease.

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[10] Jelinek: Page 276.
[11] https://en.wikipedia.org/wiki/Expanded_Disability_Status_Scale
[12] Evoked potential (EP) tests measure the electrical activity of sensory nerve pathways in the brain. They can detect the slowing of electrical conduction caused by demyelination along these pathways.

Chapter 2

Demographics

Demographics

Like all statistics, those especially related to MS have to be taken with a grain of salt. The major reason for this has to do with symptoms. Because the cause is unknown and the symptoms so incredibly varied and disconnected from one another, diagnosing MS is more of an art than a science. Different countries will no doubt emphasize different diagnostic tools, and in a country as large as the United States, it is not hard to imagine that similar symptoms will have differential diagnoses depending on the type and location of the doctor, whether neurologists, GPs, or of some other specialty, and/or whether their practice is in large cities, versus towns, villages and rural areas.

Nonetheless, we know more about the demographics of MS than virtually anything else about the disease. *This cannot and should never be underemphasized!* To find the cause—a failure for over 125 years—let alone a cure, any theory worth pursuing must take into account the MS demographics and be consistent with what we know. Regardless of the number of studies of any one theory. . . however detailed or statistically significant. . . however propounded by the most recognized authorities. . . in the most prestigious institutions. . . if the theory *cannot* explain why there are twice as many cases in region X than in region Y, or why women get MS 2-3 times more than men, it should be rejected—it could not be one of the causes!

With that caution, it is reported that in 2015, about 2.3 million people were affected globally with rates varying widely in different regions and among different populations, with Canada having over 290 incidents per 100,000.[13] Other largely agreed to demographics include:

- The disease is most prevalent between the ages of 25 and 50.
- It is at least twice as common in women as in men.
- Despite the above, the severity of the disease and the prognosis is significantly worse for men.
- The disease is not contagious or directly inherited.
- MS is far more common in people who live farther from the equator—that is in colder climates—although exceptions exist.
- It appears that people who move to a different region of the world before the age of 15 acquire the new region's risk of MS. (There is some evidence that the effect of moving may still apply to people older than 15.)

[13] https://www.healthline.com/health/multiple-sclerosis/facts-statistics-infographic#1

- Race:
    - (a) White people, particularly those of Northern European descent, are at highest risk of developing MS. People of Asian, or Native American descent have the lowest risk.
    - (b) For years it was thought Caucasians have a higher risk of MS than Afro-Americans, but recent studies question that assumption and report Afro-American women have a high risk.[14]
- Family history. If one of the parents or siblings has had MS, one has a higher risk of developing the disease.
- During pregnancy, symptoms of MS remain constant—i.e., are in remission.
- Statistics understates its prevalence, especially in recent years.

Besides the above, there is reason to believe the incidence of Multiple Sclerosis is increasing exponentially. Especially important, some recent studies have shown that both the incidence of MS, and the ratio of woman to men with MS have increased dramatically over the last 50-60 years.[15]

In fact, in an MS Hope for a Cure symposium April 7, 2019, Adam Kaplan, MD pointed out in his presentation that according to the population of the U.S. and the number of cases of MS, there are now over 300 per 100,000. I personally verified that is what he stated, when he showed me how he arrived at that figure. (Dr. Kaplan is, among other things, an Assistant Professor of Psychiatry, and Chief Psychiatric Consultant, at the Multiple Sclerosis Clinic, Department of Neurology at John Hopkins University School of Medicine.)

[14] See https://about.kaiserpermanente.org/our-story/news/in-the-news/black-women-have-higher-incidence-of-multiple-sclerosis-than-whites AND https://www.verywellhealth.com/racial-differences-in-multiple-sclerosis-2440698

[15] Rojas JI, Patrucco L, MIguez J, Sinay V, Cassara FP, Cáceres F, "Gender ratio trends over time in multiple sclerosis" patients from Argentina. *J Clin Neurosci*. 2017 Apr;38:84-86. doi: 10.1016/j.jocn.2016.12.030. Epub 2017 Jan 10.

Chapter 3

Viruses and EBV

Introduction

One of the major theories of MS is that it is caused by a virus, specifically one known as Epstein-Barr (EBV). Hundreds of research studies have been done over the last 50 years attempting to prove that relationship, with conflicting results—although it appears the majority have concluded there is some sort of relationship between the two.

Part III and IV will review studies both pro and con for EBV-MS, and in Part V the author will show why he believes a causal relationship is implausible. In this section, our goal is to try to educate the reader about viruses in general and Epstein-Barr in particular, and let the other parts speak for themselves, other than stating for the first, but by no means last, time, that all EBV-MS studies, by nature, are "observational," or more precisely, fall into the category of *epidemiological* research.

Accordingly, it is extremely important the reader understand that this type of research can never scientifically prove *causal* relationships. At best, it can show "associations" or "correlations" between variables which can be quantified. To put it another way, epidemiological studies alone cannot state A *causes* B. For B can be the cause of A, or just as likely, C, unknown and unaccounted for, could cause A *and* B! Keeping that in mind, following is a brief summary of what we know about viruses and EBV in particular.

Viruses[16]

A virus is a small infectious agent that can only replicate inside the cells of an organism. Accordingly, most scientists do not consider it a *living* entity. They can infect all types of life forms, from animals and plants to microorganisms, even bacteria. They depend on their host cell's metabolism for energy, enzymes, and precursors, in order to reproduce. As such, viruses do not *code*[17] for many of their own viral proteins, but rather, they use the host cell's machinery to produce the viral proteins they require for replication.

While not inside an infected cell or in the process of infecting a cell, viruses exist in the form of independent particles. These viral particles, also known as *virion*s, consist of: (1) either DNA or RNA, long molecules that carry genetic information; (2) a protein coat, called the *capsid*, which surrounds and protects the genetic material, and in some cases (3) an envelope of lipids that surrounds the protein coat. The shapes of virions range from simple helical and icosahedral[18] forms for some species to more

[16] Excerpted from: https://en.wikipedia.org/wiki/Virus
[17] That is, they are not genetically able to reproduce some of their DNA or RNA.
[18] An object of three dimensions, with 30 edges and 20 faces.

complex structures for others. Most virus species have virions that are too small to be seen with an optical microscope. On average, they are about one hundredth the size of a typical bacterium.

Viruses have nucleic acid *genomes*[19] based on the same genetic code that is found in our cells. They also have genetic variations and can evolve. We, like all other cell-based life, use DNA as our genetic material. Viruses, on the other hand, may use either RNA or DNA, both of which are types of nucleic acid.

We often think of DNA as double-stranded and RNA as single-stranded, since that is typically the case in our own cells. However, viruses can have all possible combinations of strandedness and nucleic acid type (double-stranded DNA, double-stranded RNA, single-stranded DNA, and single-stranded RNA).

The viral lifecycle is the set of steps in which a virus recognizes and enters a host cell, "reprograms" the host by providing instructions in the form of viral DNA or RNA, and uses the host's resources to make more virus particles (the output of the viral "program").

While their origin is unclear, they are an important means of horizontal gene transfer, which increases genetic diversity. They infect humans several ways. Influenza viruses are spread by coughing and sneezing. Herpes viruses are spread through oral and genital contact, while HIV is one of several viruses transmitted not only through sexual contact, but exposure to infected blood. The variety of host cells that a virus can infect is called its *host range*. It can be narrow, meaning a virus is capable of infecting few species, or broad, meaning it is capable of infecting many. While the method(s) viruses use to penetrate into our cells and/or evade our immune system are intrinsically interesting, they are beyond the scope of this paper.

Viral infections in animals provoke an immune response that usually eliminates them. Immune responses can also be produced by vaccines, which confer an artificially acquired immunity to specific viral infections, and several antiviral drugs have been developed that are reasonably effective. Some viruses, including those that cause AIDS and viral hepatitis, indeed EBV, can often evade the immune responses and result in chronic infections. Viruses are classified differently by different organizations and different criteria. For our purpose we will use the Baltimore Classification System that places viruses into one of seven groups depending on a combination of their nucleic acid composition (DNA or RNA), strandedness (single-stranded or double-stranded), the nature of their roles, and method of replication. They are summarized in Table 1, while Appendix II classifies them by name.

[19] Genetic material of an organism. It consists of DNA (or RNA in RNA viruses).

Table 1

Baltimore Classification System of Human Viruses

	Name	Description	Human Diseases
I	dsDNA	Double Stranded DNA	EBV, Herpes, Chicken Pox
II	ssDNA	Single Stranded RNA	
III	dsRNA	Possess Double-stranded *genomes*	Rotavirus
IV	(+)ssRNA	Single Stranded RNA genomes (+)	Polio, Hepatitis C, Yellow Fever, Rubella
V	(-)ssRNA	Single Stranded RNA genomes (-)	Ebola, Influenza, Measles, Mumps, Rabies
VI	ssRNA-RT	Single Stranded RNA via DNA	HIV, Retroviruses
VII	dsDNA-RT	Double Standard DNA via Reverse Transcription	Hepatitis B

There are close to 100 human viruses that have so far been indentified. (See Appendix I.) While there are always dozens of viruses in our body[20] (studies have shown as many 40 percent of humans have about 40+ different viruses running about at any given time) hoping to wreak havoc, our immune system does a phenomenal job seeking them out and destroying them, or at the very least, deactivating their potential for harm.

Epstein–Barr Virus (EBV)[21]

EBV is one of eight known human virus types in the *herpes* family, and is one of the most *common.*[22] It is best known as the cause of *infectious mononucleosis*. It is also associated with various non-malignant, premalignant, and malignant lymphoproliferative[23] diseases such as Burkitt's lymphoma, Hodgkin's lymphoma, and non-lymphoid malignancies such as gastric cancer and conditions associated with human immunodeficiency such as central nervous system lymphomas. [3] It is thought to increase risk of developing certain autoimmune diseases, [7] such as systemic lupus erythematosus, and rheumatoid arthritis. The above is only a partial list of diseases EBV is implicated in. Infection with EBV occurs by the oral transfer of saliva and genital secretions. Most people become infected with EBV and gain adaptive immunity. In the United States, about half of all five-year-old children and about 90% of adults have evidence of previous infection.[14] Childhood infections usually cause no symptoms or are indistinguishable from other mild, brief illnesses. When infection

[20] See: https://viralzone.expasy.org/

[21] Excerpted from https://en.wikipedia.org/wiki/Epstein-Barr_virus. For easier reading, the author has skipped text not critical or relevant, without placing the usual three to four ellipses (. . . .) normally used.

[22] When quoting a study or Wikipedia, unless specified otherwise, the emphasis are the author's.

[23] Malignant diseases of the lymphoid cells that usually occur in people with compromised immune systems, such as those with AIDS or individuals with recent transplants.

with EBV occurs during adolescence, it can cause infectious mononucleosis up to 50 percent of the time. [16] Contracted as an adult, it may cause fatigue, fever, inflamed throat, swollen lymph glands, enlarged spleen, swollen liver, or rash. [17] EBV infects B-Cells of the immune system and *epithelial*[24] cells. The mechanisms for entering these two cells are different and beyond the scope of this paper, other than to note once the virus enters the cell, its capsid dissolves and its *genome*is transported to the cell nucleus. Once EBV's initial infection is brought under control, EBV latency persists in the B-Cells for the rest of the individual's life. [13]

History

The Epstein–Barr virus was named after Michael Anthony Epstein and Yvonne Barr, who discovered and, in 1964, published its existence. [40] [41] In 1961, Epstein, a pathologist and electron microscopist, attended a lecture by Denis Burkitt, a surgeon practicing in Uganda, describing a pediatric form of an “endemic variant” of the disease that bears Burkitt’s name. In 1963, a specimen was sent to Middlesex Hospital to be cultured. Virus particles were identified in the cultured cells, and cell lines were sent to Werner and Gertrude Henle at the Children's Hospital of Philadelphia, who developed *serological*[25] markers. In 1967, a technician in their laboratory developed mononucleosis and they were able to compare a stored serum sample showing that antibodies to EBV developed. [42] In 1968, they discovered that EBV can directly “immortalize” B-Cells after infection, mimicking some forms of EBV-related infections, [45] and confirmed the link between the virus and infectious mononucleosis. [46]

A particularly complex virus, EBV is not yet fully understood. It is known EBV is composed of two major types, EBV Type 1 and EBV Type 2. Each stems from different genes. As a result, they differ in their transforming capabilities and reactivation ability. Type 1 is dominant throughout most of the world, except for Africa, where they are equally prevalent.

Once an acute infection subsides, EBV can exhibit one of three latency programs: Latency I, Latency II, or Latency III. Each leads to the production of a limited, distinct set of viral proteins and viral RNAs, which reprogram and subvert infected B-Cells to proliferate and bring infected cells to the sites at which the virus presumably persists. Eventually, when host immunity develops, the virus evades detection by turning off most (or possibly all) of its genes, only occasionally reactivating to

[24] One of the four basic types of animal tissue, along with connective tissue, muscle tissue and nervous tissue. Epithelial tissue lines the outer surfaces of organs and blood vessels throughout the body, as well as the inner surfaces of cavities in many internal organs. An example is the epidermis, the outermost layer of the skin.

[25] i.e. The scientific study of serum and other bodily fluids and the diagnostic identification of antibodies in the serum. Serological tests may be performed for diagnostic purposes when an infection is suspected.

produce fresh virions. A balance is eventually struck between occasional viral reactivation and host immune surveillance, removing cells that activate viral gene expression.

EBV and MS[26]

Although the cause of MS is still unknown, for a number of years it was believed that MS was triggered by a viral infection. In the 1960s, it was thought a measles virus was re-activated in adulthood and was the agent that brought about MS. With the "technological revolution" in medicine, immunology, and information services, the number of studies in areas of all diseases has risen exponentially, including those of an MS viral connection—focusing on EBV. Some studies suggest a viral "trigger" could cause a disease, such as lupus, rheumatoid arthritis, and Type 1 diabetes—like EBV, all in the human herpes virus family.

Meanwhile, epidemiological studies suggest that *90 percent of the general population have evidence of exposure to EBV in their blood*; that percentage is closer to 100 among people who have MS. And multiple studies suggest that Epstein-Barr virus, the most common cause of "mono," may play a role in the development of MS.

What mechanism does a virus use to produce the internal changes associated with MS symptoms? Multiple theories abound, including one pointing out that as the structure of EBV is very similar to the proteins in myelin, the EBV virus causes the immune system, especially B-Cells, to proliferate, and they then mistakenly attack the myelin thinking it is EBV.[27]

That idea is quite similar to studies at the University of British Columbia in Vancouver (UBC) focusing on "mono" and MS, to see if EBV can offer clues as to how to diagnose MS earlier and, ultimately, treat it more effectively.

> "Most of us in North America and Europe get mononucleosis as a result of exposure to EBV, typically in our teens or early twenties," says Marc Horwitz, co-leader of the Infection, Inflammation, and Immunity Research Group at UBC. "Anecdotally, we also know that people with MS typically had more severe cases of mononucleosis than those without MS. . . ."
>
> Their group identified several studies that found a link between EBV and the risk for developing relapsing-remitting MS (RRMS), although, the association with primary progressive MS (PPMS) was less clear. These studies suggest that those with RRMS have elevated levels of anti-EBV antibodies[28] in their blood, which could cause their neurologic symptoms. Other studies also found that people with RRMS had higher levels of the human herpesvirus antibody immunoglobulin M,

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[26] An important source for what follows is: "Risk Factors Associated with the Onset of Relapsing-Remitting and Primary Progressive Multiple Sclerosis: A Systematic Review." Kyla A. McKay, Vivian Kwan, Thomas Duggan, and Helen Tremlett: *Biomed Res Int*. 2015; 2015: 817238. Published online 2015 Jan 31. doi: 10.1155/2015/817238 PMCID: PMC4329850 PMID: 25802867 and FN 24.

[27] Jelinek: Page 261.

[28] Proteins used by your immune system to neutralize pathogens such as bacteria or viruses, usually are quite specific to a given infectious agent and serve as finger-prints for future attacks.

and that people with a history of another herpesvirus, commonly known as chickenpox, may be at increased risk for RRMS.

Horwitz and his team believe these findings may indicate that the immune systems of those with MS have a history of "overreacting" to their initial EBV infection, with their B cells (white blood cells that produce antibodies to fight infection) producing higher levels of anti-EBV antibodies than those of people who don't have MS. This can have benefits in fighting infections, but also poses costs in terms of autoimmune diseases such as MS.

"But you still have to be genetically susceptible to MS," Horwitz emphasizes. **"***You're not going to get MS simply because you had EBV when you were younger.* Otherwise, more of us would have MS**.** However, what we think is that EBV may provide a key to a better understanding of MS and how it develops."[29]

A different perspective comes from a study at Queen Mary, University of London of post mortem brains of MS patients, examining areas where neurological damage had recently occurred:

Lead researcher, Dr Ute-Christiane Meier explained: "EBV is quite a clever virus; when it's not growing and spreading it can hide away in our immune cells. . . . In this study we used a different technique which allowed us to detect the virus in the brains of some people affected by MS, even when it was hiding away in the cells. . . "

Dr Meier and her team of collaborators found that, although the virus was not actively spreading, it was releasing a chemical message into areas of the brain nearby. This chemical message—made up of small RNA molecules—was activating the body's immune system, causing inflammation. This damages nerve cells in the brain and results in MS symptoms. Dr Meier continued: "We have to be careful and have to study more MS brains but this is potentially very exciting research. Now we understand how EBV gets smuggled into the brain by cells of the immune system and that it is found at the crime scene, right where the attack on our nervous system occurs. Now that we know this, we may have a number of new ways of treating or even preventing the disease."[30]

Finally, according to studies from the Harvard School of Public Medicine, EBV is different from other viruses in its association with MS. Two findings were that EBV antibodies were significantly higher in people who eventually developed MS than in a matched set of individuals who did not get the disease, and the risk of MS increased significantly following an EBV infection.[31]

Impressive as the above might sound, in point of fact, despite tons of studies of a viral MS connection over the past 50 years, now largely focused on EBV, conclusions are controversial, the various theories of a viral and/or EBV mechanism have not been incontrovertibly proven, and most importantly, as you will see shortly in the following section, epidemiological studies showing associations between two variables are just that. *Cause* and *effect* are pure speculation; that is, there

[29] "What Does the Epstein-Barr Virus Have to Do With MS?" as reported at https://www.everydayhealth.com/multiple-sclerosis/what-does-epstein-barr-virus-have-with-ms/ Quotes from Dr. Marc Horwitz of the University of British Columbia are not individually footnoted.

[30] As reported in https://www.news-medical.net/news/20120106/Study-shows-how-Epstein-Barr-virus-triggers-MS.aspx

[31] As reported in https://www.verywellhealth.com/does-mono-epstein-barr-virus-cause-multiple-sclerosis-2440740

may be a number of reasons why EBV and MS seem to be associated, having absolutely nothing to do with causality.

From the author's point of view, the most amazing fact of these plethora of viral and EBV studies is that after 50 years, so little is still known, and virtually no progress has been made finding undisputed diagnostic criteria—let alone a cause—given it is not unreasonable to suppose there may be any number causes of MS. As far as treatment, none work with the bulk of the MS population—and claims that some reduce MS frequency are controversial and too often have side-effects worse than the disease.[32]

[32] See: https://www.sciencedaily.com/releases/2017/05/170515122201.htm

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PART II

Tools

Chapter 4

The Immune System

Good and Evil

While the world seems incredibly complicated to most people, in fact it is really quite simple. In some cases, a number of "universes" can be reduced to only two elements. In most other cases, four elements (i.e. east, west north, south) are all that is necessary to describe a "universe" that appears unbelievable complex, only because of the way the elements are combined. As an example of the former, the fundamentals of any digital computer system can be reduced to a "bit;" the most basic element of every single computer system. It is either "on" or "off," much like a light bulb. However, if you take two 2 light bulbs and string them together, you can easily see that the possibilities increase from two to four. That is, bulb 1 and 2 are off, both are on, bulb 1 is on and bulb 2 is off, or bulb 1 is off and bulb 2 is on. Now if you imagine 8 bulbs strung together, you would have 256 possibilities—from all bulbs being "on" to all bulbs being "off," with 254 separate sequences in between.

In computer terminology, stringing 8 bits together comprise what is known as a "byte"—which can represent anything from letters of the alphabet, numbers, special characters, functions, and data. By stringing "bytes," you can continue to add indefinitely to the possibilities. So regardless how complex our computer system *seems*—because of its incredible speed—at the basic level it can be reduced to a simple bit; either "on" or "off."

So to in life! All the *billions* of proteins in the animal world stem from only *four* basic *nucleotides*, which combine in a plethora[33] of ways. Like chess, which has only six basic pieces (king, queen, rook, knight, bishop, and pawn), the number of possibilities for any game is countless. And if you think about it for a while, you might realize cellular metabolism and a revolution in a third-world country are no different in terms of "process." In cellular metabolism, an important process known as the "sodium-potassium" pump attempts to keep the relationship between the two elements—sodium inside the cell, potassium outside—within set parameters. If for some reason, however, cells are inundated with sodium and it cannot be pumped out quickly enough to maintain equilibrium, the cell will burst. Likewise, in a country, especially one of tyranny, where the very, very few rich and powerful continually exploit the poor; physically, psychologically and financially; at some point, the country will "burst," so to speak, and a revolution will occur. The poor will get rid of its exploiters, but, alas, invariably the process begins anew, in which a select few of former revolutionaries lust for power and

[33] Every since I saw Steve Martin's, "The Three Amigos," I love that word.

exploit the rest—the French Revolution and Castro's Cuban take-over being only two of hundreds of examples.

While the forces of good and evil hold true in the world at large, in some sense they also do so at the basic cellular level (leaving morality aside for the moment), and is an apt analogy to explain the immune system. Forces of evil in our body are bacteria, viruses and other foreign objects that do not belong there (keeping in mind most bacteria are good and necessary, and even some viruses have a positive effect). These "evil" invaders—however they manage to get into our system—desire to destroy the organism—if one cell at a time.

We fight this evil with the immune system, which is made up of a number of distinct cells and methods, and overall do an exemplary job, considering that despite diseases caused by bacteria and viruses, the average American lives 75-80 years.

Now if we assume law enforcement represent "good," (liberals, this is just an analogy), and the criminal elements represent "evil;" as the former invent or discover new techniques to find and apprehend the "bad guys" in our society, criminals are also active in inventing new techniques to get through those barriers to avoid detection and capture. Similarly, entities such as banks, jewelry stores, internet sites, and others having valuable merchandise or information, devise techniques and technology to prevent crime in their domain, while the "bad guys" try to figure out ways and scams to breach their security. This is exactly what goes in our body; the good guys have techniques to identify and capture these foreign invaders, while the invaders develop their own techniques to prevent identification and continue their destruction. (I'll leave it to philosophers to comment on whether the immune system and/or the foreign invaders have consciousness or a soul.)

Who are these evil geniuses and those geniuses of the immune system in the never ending battle between good and evil, and where do they come from? We will try to answer those questions step by step, so the average reader can better understand subsequent chapters when we discuss those studies that lead many to conclude MS is caused by a virus, such as EBV.

Because good and bad guys stem from the same source—as do human good and evil—we must start with *Chromosomes*. *Chromosomes* are the base, if you will, for genetic material that is passed on from generation to generation. They reside in the nucleus of all cells and consist of DNA, or in some cases RNA, which we have no intention of defining chemically, except to say it has been known for decades that in its make-up and structure, it contains the genetic material passed on to our children and grandchildren.

As “double helix” has been associated with the shape of DNA, we should state helix is simply a three dimensional shape similar to a coiled wire (like a slinky or the railing in a spiral staircase). “Double” simply refers to the fact that each chromosome has a mate exactly alike, and slight changes in its structure, more than anything, accounts for myriad possibilities. In humans, there are 23 *sets* of chromosomes numbered from 1 to 23, making 46 chromosomes in all. Each set is identical except for number 23 which comes from the male and female of our species respectively, and named the Y (male) and X (female) chromosome. Females contain an XX chromosome and males an XY chromosome. Depending which one of the male’s 23rd chromosome is passed on determines the sex of the offspring.

You probably know that a *gene* is the biological molecule that specifically carries this information, resides on the 23 chromosomes, and through a complicated procedure which we won’t go into, is converted, split, and passed on to the offspring. What you may not know is that each human chromosome has anywhere from 50 to 2000 genes—new ones are discovered all the time—and in total, the human has somewhere around 20,000. The combinations that are possible therefore number in the hundreds of millions. However, only about 10 percent of genes in humans are considered “essential” for the species to survive. Where the gene resides on the chromosome is called the *locus*, or *loci* in plural. A simplified analogy is the NYC subway system: each line (F, D, B, 4, 5, and 6) is like a chromosome, and each stop, like a gene.

Most genes have variations—called *allele*s—in their molecular coding or structure that usually have no effect on the organism, but occasionally will result in an observable trait, such as skin pigmentation. These variations are like companies who issue variations of their common stocks, known as class A, B, and C. Each varies slightly, usually not particularly significant. Thus, if the company does well, all will go up; if the company does poorly, all will go down.

Given the number of genes and the new ones being discovered daily (or the discovery that two genes believed different are identical), a project to specifically name and account for each and every one has been under way for years, consisting of characters and numbers, including the chromosome it is found on, the physical position where it resides, a code distinguishing its alleles, if any, and sequence numbers allowing for variations.

It is now time to briefly revisit the bad guys; our focus concentrated on viruses. Most of them—unlike bacteria—are evil. We already mentioned there are close to 100 known viruses that affect humans (see Appendix I), and that at any given time, dozens of viruses are in our body hoping to wreak havoc in one form or another. We discussed the Baltimore System as the most helpful and used classification system, further broken down into “families,” which are primarily based upon whether the

virus is DNA or RNA, and whether it composed of a double or single helix—or more precisely, a double or single strand. (See Appendix II.) Since this paper is about Epstein-Barr, we will mention that EBV is a Baltimore Class I virus known as a Herpes *viradae* (a taxonomist's term for classifying viral families), and is double stranded. We also might mention there are 20 viruses in Class I, under five different family names.

Like the endless battles between husband and wife, Democrats and Republicans, and prosecutors and defense counsels, an internal battle rages every second of every day inside each of us between the viral foreign invaders and our immune system. The latter is victorious 99.9 percent of the time, but unfortunately, the .01 percent of the time in which the invader prevails results in one disease or another; some quite dangerous, even fatal—unless some outside help is given.

This section more any other puts my ability as a writer to the test, for in order for lay stakeholders to understand the detailed EBV-MS studies at the cellular level, one must have some basic understanding of the immune system. And while there is much we know about it, what we don't know is far, far greater, and changes daily. Indeed, our immune system is quite complex with many different players, functions, structures, and processes; with unusual terminology, often confusing and in flux. The author could not find one source that could explain the immune system logically so that it would be understandable to him in one or two readings, and fears he may not succeed, as well; that he will not necessarily be clearer than other explanations.

However, that is no excuse for not trying.

Major Histocompatibility Complex (MHC)

Since humans love to classify, any discussion of the immune system should begin at the very top, so we introduce the Major Histocompatibility Complex (MHC). The term itself already bears out what we just stated, but in reality it is nothing more than an overall *descriptor* of the immune system in vertebrates. It is a term a human made up; an umbrella if you will similar to the National Collegiate Athletic Association (NCAA), which denotes nothing unless you know (among other things) it supervises collegiate sports, and is made up of three divisions, depending mostly on how many students an individual college has.

So while MHC sounds like some extremely complicated cell or protein, it is nothing tangible or physical, and by itself is meaningless. It could easily have been named the Vertebrate Military Policing System (VMPS). However, we do know the vertebrate immune system has many common components among its membership: how its immune system reacts to foreign invaders, its structure, and the basic "units" that are involved in defense of the organism.

Yet within each species, there are certain differences that make it convenient to sub-divide MHC into three classes. Accordingly, MHC has three divisions known as MHC-I, MHC-II, and MHC-III—the details which we leave in abeyance for the moment. Frankly, we wouldn't even bother with MHC, except it is mentioned again and again in EBV studies. More relevant is another high-level category, denoting the immune system in *humans*. That is known as the *Human Leukocyte Antigen* (HLA) System. Like the MHC, it is just a category—another descriptor and nothing tangible.

Although HLA is a subset of MHC for humans, for whatever reason, much literature relating to the immune system refers to MHC-I, II, and III. Nonetheless, we cannot totally ignore HLA, and it is easy enough to state a *Leukocyte* is simply the technical term for a *white blood cell*. But the term *antigen* puts us at our first crossroads. Normally, we would wish to discuss the three divisions of MHC/HLA—what they do, and how they differ. But doing so, the term *antigen* would be left hanging for quite a while. We would have no idea what it is, or why it used in a high level description of the human immune system. I believe, therefore, we have little choice but to get "down and dirty," leave high-level descriptions alone for the time being, and go to the District Attorney's office, ask for immunity—pun intended—tell him what we know, and let him figure out who to indict.

Antigens

Accordingly, let us journey down and deep within our immune system (don't forget your face mask), and describe *antigens*. In simple terms, then, an *antigen* is a special type of protein that will eventually reside on the membrane of a cell, foreign or our own, provide information of the type of cell it is, allow other molecules to identify the cell, and, if necessary, provide a gateway for other molecules to adhere to. Note antigens are *not* cells themselves, with a nucleus and other goodies, but molecules of protein whose shape differs ever so slightly for each type of cell, to account for thousands, indeed millions, of foreign substances which can enter our body without belonging there, in the never ending battle between good and evil.

Two questions that immediate arise are: (1) Where do antigens come from and (2) how do we recognize them? Unfortunately, we cannot answer this question without taking another detour, to define our immune system by how "smart" it is.

The Non-Specific Immune System

One of several ways of looking at immune systems is by the use of the terms *innate* and *adaptive.* The former means that warriors assigned to that division attack foreign invaders without knowing, or frankly caring, who they are, while in the adaptive system there are officers to guide the enlisted men so each sub-unit attacks very specific invaders, and only those.

This naming convention, however, leads to confusion, since, in fact, the *adaptive* immune system is also *innate*. Far better would be to refer to these two types as *Non-specific* and *Specific*—perhaps not as sexy, but at least more meaningful. As an analogy for the non-specific system, assume you are a 25 year-old male seeking a female for marriage—you might go to a dance, recognize the difference between males and females and approach one of the latter knowing nothing about her, other than you are attracted to something external—her smile perhaps, or her figure—and try to make her acquaintance. And so with our non-specific system. Certain cells known as *phagocytes* recognize foreign objects floating around inside us called *pathogens*—say a virus or bacterium—and go over to make their acquaintance, knowing only they do not belong in the body. These phagocytes (with some exceptions) will essentially surround the pathogen with its membrane, engulf it, "strangle" it to death, and "digest" it. (Being a male at a dance, hopefully you only wish to enfold the female; not strangle her.) But they do a bit more by expropriating some of the "spoils;" that is, protein inside the *pathogen*, and attach it to certain proteins they already have, creating a new molecule. I believe every reader has guessed by now what the new molecule is. You are correct: *that* is "*an" antigen*. (We need to emphasize "an" since we will see later that antigens derive from another source.) In short, antigens consist partly of "stuff" from the pathogen, and partly of "stuff" from the phagocyte. Finally, the phagocyte, who has proven to be a worthy warrior, transports the antigen from inside its cell—don't bother to ask how, I wouldn't tell you even if I knew, which I don't—and attaches it to its surface, or cell membrane.

Like Paul Revere did 250 years ago, the phagocyte then calls out, "The viruses are coming; the viruses are coming, " giving notice to our immune system that we have detected a new invader and we better make plans to neutralize it. We should mention there are different sub-types of phagocytes, and they kill invaders in a couple of ways, but we wish to keep things as simple as possible and there is nothing much to be gained by discussing those. However, because it is funny, I will mention the phagocyte above is known as an "*Antigen Presenting Cell."* At least I think it is funny. I imagine the yearly king's ball at which the guests are introduced and Sergeant at Arms enters, stating, "Your Majesty, I'd like to present the Duke of Antigen and the Duchess of Pathogen who recently married and pledge allegiance to the realm."

Natural Killer Cells

Another cell that is part of non-specific immune system is known as a Natural Killer Cell or NK Cell. They are like armies in reserve. Once the battle gets going and is made known, NK troops are deployed to the scene—the site of the infection—within a couple of days. NK cells are especially

unique and valuable as they don't need specific markers such as antigens, and function in the absence of antibodies (we will get to their definition shortly), allowing for a much faster immune reaction. NK cells are best known for killing virally infected cells, and detecting and controlling early signs of cancer. There is much research going on related to NK Cells because of their recently discovered "inhibitory" and "excitatory" properties, which we need not go into.

Returning back to the MHC/HLA subject matter, we can now relate that the proteins phagocytes add to the spoils of the invader to produce antigens are from MHC/HLA Type II, which are genes or alleles of genes, found on chromosomes that are responsible for the immune system. In fact, technology is advanced enough to know that in humans, virtually all the MHC/HLA defenders reside on Chromosome 6. They have mapped it and know where on the chromosome they reside, and how much space they take up, but that information serves no purpose here—not to mention I couldn't explain it anyway.

What hopefully *is* clear by now is why it is so difficult to understand the immune system without a lot of going back and forth. Also, we can see that there is no clear dividing line between the *non-specific* and *specific* immune system; they sort of meld together. Thus the original "dumb" phagocyte—at least regarding who he enfolds and kills—worked hard to earn his B.S., which he proudly wears on his head as an "antigen presenting" cap, if not gown, indicating he has identified the evil pathogen for his mates to gather their forces and go on the attack.

But to continue with our story. . .

The Specific Immune System.

We now describe the *specific immune system*—which is even more complex as it can identify and neutralize millions of foreign agents in the constant battle between good and evil. Here the analogy is going on a blind date that your friend set you up with. He told you enough about the girl to interest you, so you go to her residence at a specific address in a big city, take the elevator up to a specific apartment number, pick her up, take her to dinner, and afterwards hope she invites you in for coffee—perhaps even dessert. In the body, the specific immune system is made up of several white blood cells called *lymphocytes*, which includes *B-Cells* and *T-Cells.* Each has one or more functions, structures and abilities—both separately and working in tandem—to seek out and—almost always—destroy "foreign" invaders.

B-Cells

B-Cells are "recruited" from bone marrow and are specialists in the *humoral response*, which simply means finding viruses or bacteria floating around in our body fluids that, as yet, have not

infected any cells. After they mature and develop their skills, amazingly, on *each* B-Cell membrane surface are thousands of protein complexes shaped like a Y, with both a fixed and variable portion. Interestingly, the variable part of the "Y" results because the DNA inside the B-Cell does the "twist," the "shuffle," the "hokey pokey" or whatever dance tends to shake it up, and the resulting molecules have a slight, but significant variation from its neighbor. Wouldn't you know it? It is these "Y" shaped protein complexes that are known as *antibodies*! Although the general structure of all antibodies is identical, those small regions at the tip of the protein are extremely variable, which results in millions of antibodies with slightly different structures, or *antigen-binding* sites. Each of these "tips" or variants is called a *paratope*. Again, antibodies are proteins, not cells. The one described above is attached to a B-Cell; there is also a "free floating" version in the body fluids that we will explain shortly.

The enormous diversity of antibodies allows the immune system to recognize an equally wide variety of antigens. They are like the informers in cop and robber movies, pointing out the bad guys. In the body fluids, if any B-Cell's antibody matches the part of the pathogen's membrane known as an *epitope*—it sometimes becomes like Popeye the Sailor Man after eating his spinach. Like a phagocyte of the non-specific immune system, the B-Cell engulfs the pathogen and does away with it. And again, like the phagocyte, the B-Cell also takes spoils or some proteins of its victim, attaches some of its own protein, and creates an antigen for that particular invader which it places on its membrane as a marker for more of those pathogens. Besides all that, there are times the B-Cell is so thrilled by finding a match, that it becomes a "wild and crazy guy" and starts cloning or replicating itself in the tens of thousands, with the antibodies on its surface that fit that particular pathogen. If you are ready to award the B-Cell the Silver Star, wait, because it also differentiates into two different types. One, known as *Memory B-Cells,* hangs around in the body for years as sentinels, in case the virus is dumb enough to try another attack; in that event it would identify it quickly and help get rid of it expeditiously. The other type is called the *Effector* B-Cell, technically known as a *Plasma Cell* (not to be confused with the blood plasma) which become the YouTube of the immune system—a manufacturing plant churning out thousands and thousands of antibodies every minute—although unlike like the 300,000 videos added to YouTube every day—each antibody is *exactly the same*, so that released into the humeral fluid, it can start binding to those *pathogens* that invade the body. Perhaps the B-Cell deserves the Medal of Honor!

Although the above is accurate as far at it goes, most of the time the B-Cell needs a boost or catalyst before it becomes a "wild and crazy guy." That is the responsibility of the T-Cell, which also originates in the bone marrow, but goes thorough basic training in the *thymus* gland. There are several

different types of T-Cells, which like B-Cells are lymphocytes—or white blood cells—and intimately involved in what is known as the *Mediated Immune Response*.

T-Cells

The T-Cells that help "activate" the B-Cells are called *Helper T-Cells.* The Helper T-Cell, like the B-Cell, has a receptor on the outside—in this case *not* an antibody—which is quite specific, but different than its neighbor's T-Cell, and sooner or later, one of these Helper T-Cells will eventually "bump" into and attach its receptor to the Antigen Presenting Cell corresponding to the phagocyte we discussed above.

That activates the T-Cell and causes it to duplicate and differentiate into *Memory* and *Effector* T-Cells. *Memory T-Cells* will hang around for years, as do Memory B-Cells, to give early warning if the same invader comes back, while the Effector cells go on patrol. Eventually an Effector T-Cell will bump into and attach its cell membrane molecule to the antigen on the surface of a B-Cell that has not yet become "a wild and crazy guy." That will activate the latter so it can proliferate and differentiate.

This brings us to a critical junction, when there is no simple explanation of what happens next. If I can guide you past this point with some understanding, you should be able to assess most EBV-MS studies, even at the cellular level. We started off with MHC and indicated it is a high level descriptor of the vertebrate immune systems, divided into three divisions. That is true. We don't need to consider MHC-III, but we do need to differentiate between MHC-I and MHC-II. In fact, those two types have more similarities than differences and it is possible, if not likely, ten years from now the classification system will no longer be useful and a new one developed, but for now both classes consists of molecules—better clusters of genes—that have some relationship to the immune system. Despite so much in common, the major reason molecules are placed in Class I or II seemingly has to do with how they are identified by the immune system, specifically T-Cells. As an analogy, consider two types of vehicles, one with a gas engine and one with a diesel engine. The former is part of the "Vehicle Fuel Type" (VFT), to make up a name, Class I, while the diesel engine is VFT Type II. This despite the fact those vehicles have far more in common than the type of fuel they use.

As it turns out, every human cell expresses on its membrane a protein that is part of MHC Type-I, while phagocytes and B-Cells display proteins on their membranes from MHC Type-II. This is a key difference, because it is the T-Cells that do the bulk of seeking and destroying pathogens that have invaded our cells—whether externally or perhaps through an internal mutation such as a cancer cell—and unlike B-Cells, only they can find and bond to MHC Type-I molecules.

These T-Cells are called C*ytotoxic T–Cells* since they must have no mercy, but kill the infected cell along with the pathogen—whatever it is, for the sake of the organism. Once activated, Cytotoxic T-Cells replicate as fast as possible to "seek and destroy"—for it is true, "a rotten cell spoils the bunch." But don't let that worry you, since the average adult human loses b*etween 50 and 70 billion cells each and every day for one reason another*.[34]

There are a couple of loose ends that need to be tied up. One is that the proteins, or better genes, from which the immune system gets its ability to destroy foreign pathogens, are quite robust and variable—or *polymorphic*, which means that these genes have many different alleles, allowing the fine-tuning of the specific immune system to adopt to virtually anything invading our sacred space. For many reasons it is important that each variation or allele be identified and accounted for, so in the late 1960s, the World Health Organization (WHO) centralized gene identification and eventually set up a classification system to describe each gene as accurately as possible. Normally this would not be our concern, except it became evident the indexing system prior to 2010 was not robust enough to distinguish new gene discoveries and variations between gene molecules, and so was expanded. We mention this for stakeholders who are scientists, since Epstein-Barr studies prior to 2010 used the earlier coding scheme, while later and current studies use the 2010 classification, causing some difficulty and confusion when referencing and comparing studies in those two time periods. One can go to https://en.wikipedia.org/wiki/History_and_naming_of_human_leukocyte_antigens, for more information.

Another loose end we would prefer to skip but need to mention, because one sees them often in EBV-MS studies is the type of protein T-Cells use to bind to another cell—whether friend or foe. They are CD4+ and CD8+, respectively. What differentiates them is that the former can only bind to MHC-II type receptors, while the latter can only bind to MHC-I receptors. Accordingly, *Helper T-Cells* are CD4+, while *Cytotoxic T-Cells* are CD8+.

We haven't discussed so called "auto-immune" reactions, because—we assert—they and a viral or EBV cause of MS are mutually exclusive. But if you forgive another brief "soap box" digression—the author is convinced too many diseases are given the "auto-immune" label for political and economic reasons. It is much easier to get funding for alleged AI diseases, because like AIDS, it scares people, not to mention the tens of billions drug companies are making for treatment of MS as an auto-immune disease. Whether MS is truly an auto-immune disease as most of the literature claims is beyond the

[34] https://en.wikipedia.org/wiki/Apoptosis

scope of this paper, but for the record, the author believes that designation is based upon politics, not science.

Indeed, the author himself had a *true* auto-immune disease and is fortunate to be alive! My mother's blood type was Rh- Negative, and during pregnancy with her first child, my sister born eight years before me, antibodies built up against the Rh+ Positive factor she inherited from our father. By the time I was born, those antibodies were in my system, and began attacking my red (Rh+) blood cells. I needed not one, but two, complete blood transfusions to survive.

Chapter 5

Research Methodology

Introduction

Before beginning our survey and evaluation of EBV-MS research, we believe it is important to acquaint the lay reader with the types of studies typically used by researchers for MS, so they better understand the logic the behind the studies, their efficacy, if any, and our evaluation and criticism. We especially believe stakeholders involved in various aspects of MS—for example, funding new research—should have enough sophistication to evaluate new research projects, have the principals explain their goals/methods, and defend their approach where warranted.

In fact, there are a number of approaches to studying relationships between two or more variables—which vary tremendously in terms of time, cost, required data, and relevance of findings. We have found that too often, researchers take previous conclusions at face value, refer to it briefly in their paper, and use them as the rationale for their own studies, when, in fact, many of those prior studies have substantial flaws which bring into question their findings. We have, unfortunately, found this especially true in the EBV-MS studies we have reviewed.

Be that as it may, volumes have been written on the art/science of research, which, oversimplified, fall into three major categories: *sampling*, *observational studies*, and *experiments*. Needless to say, the purpose of research is to gather information regarding the relationship between two or more variables, such as events, characteristics, traits, motivations, processes, and a host of other factors, for reasons from gaining knowledge, to providing ideas for further research; from predicting voter preferences in an election, to changing a manufacturing process so it becomes more efficient; from determining the best method of teaching mathematics, to, in the case of medicine, finding factors associated with diseases. *Sampling* of voters' preferences, for example, attempts to predict results for a federal, state, city or county election. It does not attempt nor can it identify cause and effect, and can be dispensed with for the purposes of this paper. *Experiments* are the most robust of studies, and the only category that it may be possible to state X causes Y. *Observational Studies*, which are what we are concerned about, lie somewhere in the middle of the research continuum. At best, they can show an association or a correlation between two variables—*but never a causal relationship*. However, their criteria are in dispute, so that they often are confusing not only to the laity, but even to some researchers, as we will show in Part III. We hope that referencing this section will clarify the benefit of these types of studies, what they can and cannot accomplish, and the confidence one can have in their conclusions.

Experimental Studies: (ES)

Both for comparison and because much of the terminology is the same as for Observational Studies, a brief explanation of experimental studies is in order. In general, an *experiment* is usually short-term, variables are under fairly strict controls, and typically the attempt is to scientifically discover whether one variable has an effect upon another—and how much. In a well-designed experiment, one can usually have confidence in the results, which are measured statistically by one of any number of methods.[35] A hypothetical example of a medical experiment follows*:*

Let us say a new company has developed eye drops it claims "reduces" cataracts significantly, obviating the need for surgery. They set up an experiment using volunteers who have cataracts, varied as much as possible to be representative of the cataract population at large (age, ethnicity, etc.) and separate them into two groups. One group, called the *experimental group* would get the eye drops of the new company. The other, known as the *control group,* would get eye drops with a water solution—known as a *placebo.*[36] Two other terms often used are *independent* and *dependent* variables; the former being the eye-drops, the latter, the cataracts—or more precisely, their reduction, if any. Particularly important is that all subjects do not know whether they are getting the medicine or water solution, nor do those administering them. After a period of time, say two months, each group would be evaluated to determine cataract improvement and the data would undergo statistical analysis to determine if the result indicated the medicine reduced the cataracts to a *significant degree*[37] between the experimental and control group.

Observational Studies (OS)

Observational Studies attempt to draw inferences, determine associations, and/or compute correlations between one or more variables in related populations, and are a mainstay of social sciences, psychology, and statistics. When the field is health and medicine, these studies are called *epidemiological*. Critical to all observational studies, and unlike the experimental study above, the independent variable is *not under the control* of the researcher. As a result, OS in general, and

[35] Statistical analysis is a major subject matter, way beyond the scope of this paper, and unnecessary to go into, with one exception, in Part V.

[36] Because the mind/body connection is well-known, a positive or *placebo effect* often occurs if an individual *believes* he is getting medicine, even when not. Accordingly, somes studies would use three groups, the third getting nothing for their cataracts.

[37] In virtually all experiments, by convention, the probability that the independent variable (eye-drops) had an effect on the dependent variable (cataracts) must be <.05 (and for some experiments: <.01) to conclude the results could not have occurred by chance.

epidemiological studies in particular, are deemed never to be able to state *unequivocally* one variable is the *cause* of another.[38]

While we agree in principle with the above statement, there are those that state epidemiological studies can, in fact, determine "cause." Accordingly, this section of our paper is extremely important for the reader to grasp, since many EBV-MS studies have, in fact, stated EBV *is the* cause, or at least *a* cause of MS.

As an example of an Observational Study,[39] in a certain village an unusually number of ear infections break out in children between 4 and 7. As the village uses two water supply systems, A & B, researchers set up an OS creating two populations—by address or some other means, to determine where each group gets its water supply. After six weeks they compare new cases of ear infections with the water supply families used. If the difference between the two was significant, one might conclude the water supply associated with the infections was the *cause*.

The problem, however, with using the term "cause" is that the water supply itself does not cause the infection; rather it is some bacteria within. Besides, any town official with brains would not have waited six-weeks, but simply had both water supplies tested for bacteria that would produce ear infections. Well, better late than never, so they do the test, but find no bacteria that would cause ear infections. Rather, it was within the piping that brought water from supply A to the homes where the bacteria thrived. Technically, while the water supply was associated or correlated with ear infections, it was not actually the cause, although the study did lead to the ultimate solution.

It is extremely important to keep this mind when evaluating studies that often present statistics and conclude EBV is the/a cause of MS—when, at most, it can only demonstrate an association.

Epidemiological Studies (EPS)

Epidemiological Studies measure *outcomes* (diseases) in a population related in one or more ways that is thought to be associated with one or more variables, and/or seek to understand how often diseases occur in different populations and the factor(s) that might give a *clue* as to the reasons. Information gathered is used to plan and/or evaluate strategies to reduce/prevent the illness, and to guide the management of patients in whom disease has already developed. Like clinical findings and laboratory tests, such as MRIs and blood samples, the epidemiology of a disease is an integral part of its basic description. EPS has special techniques of data collection and interpretation, and its own

[38] See Kahn Academy Video:
https://www.khanacademy.org/math/statistics-probability/designing-studies/modal/v/correlation-and-causality

[39] Based upon an example by Annette Gerritsen, Ph.D. at https://www.theanalysisfactor.com/cohort-and-case-control-studies-pros-and-cons/

vocabulary. This chapter describes the major types of epidemiological studies, its terminology, and methods, since most studies of EBV-MS use them as their discipline.

Again, we remind the reader, at best they can provide clues for possibilities, and only further experimentation can verify whether their conclusions are valid. While they present more difficulties and are considerably less reliable than experiments, when used correctly, they can be most important in narrowing down potential reasons (i.e., variables) for disease—and equally important, in ruling them out—to be followed up by more precise studies. For example, through EPS we learned that smoking and lung cancer have an extremely high correlation.

For our purposes, EPS can be divided into four major categories, with different design methodology and the type of conclusions that may be warranted. They are *cluster, ecological, cohort,*[40] *and case-control* studies. Note the above is only one of multiple frameworks that are used to describe this field. Indeed, the definitions/criteria of the last two are not universally agreed upon—some would take issue with our breakdown and examples—and therefore result in much confusion. Nonetheless, we believe our understanding is as reasonable as any,[41] and more importantly, we will be consistent in using it. While concentrating on *Cohort* and *Case-Control* studies, for completeness a short explanation of the first two follow:

Cluster Studies

Cluster Studies are a common technique for statistical data analysis in a number of disciplines. In medicine, they can be used for PET scans to differentiate between different types of tissue in three-dimensional images, and to classify antibiotics according to their antibacterial activity.

In general, cluster studies involve comparing the observed number of cases with the number expected, based upon size, age and other demographics of a population. They also can be used to investigate an unexpectedly high prevalence of a disease in a location, by time of year, or because of an unusual event in a defined area, such as a state, city, town or even neighborhood. A well known example of a cluster study is Legionaries Disease (a form of very virulent pneumonia) that broke out in Philadelphia during the American Legion's annual three-day convention in 1976. Eventually the cause—staying at the Bellevue-Stratford Hotel, whose cooling tower of the hotel's air conditioning system bred the bacteria—was discovered.

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[40] Cohort refers to a homogeneous group having one or more characteristics in common. It is derived from the Roman Empire military, in which ten cohorts (about 450-650 men each) made up a Roman Legion.

[41] The author has been a systems analyst for a number of years, written both technical and user manuals, and with a M.S. in experimental psychology, asserts he is more than qualified to categorize epidemiological studies based upon logic and common sense.

Ecologic Studies

Ecologic Studies evaluate the relationship between one or more environmental agents and disease in a specific population (or *aggregate*), such as in a large city, a rural area, a county, or even a community. Average measures of exposure of some variable, and disease frequency are obtained for each aggregate, and the analyst tries to determine whether or not high levels of the variable are associated with high disease rates. Often the information about disease and exposure is abstracted from published statistics, and therefore does not require expensive or time consuming data collection.

As an example, unusual mortality from coronary heart disease in areas of England and Wales was correlated with neonatal mortality in the same areas 40-50 years earlier. This observation generated the hypothesis that coronary heart disease resulted from poor nutrition in early life that impaired development of blood vessels in the fetus and in infancy, so that survivors were much more prone to disease.[42]

Especially interesting are the biases that can occur in these types of studies and lead one astray, as they foreshadow those we found in a number of EBV-MS studies. Thus, medical consultation for back problems was much higher in the north of Great Britain than in the south. Initially, it was believed that there was some causative agent or life-style activity in the north that would explain this discrepancy. Turned out it was nothing more than residents' medical consultation patterns that varied. In the north, a greater percentage of the population tended to go to doctors when they felt ill.[43]

Cohort and Case Control Studies: (CS and CCS)

Most EPS studies relating to EBV and MS fall under the Cohort or Case-Control banner. In theory, they can be *prospective* or *retrospective*; each having pros and cons, and different design methodologies. Unfortunately, as we earlier noted, there are far too many differences of opinion as to what constitutes each, making these studies especially confusing. There are those who state cohort studies can *only* be prospective, while case-control studies can be *only* retrospective.[44] But worse, from our point of view, are those who state that both are capable of determining a *cause.*[45,46]

This state of affairs is truly unfortunate, in that much of MS research, whatever its focus, is ill-conceived, biased, and based upon unsound prior studies making claims that have no basis. Much worse, of course, are studies paid for by drugs companies who expect results consistent with their

[42] https://www.sciencedirect.com/science/article/pii/S0140673686913401
[43] https://www.bmj.com/about-bmj/resources-readers/publications/epidemiology-uninitiated/6-ecological-studies
[44] https://www.differencebetween.com/difference-between-cohort-and-vs-case-control-study/
[45] For example: https://www.theanalysisfactor.com/cohort-and-case-control-studies-pros-and-cons/
[46] See footnote 38 for a clear negation of that belief.

products.[47] It is no wonder that after 75 years, we are hardly closer to the true cause of MS—with treatment and drugs often based upon fallacies, or worse, greed, considering the billions of dollars made by drug companies and others, using theories in which the evidence is virtually non-existent.

If MS suffers are angry at the "system," it is understandable—for with some exceptions—the "system" too often takes advantage of the victims' desperation, by hawking all sorts of treatments and cures, exaggerating their benefits, while neglecting to reveal their side-effects—often, worse than MS itself. I hope the reader will forgive me for this brief "soapbox" detour, but my wife has MS, so I know first hand about suffering.

One additional point needs to be stated clearly, since there are numerous researchers whom we assert misinterpret their very own studies. We argue *prospective* and *retrospective must* be defined based upon the date that the *new* research begins! One cannot state he/she began a "prospective" study in 2015 using data collected from 1985 to 2005 from 100,000 subjects. Regardless whether the subjects in 1985 had the disease or had the risk-factor, since the 2015 study is going back in time, it should be treated as *retrospective*.

Prospective Studies

A *prospective* study involves identifying subjects who were *exposed* to any variable—or variables—that is thought to be associated with a certain *outcome* (i.e. disease) and followed over a given period of time—minimally years, if not decades—to see, if indeed, the *outcome* occurs at a statistically significant level to suggest it is related to the exposure(s). *The distinguishing feature of a prospective study is that at its outset, all subjects are free of the outcome*![48]

Baseline information should be gathered using identical collection methods for all subjects. Often blood samples will be taken and frozen to be available years later for inspection. For example, one could identify individuals with high cholesterol readings at baseline, and compare their subsequent incidence of developing heart disease to the population at large.

Like all studies, there are certain issues that must be addressed: They include, but are not limited to:

1. Significant numbers of subjects need to be followed.

2. The study can become quite expensive, time consuming, and often take years until a result is obtained.

3. They are not particular appropriate for diseases with a long latency (which includes MS), as any information, however helpful and reasonable for follow-up research, will not be available for years.

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[47] https://drugs-forum.com/ams/researchers-expose-pharmaceutical-industry-misconduct-and-corruption.27724/ http://medicinesocialjustice.blogspot.com/2018/09/baselga-graft-and-corruption-in-medical.html

[48] See: http://sphweb.bumc.bu.edu/otlt/MPH-Modules/EP/EP713_AnalyticOverview/EP713_AnalyticOverview3.html

4. Loss of subjects because of death, moving, desire to no longer to participate, or any number of other reasons can introduce significance bias in the study.

5. The above also applies to researchers; some of whom by its end may no longer be associated with the project; replaced by others with differences in perspective, expertise, etc.

Retrospective Studies

A *retrospective* study examines *outcomes* that *have already occurred*, and look backwards to factors (exposures) that they believe were related to those outcomes. For example, a retrospective study might look at individuals who have diabetes and attempt to determine what factors (smoking, drug use, excessive weight gain) were associated with it. In these studies, historical data is typically a database that has been in existence for decades, created by some other entity and used for other reasons. Data would include clinical, educational, demographic records, and may even include blood or tissue samples that were frozen for future analysis.

Retrospective studies are much shorter, and less expensive, as the data for the population has already been gathered, but they are much less scientific, since the original research usually has little, if anything, to do with the current focus—in our case: EBV-MS. Accordingly, data important for the study may not have been gathered and is unavailable. This is especially problematic when exposure status is not clear, and the data is not able to clarify those questions. Additionally, *multiple exposures* may make interpreting the results difficult, as well as *co-variants* (i.e. other "risk factors") that confound the study, such as whether the subjects smoked, drank, or ate certain food, not stored in the data base.

Issues especially relevant to retrospective studies include:

1. Records and data were not designed for the current study and may be of poor quality.

2. There is no way of retrieving absence of data for potential confounding factors.

3. Differential Loss[49] to follow-ups will bias the results.

4. Blood and/or tissue samples taken decades before the current study may have deteriorated far more than suspected and significantly compromise the findings.

5. For data gathered over the past two decades, stronger laws of *privacy* may make it more difficult to verify data, and/or some data may be restricted.

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[49] In epidemiological studies, one often will come upon this important concept. It is simply the fact that participants may be lost because they do not wish to take part in the study anymore, died, moved and cannot be located, or for a myriad of other reasons. Clearly, when the observation period spans many years, it will be difficult to track all subjects for the entire study. Minimizing these losses is crucial, because by reducing the number of participants, differences between the experimental and control groups are much less reliable.

Cohort Studies

A *cohort* is a group of individuals who share a common characteristic or experience, such as being a certain age, living in a certain area, treated with a certain drug or vaccine, being exposed to one or more pollutants, or having undergone a particular medical procedure. They are then followed for a defined period of time which could be years or even decades, to see if the exposure, characteristic, or experience results in some *outcome* (i.e. disease).

Cohort studies represent one of the fundamental designs of epidemiology which are used to discover and analyze "risk factors" that could affect the incidence of disease. Unlike experimental studies, no intervention or exposure is administered to participant*s*. Rather, the data is taken from the life styles/histories of segments of populations that the investigator is interested in.

A comparison group—or groups—are taken from the same population of which the cohort is drawn—a similar cohort having had no exposure, treatment, or characteristic of the former. While the cohort design does not have the detailed controls of an experiment, the comparison group's incidence of the disease is statistically compared to the cohort, and functions as the control.

As we alluded to earlier, there is disagreement whether cohort studies can be *retrospective*—looking back in time by using existing data such as medical records—as many in the literature assert, or just *prospective*.[50] As far as we are concerned, the answer is the latter. We believe the major value of the cohort study is the fact they are more rigorous in that they measure potential exposures *before* disease has occurred, and therefore can demonstrate "associations" between the X and Y more reliably. Looking "backwards" has it benefits, but it can never be as "pure" as the true cohort study, as in retrospective studies the researcher is totally dependent upon data he had nothing to do with, cannot determine how precise or reliable it is, the time between follow-ups, and a host of other factors that can easily confound or bias his study.

[50] As an example of the former, see https://www.nwcphp.org/docs/study_types/study_types_transcript.pdf Page 7.
Of the latter see: https://www.differencebetween.com/difference-between-cohort-and-vs-case-control- study/

Case-Control Studies: (CSS)

Case-control studies are often confused with cohort studies so it is important to be clear about the differences. Most importantly, CSS defines two groups at the *start*:[51] one with the outcome/disease (the case group) and one without (the control group). Researchers then look *backwards* to a specific *exposure* or *risk factor* to assess whether there is a statistically significant difference between the two groups. Unlike cohort studies, CSS are *a*lways *retrospective*[52] because the outcome has already occurred. (While some literature states there is no *control group* for these type studies,[53] we disagree. At worst, it is a matter of semantics.)

Typically, the researchers rely on both medical records and patient recall for data collection. Cases are usually selected from a presumably reliable source, such as a disease registry. Controls should be matched on the basis of age, sex, ethnicity, etc., to ensure differences do not confound the study. For studies involving physiological "markers," researchers are often able to procure blood or even tissue samples taken from the registry at the start of data gathering and frozen so they can be analyzed years later.

CSS have specific advantages compared to other study designs. They are comparatively short-term and inexpensive. Since they start with people having the outcome (rather than starting with a population free of disease and waiting to see who develops it), it is possible to enroll a sufficient number of patients with a *rare disease.*[54] The practical value of producing rapid results or investigating rare outcomes often outweighs their limitations. They are also a good choice for preliminary investigation of a suspected risk factor for common conditions; conclusions may be used to justify a more costly and time-consuming longitudinal study later. Finally, one can assess *multiple* exposures for the defined outcome/disease.

On the negative side of the ledger, CSS studies are more prone to bias. For MS studies, always at issue is that a certain proportion of those with the disease might not have had a formal diagnosis, may not presently be under medical care, or may have been misdiagnosed, to name a few. Often these

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[51] See https://www.students4bestevidence.net/case-control-and-cohort-studies-overview/ OR https://www.differencebetween.com/difference-between-cohort-and-vs-case-control-study/ Page 3.

[52] This is another area of confusion. Some theorists would state certain CCS studies can be prospective because not all the cases have occurred at start and when new cases arise during the years of the registry, they are added to the data base. We disagree—that only confounds the study.

[53] For example: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2998589/ [Page 7, 2nd Paragraph]

[54] From a lay standpoint, MS is obviously not a rare disease. Indeed there are good reasons to believe its prevalence is vastly under-estimated. However, from a scientific standpoint, the fact that it takes years to manifiest clinical or physiological symptoms is decisive. In that sense it is "rare," and *prospective* studies taking years would have virtually no benefit.

studies require the participants to self-report their exposure to the risk factor. *Recall bias* is the systematic difference in how different subjects in the study recall past events.

While CSS can suggest associations between the risk factor(s) (RF) and the disease, no definitive causality can be drawn. Rather, results may be used to calculate correlations or the *Odds Ratio* (OR) of getting the disease, given the specified risk. But in these studies, one must always be aware that subjects with the disease who are found to have significantly greater "exposure" levels than those without the disease may be explained not because the exposure contributes to the disease, *but because the disease contributes to the exposure*! In short, CSS *cannot* differentiate between cause and effect![55]

Nested Case Control Studies: (NCSS)

One important subtype of CCS is the "Nested Case-Control Study." As in the typical CCS, the investigator identifies cases of disease that occurred in the entire population, as well as identifying disease-free individuals within the group to serve as controls. Using the collected data and often able to obtain available biospecimens, the investigator compares the exposure frequencies in cases and controls as he would in a non-nested case-control study. For NCSS, however, the investigator does not use the *entire* population as the control, but a *representative sample*.

Because the entire population is not used, statistical analysis to determine whether a greater degree of exposure is significant in leading to the disease is different from the typical CSS. This type of study is often used often in MS research, so it is worthwhile to go through a *hypothetical* example to make it clear to the reader what a NCCS consists of.

***Example 1 – DDT Exposure Related to Breast Cancer (Hypothetical)*[56]**

Suppose you were asked to conduct a study in a medium-sized town in which the rate of breast cancer was considerably higher than average. It was also known that the pesticide DDT was used in a number of areas until it was banned 10 years earlier. Fortunately, about that time, that town was selected as a representative U.S. town for a number of studies, which included gathering data for 50,000 women as well as blood samples that were frozen at the outset. It is now twelve years since DDT was banned and two years since all the previous studies had concluded.

Searching the data base, you find 900 woman contracted breast cancer. Had you assayed the blood samples for all 50,000 women, you would have found the following, as shown in Table 2.

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[55] Because these are observatonal studies, not experimental, whatever the results, the exposure can never be scientifically or logically deemed the cause. Other—confounding—factors may prove to the true reason why the outcome and exposure are seemingly related. At best one can say there is an association or correlation. For more information about this extremely important point, see the video at The Kahn Acadamy website related to "Study Design." https://www.khanacademy.org/math/statistics-probability/designing-studies.

[56] Adapted from http://sphweb.bumc.bu.edu/otlt/MPH-Modules/QuantCore/PH717_Case-ControlStudies/PH717_Case-ControlStudies3.html Boston University: School of Public Health: Wayne W. LaMorte, MD, PhD, MPH

TABLE 2

Breast Cancer among Women for the Entire Population

Sample: Entire Population (50,000)

DDT	Breast Cancer	No Breast Cancer	Total	OR Odds Ratio
EXPOSED	300	7,700	8,000	3.75
UNEXPOSED	600	41,400	42,000	1.43
SUBTOTAL	900	49,100	50,000	1.80

The *Odds Ratio* (OR) of each subgroup getting cancer is easy enough to calculate by simple division. For those women exposed to DDT it was 3.75 percent—far above the national average. For women not exposed to DDT, the OR is 1.43 percent—consistent with natural average. However, just living in that town made the OR of getting cancer somewhat higher at 1.8 percent.

Since the entire population was tested, the *Risk Ratio* (RR) of contracting cancer can easily be calculated, and is **2.32** (3.75-1.43). Just to be clear, RR represents the probability of women getting breast cancer, *above and beyond* the typical odds—other things being equal. That is by far the most important finding. Note that 2.32 is not the likelihood of getting cancer, but is rather like horserace odds, such as a horse going off at 2-1 (which means receiving 2 dollars for every 1 dollar you bet should he win). Here, 2.32 states that the probability of getting cancer in that hypothetical town is 2.32 *times* greater than the national average—not particularly good news for women living there. However, expenses in doing this study are prohibitive as the researcher is informed that each blood assay is $36.00, which for the whole population of 50,000 would run $1,800,000.00.

Accordingly, a Nested Case-Control Study is called for. All the blood samples from the 900 women who developed breast cancer would be analyzed, while only a fraction of the 50,000 would serve as the control. In this case, the investigator uses 2 healthy women for every subject with cancer.[57] Accordingly, 1800 *control* subjects are matched as much as possible for age, ethnicity, marital status, etc.

[57] In EBV-MS studies, the controls can range from 1-1 to 4-1: (i.e. Controls per MS Subject.)

The results of this sample are shown in Table 3.

TABLE 3

Breast Cancer among Women for Nested Cases

Sample: Nested Case-Control @ 2-1 (2,700)

DDT	Breast Cancer	No Breast Cancer	Total
EXPOSED	300	317	617
UNEXPOSED	600	1483	2083
SUBTOTAL	900	1800	2700

At first glance the data looks weird, as half of the women exposed to DDT contracted cancer. However, because we are sampling only a subset (*wherein lies the meaning of Nested*) of 50,000 individuals, we calculate the Risk Ratio quite differently by using the formula:

$$RR = \frac{\text{BC Exposed} \div \text{BC Unexposed}}{\text{No BC Exposed} \div \text{No BC Unexposed}} = \frac{300 \div 600}{317 \div 1483} = \frac{.50}{.21} = \mathbf{2.38}$$

Using this approach, the Risk Ratio obtained from only a small sample of the entire population with little loss in precision, cost only $97,000.00 compared to 1.8 million if the entire population was assayed. Here, a CSS strategy was used, but it was *nested* within the population already defined.

Covariance

The last concept we should explain is *covariance*, as you will come across it often in epidemiological studies. Formally, it can be defined as the measurement of the relationship between one variable and another. Specifically, covariance measures the degree to which two variables are linearly associated. If two variables are independent, their covariance is 0.

Covariates may affect the outcome in a study. For example, assume you are running an experiment to see how corn plants tolerate drought. The level of drought is the actual “exposure,” but it isn’t the only factor that affects how plants perform: size is a known factor that affects tolerance levels, so you would also use plant size as a covariate. A covariate can be an independent variable (i.e. of direct interest) or it can be an unwanted, confounding variable. Similar to an *independent* variable, a covariate is also deemed to have an effect on the *dependent* variable (i.e., outcome). If measurable, it needs to be taken into account. In our hypothetical example, women smokers would be a covariant and should be added to the study.

PART III

Studies Pro EBV-MS

Chapter 6

EBV-MS Studies Claiming a Relationship – Study I

Introduction

Part III reviews four studies which conclude results shows a relationship between the Epstein-Barr virus and MS. Because of the author's predisposition to discount viruses in general and EBV in particular as the cause of MS, the first three studies, at least, were not chosen at random or because a brief review found them especially wanting. Rather, they were referenced in George Jelinek's book, "Overcoming Multiple Sclerosis," as three studies indicating MS could be caused by EBV.[58]

Note that Dr. Jelinek by no means asserts the MS *is* caused by a virus or EBV. Rather he is reviewing various theories, and his references relate to the particular theory he is discussing. As a medical professional and researcher who himself contracted MS ten years before the first publication of his book (2009, with a newer version in 2016), and disease free since then, he largely is concerned with "Evidence Based" treatment options. He is neither pro nor con regarding EBV; indeed any theory, as far as I can tell. (I might suggest his book should be on the shelf of every MS patient.)

Also, to be as fair as possible, each of the first three studies was evaluated separately in the order referenced by Dr. Jelinek, and before evaluation of any study in Part IV, which shows no relationship between EBV and MS. As the reader will see later, a number of the author's points regarding the first four studies were echoed by Study V in Part IV, without his prior knowledge.

When reviewing Parts III and IV, the author reminds the reader that italics in the body of the studies are his, unless indicated otherwise. Also, footnotes that are subscripted in the study's body are the author's as well, to explain technical terms used in the study that the non-scientific reader may not be familiar with.

[58] Jelinek: Page 259.

Study I: ***Multiple Sclerosis and Epstein-Barr Virus (2003)***[59]

Context:
Infection with Epstein-Barr virus (EBV) has been associated with an increased risk of multiple sclerosis (MS), but the temporal relationship remains unclear.
Objective:
To determine whether antibodies to EBV are elevated before the onset of MS.
Design, Setting, and Population:
Nested case-control study conducted among more than 3 million US military personnel with blood samples collected between 1988 and 2000 and stored in the Department of Defense Serum Repository. Cases were identified as individuals granted temporary or permanent disability because of MS. For each case (n = 83), 2 controls matched by age, sex, race/ethnicity, and dates of blood sample collection were selected.
Main Outcome Measures:
Antibodies including IgA against EBV viral capsid antigen (VCA) and IgG against VCA, nuclear antigens (EBNA complex, EBNA-1, and EBNA-2), diffuse and restricted early antigens, and cytomegalovirus (CMV).
Results:
The average time between blood collection and MS onset was 4 years. The strongest predictors of MS were serum levels of IgG antibodies to VCA or EBNA complex. The risk of MS increased monotonically[60] with these antibody titers;[61] relative risk (RR) in persons in the highest category of VCA (> or =2560) compared with those in the lowest (< or =160) was 19.7 (95% confidence interval [CI], 2.2-174; P for trend =.004). For EBNA complex titers, the RR for those in the highest category (> or =1280) was 33.9 (95% CI, 4.1-283; P for trend <.001) vs. those in the lowest category (< or =40). Similarly strong positive associations between EBV antibodies and risk of MS were already present in samples collected 5 or more years before MS onset. No association was found between cytomegalovirus antibodies and MS.
Conclusion:
These results suggest a relationship between EBV infection and development of MS.

Evaluation:

***This study must be totally discounted as it was retracted two years later*!**

As the authors state:

> In this issue of JAMA, Ascherio and colleagues request retraction of their article titled "Multiple Sclerosis and Epstein-Barr Virus," which was published in the March 26, 2003, issue. As the authors explain in their letter to the editor, an inadvertent error occurred in sorting of one of the data files used in their nested case-control study. This error led to incorrect matching of the serologic findings from the laboratory analysis and the clinical data, including incorrect assignment of several dates of blood collection. Once the authors discovered this error and reanalyzed the data with the correctly matched samples, it was apparent that many of the data points reported in the original article were incorrect. *Even though the main finding of the study was essentially unchanged*,[62]

[59] "Multiple Sclerosis And Epstein-Barr Virus," Levin Li1, Munger Kl, Rubertone Mv, Peck Ca, Lennette Et, Spiegelman D, Ascherio A. *Clin Exp. Immunol.* 2003 Apr; 132(1): 137–143. In: JAMA. 2003 MAR 26;289(12):1533-6.
[60] A monotonic relationship is a relationship in which: (1) as the value of one variable increases, so does the value of the other variable; or (2) as the value of one variable increases, the other variable value decreases.
[61] Titer measures how much antibody an organism has produced that recognizes a particular epitope.
[62] Author's Emphasis.

> correcting the data errors completely would have required publication of an extensive correction, including republication of correct data points in the abstract and throughout the text, along with republication of corrected tables and figures.[63]

We certainly must give credit to the authors for admitting there were *major* errors in their study and retracting it. They could have kept silent, as it would have been virtually impossible for anyone not associated with the study to find those errors. But humans, being what we are, they could not resist stating despite retracting, "*The main finding of the study was essentially unchanged*." That statement is *inappropriate* and *misleading*. Rather, they should have stated, given the errors, no conclusion can be drawn, one way or another. Instead, they opened the door even wider for future researchers to cite it as evidence for the EBV-MS hypothesis, who are not aware of their retraction. This is no idle speculation. Using Google's "Scholar," it is easy enough to search any article, and get back a list and the number of citations. The retraction was published in 2005. Nonetheless, from 2006 to the present (2019), the study was cited 145 times!

While the retraction speaks for itself, there are several issues the reader should be aware of, as they are prevalent in many EBV-MS studies—indeed in research of any nature.

First, in their study, 19 prior references were cited. This is at the low end. EBV-MS studies typically cite 30-50 prior studies. But do the researchers actually read and *evaluate* the *details* of each. We believe this is extremely unlikely, as it would be prohibitive. In the best case, they would simply read the "abstract," although frankly I would bet many, if not most, studies don't even go that far, but take the word of a third study by using it as its reference.

This is all too common in research. Thus many directions and threads are built upon a house of cards, because even one flawed study in a chain of a research thread can topple the premise that future studies operate under. It is unfortunate, therefore, that, as we just noted, the retracted study is still one of the most quoted studies in the EBV-MS literature.

Second, consciously or not, we believe many researchers are blinded by studies as this, in which there is a three million+ data base from which to procure subjects. It gives the false impression of an enormously "large subject body." But that has absolutely nothing to do with the results. Only 83 individuals were used for the experimental group, and 166 for the control group, yet the three million figure could easily become salient, appearing extremely impressive, when in reality it is irrelevant.

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[63] JAMA. 2005;293(20):2536. doi:10.1001/jama.293.20.2536May 25, 2005 "Correcting the Literature—Retraction and Republication" JAMA. 2005;293(20):2536. doi:10.1001/jama.293.20.2536

Another issue that is common to a number of EBV-MS studies is "sample" bias relative to the population at large. In this study there are three, each quite significant, that one needs to be aware of. First, the study is skewed toward *younger males*, when the demographics of MS clearly indicates the age most prevalent for the disease is 25-50, with the mean of about forty. Second, females contract MS 2+ times more than males, yet the study used 2 males for every female. Third, even using a conservative figure of 100 MS cases per 100,000 in the U.S—there is evidence it is much greater—one might expect 3,000 MS cases in a 3 million subject data base.[64] To "find" only 83 diseased individuals needs explanation!

Finally, using a control group twice the size of the experimental group provides no particular advantage. Doubling or tripling it makes the study longer, costs more money, increases the probability of error, especially when there are tissue or blood samples that need to be assayed. Yet, EBV-MS studies consistently use control groups 2 to 4 times the experimental group, which, as we shall see in a later study, had dire consequences.

[64] Accounting for the lower average age, and the over representation of males in the army data base, there still should be a minimum of 1,500+ individuals with MS.

Chapter 7

EBV-MS Studies Claiming a Relationship – Study II

Study II: *T-Cell Responses to Selected Epstein Barr virus Immunodominant Epitopes in Patients with MS (2003)*[65]

Abstract:
An increased frequency of antiviral CD8+ T-Cells is seen in chronic viral infections. During herpes virus infections the expanded CD8+ T-Cells are thought to control the reactivation of the latent infection. Because multiple sclerosis (MS), a presumed autoimmune disease of the central nervous system, has been associated with a late Epstein–Barr virus (EBV) infection, we wished to examine whether the CD8+ T-Cell response to EBV epitopes differed between MS patients and healthy controls. Here we report an increased frequency of CD8+ T-Cells responding to EBV epitopes from nuclear antigen 3 A (HLA-A2/CLG) and latent membrane protein 2 (HLA-B7/RPP) in MS patients. Noticeably, the altered CD8+ T-Cell response occurred to some but not all EBV epitopes and did not reach the high level seen during acute infection. The responses towards two immunodominant epitopes from human cytomegalovirus (HCMV) were similar in MS patients and normal controls. Together, our data demonstrate the presence of an increased frequency of CD8+ T-Cells reacting with two epitopes from EBV in patients with MS. The altered response to only two of the tested EBV epitopes would be consistent with the presence of cross-reactive epitopes.

Evaluation:

Unless you are an immunologist, this study, at first glance, is quite difficult to understand, even for veteran stakeholders. It is extremely detailed and assumes at least some knowledge of immunology, not to mention statistics. Hopefully, our review in Chapter 5 will give you, the reader, the background, and with common sense and definition of a few terms, make it understandable to the non-technical stakeholder, especially those who are instrumental in funding MS studies or those in some management capacity. Because this study has many of the pitfalls of MS research, we will review it in some detail, not only to point out the types of errors that are prevalent in studies of this type, but hopefully educate stakeholders and make them much more aware of how to evaluate MS research—what to take seriously and what to discount.

In short, the authors wish to determine whether a certain type of immune cell (a CD8+ T-Cell) has an *association* with the several variations of the EBV virus in MS patients vs. controls. As we described in Chapter 4, T-Cells fight foreign human invaders, such as viruses and bacteria, scientifically referred to as *pathogens*. We delineated the various types and subtypes of T-Cells, which have different functions including the CD8+ subtype which has a protein that is attracted to MHC-1

[65] "Altered CD8+ T cell responses to selected Epstein-Barr virus immunodominant epitopes in patients with multiple sclerosis." Höllsberg P, Hansen HJ, Haahr S. *Clin Exp Immunol*. 2003 Apr; 132(1): 137–143. doi: 10.1046/j.1365-2249.2003.02114.x

(Major Histocompatibility Complex) type molecules, which are on all cell membranes in humans. If you recall, CD8+ T-Cells are *cytotoxic*, because they destroy cells that have been infected by a virus and whose antigen is on the cell membrane, which the T-Cell, by now, recognizes.

Just as astrophysicists name stars, cellular biologists name antigens. While we mentioned the Human Leukocyte Antigen (HLA) System Complex—essentially equivalent to MHC in humans—we did not discuss the details, as we did not wish to overburden the non-technical reader.

At this point, however, it is necessary and should be understandable to relate that HLA-A and HLA-B are simply two of three major types of human MHC-I *antigens* or cell surface receptors, and HLA-A2 and HLA-B7 are simply two subtypes of each. If the above is not quite understandable, that is no problem, as errors with this study relate to much higher level issues.

In any event, this study was *not* designed to determine whether EBV is the *cause* of MS. At best, all it could accomplish is to associate a given immune response to two subsets of EBV in MS subjects versus controls. But there are reasons why CD-8 T-Cells might bind to the EB virus, having nothing to do with MS, but simply a result of various MS symptoms activating the immune response to a much greater degree than a non-MS control. In fact, the authors themselves state, *"Noticeably, the altered CD8+ T-Cell response occurred to some, but not all EBV epitopes.* (If you recall, the *epitope* is the part of the antigen that is recognized by the immune system to which the T-Cells bind.) *and did not reach the high level seen during acute infection."*[66] They also state, *"The responses towards two immunodominant epitopes from human cytomegalovirus (HCMV) were similar in MS patients and normal controls."*[67]

Additionally, Table 4 below (taken from their study) shows there was no major difference between the MS and the control group; as one can see, differences are minor, and the authors state, *"HLA-A2 and HLA-B7 between healthy controls and MS patients did not reach statistical significance."*[68]

Table 4

Expression of HLA-A2 and HLA-B7 Genes in MS patients and healthy controls

	HLA-A2			HLA-B7		
	Positive	**Negative**	**% Positive**	**Positive**	**Negative**	**% Positive**
CONT	35	31	53·0	24	42	36·4
MS	28	41	40·6	28	41	40·6

[66] Page 137.
[67] Page 137.
[68] Page 139.

Continuing our review, let us look at the subject pool:

> **Subjects:** Consecutive MS patients and controls were tested by PCR for the presence of HLA-A2 and HLA-B7 alleles.[69] Thereby 33 MS patients and 33 control individuals were selected for the antigen specific T-Cell frequency analysis. The average age for the MS patients was 47.2 years versus 40.9 years in the control group. The female–male ratio was 1 to 5 in the MS group versus 1 to 1 in the control group. Thus, the MS group was on average 6.3 years older than the control group. *Similarly, the female–male ratios reflected the expected ratio for MS patients and healthy controls, respectively.*[70]

Those who are familiar with the demographics of MS should be able to discern three significant problems right off the bat, any of which should effectively invalidate the study!

First, it is well known that the ratio of women to men who get MS is two and often as much as three times higher depending on the location; certainly not 5 males to 1 female, as in the study. *Moreover, the control group was 1 male for 1 female* without any reason given! These are *major design flaws*! Besides being totally skewed, there is no logical reason that MS patients should be divided that way by gender, or that controls should have a totally different ratio than the MS group. Moreover, it is also well established that the *severity* of MS is much greater in males. Why does the study use five times as many males? As the immune system is overloaded fighting the severe MS symptoms, EBV, like many viruses, would have free reign and be expected to show up much more in men, than in healthy controls, or even in women, who would have a milder form of the disease.

Second and related, the idea of using *healthy controls* significantly biases the study. We have demonstrated in Chapter 3 that EBV is a particularly powerful virus and causes all sorts of havoc in the body. It is implicated in various diseases in individuals, starting at an early age and building up and multiplying. It is known to hang out for years, even if latent. Controls should have been selected *randomly* as long as they were not diagnosed with MS. Not doing so is a type of *sampling bias* that confounds the study, as there might be many more controls with EBV showing positive reactions to its HLA-A2 and HLA-B7 "offspring," and affecting statistical significance. In short, *using healthy controls stacks the deck in favor of an EBV connection*.

Third, and of most concern, the average age of the controls is 6.3 years less than those of the MS group. Superficially, the difference may not seem like a big deal. But in fact, it is very misleading! It is wall known the overwhelming majority MS cases occur between 25 and 50 years of age. Given the age of MS onset and its range, it turns out that the MS group *is over 40 percent older* **t**han the control group, and much more likely to have antibodies to EBV. The math should be easy enough to follow.

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[69] An allele is a variation of a gene.
[70] Page 138. Author's Emphasis:

Using birth as the starting point or base, the 6.3 year difference between the MS subjects (average age 47.2) and controls (40.9) means MS subjects are 15.4 percent older than control subjects. We derive that by dividing the difference (6.3) by the average age of the controls (40.9). Other things being equal, we can give the researchers the benefit of the doubt. However, based upon the MS demographics and much more logical, 25 *should* be the base—when MS really begins to make its appearance—not birth. The average age of the controls would then be 15.9, while the average age of the MS subjects would be 22.2—still a 6.3 year difference. However, dividing 6.3 by 15.9 results in .396. In other words, based upon well-accepted demographics, the MS patients are—relatively—almost 40% older than the controls! It is unfortunate the design did not consider this important variable.

All the above makes the results unreliable. As an analogy, it is like comparing a 6 year old automobile to one 12 years old; both never having been serviced and seeking leaks in the transmission, radiator, gas tank, engine and other parts where fluid is integral. Both will have leaks, but the 12 year old car will be in far, far worse shape.

Fourth, looking at the part of the procedure to determine antibody presence we read:

> . . .Flat-bottomed 96-well Maxi-Sort plates (Nunc, Roskilde, Denmark) were coated with 4 mg/ml of anti-IL4 (MAB 604, R&D Systems, Abingdon, UK) or anti-IL-10 (MAB 217, R&D Systems) in a phosphate-buffered solution containing 15 mM Na2CO3, 35 mM NaHCO3 and 0•2% sodium azide. Plates were washed three times in PBS with 0•05% Tween 20 (Sigma, Bromma, Sweden) and nonspecific binding blocked by incubating with TBS with 0•05% Tween-20 and 1% BSA (Sigma) for 2 h at 37∞C. The plates were washed three times and incubated with 50 ml of the sample in duplicate overnight at 4∞C. Following three washes, the plates were incubated with biotin-conjugated anti-IL-4 (BAF 204, R&D Systems) or biotin-conjugated anti-IL-10 (BAF 217, R&D Systems). Finally, the plates were washed three times and incubated with streptavidin-HRP (P0397, Dako, Glostrup, Denmark) for 20 min at RT followed by the addition of developing reagents (Bio-Rad, Hercules, CA, USA). Reactions were stopped by the addition of 1 M H2SO4 and analyzed on an ELISA-reader at 450 nm.[71]

The author admittedly has little understanding of the assay details and we doubt the reader does either. And in fact, that part of the analysis may have gone perfectly well. However, it is important to recognize that it is easy enough to make mistakes in a procedure so incredibly detailed, and even two or three assays with the wrong amount of solution, at the wrong time, with the wrong samples could throw the result off significantly. We are by no means implying mistakes *were* made, but we need to be really cautious about studies so detailed. We all know how often doctors, especially in hospitals, prescribe medicines that are incompatible with others a patient is taking and become a serious threat. And that is pretty straightforward. How much more so can problems crop up in such detailed analysis.

[71] Page 139.

Fifth relates to the following:

> *CD8+ T-Cell responses to EBV epitopes in MS patients:* The CD8+ T-Cell recognition of EBV epitopes presented by HLAA2 or HLA-B7 was investigated in MS patients and healthy controls. Serum from all individuals was tested initially by ELISA for the presence of EBNA1 and HCMV IgG, an indication of previous infection. Whereas 100% of the MS patients had EBNA1 antibodies, 13% of the healthy controls were excluded on the basis of a seronegative ELISA result (data not shown). Analysis of IgG antibodies to HCMV showed that 56% of MS patients and 41% of the controls were seropositive, consistent with a higher average age in the MS group.[72]

We believe it is a serious flaw to eliminate 13% of the control group on the basis of a negative test for antibodies. Once the subjects are decided upon, they should remain in the study and their data used, unless there is an extremely good reason, such as they die before the study is completed, they move and cannot be located, or they simply refuse to continue.

Otherwise, there is no reason why any subject should be eliminated. Results of studies cannot be presumed beforehand and one cannot predict the effect of a 13% reduction of the controls. One might argue that those lacking a previous infection (whatever that is supposed to mean) and keeping them in the study would favor the study's hypothesis, as they would be less likely to have EBV and might make the result more compelling. But that is exactly why studies are done. The design should be adhered to; it should be as simple as possible, and subjects should not be removed in the middle, arbitrarily!

Finally, returning to the demographics of MS, which the author believes is where true progress will be born, we can see one of the most compelling arguments against a viral "cause."

> Viral infection has been associated with multiple sclerosis (MS), a *presumed autoimmune* disease of the central nervous system. [Also] epidemiological observations on migration demonstrate that the risk of acquiring MS diminishes by moving before puberty to an area with low prevalence of MS [17]. *This is consistent with a ubiquitous infectious agent that predisposes to MS in genetically susceptible individuals, if the infection occurs around puberty or later*. Epidemiological and serological analysis indicate that EBV may fulfill these requirements. [18][73]

First, as related in Chapter 3, EBV is *contagious* not to mention found in 90+ percent of the general population. By stating "*this is consistent with a ubiquitous infectious agent that predisposes to MS if occurring around puberty or later*," the authors are ignoring at best and denying at worst what is so obvious. It is well-established that MS is not contagious. If EBV were associated with MS, then the latter *would* also be contagious and the disease would become a serious epidemic. And moving to a

[72] Page 139.
[73] Page 138.

low or high MS area would be irrelevant. If the subject already had EBV as 90 percent do, he/she would contract MS wherever she/he moved.

Those two facts (MS is not contagious; viruses are) are mutually exclusive, and is major inconsistency in all viral MS hypotheses. In Part V, we will consider this in much more detail, but any change is the probability of contracting MS when moving could not possibly be due to a virus, but must be due to other causes! (There are several reasonable explanations for that result, but that is beyond the scope of this paper.) And as the authors themselves admit, "While our data demonstrate an association between the CD8+ T-Cell response to EBV epitopes and MS, we do not know whether the association is *causal*."[74]

To sum up, this study has the type of problems most observational studies are subject to, especially relating to EBV and MS. It produced no credible evidence that EBV is even associated with MS, let alone a cause; it makes assumptions that are speculative, at best, and counter to known demographics; its design is flawed and not well-thought out, and the study was altered in ways counter to true science.

Finally, it is important we recognize like many EBV studies, it stated above MS is a "presumed auto-immune disease." But, ironically, if true that would be incompatible with a viral cause. And of course, one needs to ask, "presumed by whom?" Not by the author! Given the subject matter of this paper and taking into account his wife has MS, the author must limit himself to stating there is really *no* evidence that MS is an auto immune disease—Thank God! Otherwise, those that contracted MS would die in a relatively short period of time.

[74] Page 142.

Chapter 8

EBV-MS Studies Claiming a Relationship –Study III

Study III: *Epstein-Barr Virus Antibodies and Risk of Multiple Sclerosis: A Prospective Study (2001)*[75]

Abstract

Context

Epidemiological studies suggest an association between infection with Epstein-Barr virus (EBV) and risk of multiple sclerosis (MS).

Objective:

To determine whether elevation in serum antibody titers to EBV viral capsid antigen (VCA), nuclear antigens (EBNA, EBNA-1, and EBNA-2), and diffuse and restricted early antigen (EA-D and EA-R) as well as to cytomegalovirus (CMV) precede the occurrence of MS**.**

Design, Setting, and Subjects:

Prospective, nested case-control study. Of 62,439 women participating in the Nurses' Health Study (aged 30-55 years in 1976) and Nurses' Health Study II (aged 25-42 years in 1989) who gave blood samples in 1989-1990 and 1996-1999, respectively, and were followed up through 1999, 144 women with definite or probable MS and 288 healthy age-matched controls were included in the analysis.

Main Outcome Measure:

Serum antibody titers to the specific EBV and CMV antigens were compared between cases and controls.

Results:

We documented 18 cases of MS with blood collected before disease onset. Compared with their matched controls, these women had higher serum geometric mean titers (GMTs) of antibodies to EBV but not CMV. Elevations were significant for antibodies to EBNA-1 (GMT, 515 vs. 203; P =.03), EBNA-2 (GMT, 91 vs. 40; P =.01), and EA-D (15.9 vs. 5.9; P =.04). The strongest association was found for antibodies to EBNA-2; a 4-fold difference in titers was associated with a relative risk (RR) of MS of 3.9 (95% confidence interval [CI], 1.1-13.7). The corresponding RRs were 1.6 (95% CI, 0.7-3.7) for VCA, 2.5 (95% CI, 1.0-6.3) for EBNA, 1.8 (95% CI, 1.0-3.1) for EA-D, and 1.0 (95% CI, 0.6-1.7) for CMV. Significant but generally weaker elevations in anti-EBV antibodies were also found in analyses of 126 cases of MS with blood collected after disease onset and their matched controls.

Conclusions:

Our results support a role of EBV in the etiology of MS.

Evaluation: We believe there is a major flaw from the get-go, which comes directly from the title: "Epstein-Barr Virus Antibodies and Risk of Multiple Sclerosis*: A Prospective Study."* In fact, according to our definition in Chapter 5, this is *not* a *prospective* study, but a *retrospective* one. This distinction is too important to mis-categorize, and leaves us no choice but to question any claims it makes.

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[75] "Epstein-Barr Virus Antibodies and Risk of Multiple Sclerosis: A Prospective Study" Alberto Ascherio, MD, DrPH; Kassandra L. Munger, MSc; Evelyne T. Lennette, PhD; et al Donna Spiegelman, ScD; Miguel A. Hernán, MD, Dr PH; Michael J. Olek, DO; Susan E. Hankinson, ScD; David J. Hunter, ScD. December 26, 2001 *JAMA*. 2001;286(24):3083-3088. doi:10.1001/jama.286.24.3083

The reader is advised to briefly review Chapter 5 on Epidemiological Research. He/she will find that first and foremost, a true *prospective* study is one that *must* be based on data collection and following subjects at the *beginning of the reported research paper*. This study, published in 2001, uses data gathered 25 years earlier by another organization, for other purposes, ending before the start of the study, which results in having no control of the available data. Clearly this study has *gone back in time* by searching through a data base of almost 240,000 individuals; finding those that succumbed to MS, and then extracting a subset of those who had blood drawn prior to an "official" diagnosis, and using them as the experimental group.

Other issues as well, including and in addition to those we brought out earlier, need to be addressed, if only so stakeholders can evaluate other MS research projects—whether old or in the future. In no particular order:

1. We will repeat the following again and again, because it is decisive in our view relating to an EBV-MS connection, seconded in Part V by one of the foremost organizations and its head in MS research. It is well-known that EBV is a herpes virus that *affects 90 percent of the population*. If so, what is the rationale for these studies? While the prevalence of multiple sclerosis has been rising rapidly over the years,[76] even 150 cases per population of 100,000 in the U.S.—significantly higher than earlier estimates—is only .0015 percent of the population. If EBV was implicated, the disease would be out of control, because EBV is a very powerful virus, and contagious enough to affect 90 percent of the population. This is common in EBV-MS studies, ignoring large scale demographical data and doing research whose rationale has little if any basis.

2. In a data base of more than 238,000 subjects, only those that provided a blood sample (62,439) were considered viable:

> The study base for this investigation comprised the subsets of participants who provided a blood sample in 2 large ongoing US cohorts, the Nurses' Health Study (NHS) and the Nurses' Health Study II (NHS II). The NHS was established in 1976, when 121,700 female registered nurses aged 30 to 55 years from 11 states responded to a mailed questionnaire about disease history and lifestyle items. The NHS II was established in 1989, when 116,671 female registered nurses aged 25 to 42 years from 14 states responded to a similar questionnaire. All participants in both cohorts were asked to provide a blood sample. 32,826 participants in the NHS and 29,613 women in the NHS II did so Follow-up questionnaires were mailed every 2 years to update information on potential risk factors for chronic diseases and to ascertain whether major medical events had occurred.[77]

[76] For exmples: https://www.ncbi.nlm.nih.gov/pubmed/27682228; https://www.ncbi.nlm.nih.gov/pubmed/28087187
[77] Page 3084.

In short, only 26 percent of the cohort population was considered for the study by providing blood sample at the start. There may be as many as a dozen reasons why such a high percentage of nurses failed to give blood, which the study did not address, but would bias it. First, it is more than reasonable to assume a significant number of nurses had an illness at the time of blood sampling, which they were keeping under wraps fearing they would lose their job if their illness was detected. Other nurses might have been ill with a cold or flu at the time blood was taken, so they would be likely not give blood, while a significant number of nurses could have had herpes at one time or another in their lives, and knew EBV which causes it, would be detected and they might suffer embarrassment. Regardless of the reason, for 74 percent of a population declining to provide blood, there is reason to believe the other 26 percent were relatively more "healthy," had less EBV antibodies or infections, and would make the comparison with MS subjects much more significant. On the other hand, if the control group represented the original 238,000 cohort, EBV markers may have been much closer to those found in the MS subjects, and differences, if any, not nearly as significant. The above is an example of *selection bias*.[78]

3. Turning to the collection and storage of blood.

> Blood was obtained using collection kits that were returned to our laboratory via overnight courier. Approximately 97% of the samples arrived within 26 hours of being drawn. On arrival in our laboratory, the blood samples were centrifuged and the blood components aliquotted into cryotubes and stored in liquid nitrogen freezers until laboratory analysis. Serum samples from MS cases and controls were sent to the laboratory in triplets containing the case and the 2 matched controls in random order.[79]
>
> *Some degradation of the IgG antibodies or desiccation of blood samples[80] may have occurred during shipping and storage*, but these are probably modest because most samples reached our serum bank within 26 hours and were kept in closely monitored liquid nitrogen freezers. Most importantly, blood samples from cases and controls were handled in the same manner throughout the study, and triplets composed of a case and 2 matched control samples were assayed in the same run and in random order by technicians who were blind to disease status. Under these conditions, any laboratory error should be unrelated to disease status and would attenuate the difference in antibody titers between cases and controls.[81]

Clearly all viruses or pathogens are not alike, ranging in strength and virulence from one extreme to another. Every year, doctors and drug companies tell us about a new virulent flu that we should take shots against. We do know that EBV is an extremely robust virus wreaking havoc upon the human

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[78] A much sounder design would have been to contact those nurses who contracted MS, but had not given blood intially and request a blood sample when the 2001 study began. Those that agreed could then be part of the MS population and controls could have been drawn for the full cohort of 240,000.

[79] Page 3084.

[80] Extreme dryness, or the process of extreme drying. A desiccant is a substance that induces or sustains such a state in its local vicinity in a moderately sealed container.

[81] Page 3086.

body as we documented in Chapter 3, and found, as just stated, in over 90 percent of the general population. The "degradation" referred to above could easily have resulted because "markers" for the virus in "healthy" individuals—smaller amounts of EBV antibodies in their system—were below the threshold of the assays' ability find them, where in MS subjects, with all sorts of tissue loss, and other inflammatory issues, there would still be enough EBV antibodies hanging about for the assays to find. This would result in an artificially inflated figure.

4. Another significant problem relates both to the date of onset and the diagnosis.

> The date of onset of the disease was determined by asking both women with MS and their neurologists for the date of first neurological symptoms. *When the 2 dates were discordant, the earliest date was considered valid.* A total of 149 incident cases of definite and probable MS were documented between baseline and December 1999 among women with available blood samples. For each case, we randomly selected 2 women without MS, matched by year of birth and study cohort. . . . *In 18 of the women with MS, the onset of neurological symptoms occurred after blood collection (median, 1.9 years; range, 2 months–6.5 years*). . . . the diagnosis was reported as *definite MS in 12 women* and *probable MS in 6.* Because of the age distribution of the cohorts at the time of blood collection, most of these women had a late onset of MS (median age, 52 years; range, 39-66 years).[82]

The fact that different neurologists decided whether a subject had MS or not is likely to be a *confounding* variable. Neurologists are human, and the criteria and time it takes to decide the diagnosis can vary tremendously between them. Additionally, as 85 percent of MS is "Relapsing Remitting" (RRMS), a significant number of nurses may have had MS for several years prior to a diagnosis, the symptoms were bearable, attributed to stress or other causes, went away after a couple of weeks, and they only went to a neurologist years later when symptoms became worse and chronic.[83] In short, it is hard to have confidence in the data when the *major* variable—the MS diagnosis—is uncontrolled.

5. More problematic is the fact that six subjects or one-third of the experimental group had only *probable* MS. That is an extremely large percentage and those individuals should have been eliminated from the study. Consider if even half of the 6 subjects truly did *not* have MS, how different the results would be; most likely, insignificant. MS cannot be diagnosed with certainty, as say tuberculosis or diseases that have largely been eradicated, such as polio. This is another instance of *selection* bias, which occurs in most EBV-MS studies. *Probables* should not be included in any study! But sadly, as Dr. Jelinek pointed out, researchers have significant bias according to their belief system.[84] Indeed,

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[82] Page 3084.
[83] See: Swank, Roy L. MD., Ph.D. *The Multiple Sclerosis Diet Book* (Garden City: Doubleday, 1977) Pages 15-22, for more information on unrecognized MS.
[84] Jelinek: Pages 33-41.

bias comes in all forms in most medical research, while MS research—given the nature of the disease—is especially prone to it, as we will detail in Part V.

6. Another issue the reader should be aware is:

> *A potential limitation of our study is the relatively short period between blood collection and onset of MS (median, 1.9 years).* The onset of the *autoimmune process* that leads to demyelination may precede the recognition of neurological symptoms by several months [why not years?] and so may the immune dysregulation that accompanies the disease. Thus, the elevation in anti-EBV titers could be an early manifestation of the preclinical phase of the disease.[85]

The above is difficult to understand. MS typically makes its appearance decades after EBV is contracted, so the short period referred is grounds for extreme caution. Additionally, we must repeat it seems illogical to imply MS is an auto-immune disease (which many EBV-MS studies state), while doing a study to show it is EBV related. To us, they are mutually exclusive! And, we will keep on stating it: The evidence MS is an auto-immune disease is extremely speculative. It is merely a theory.

> *Evidence on potential mechanisms by which EBV could be causally related to MS is limited. The failure to demonstrate EBV in MS plaques by in situ hybridization or polymerase chain reaction [38] suggests that direct central nervous system infection is not involved.*[86]

7. If we know anything, it is that MS is a disease of the Central Nervous System, yet the authors state the virus does *not* affect the CNS, and "*mechanisms by which EBV could be causally related to MS is limited*." It would appear from this statement that the study, in effect, is *anti EBV-MS.*

In short, there are just far too many issues to accept the results as stated. That would be bad enough, but far worse is what is stated in the "Abstract:" *"Our results support a role of EBV in the* ***etiology*** *of MS."*[87]

*A*ccording to Mirriam-Webster, *etiology* is defined as "the cause of a disease or abnormal condition." To place that statement *in and only in the "Abstract,"* which is probably the only part most researchers read for references is totally unwarranted. And as this study, according to Google, *has been cited 501 times*—one can see why so little has been accomplished in all these years. One research project after another is based on speculative conclusions perpetuated by non-scientific studies. Researchers need to be re-educated in the entire area of epidemiology, standards need to be set, and everyone made aware that Cohort and Case-Control Studies cannot prove *causation*—even for well designed and implemented studies, let alone studies with as many problems as we have pointed out.

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[85] Page 3086.
[86] Page 3087.
[87] Page 3083.

EBV-MS: Additional Issues for Stakeholders Awareness

Although there is one study left to review, we have already delineated numerous errors in the three previous studies. Because they are typical of many EBV-MS studies in particular, and epidemiological research, in general, now would be an opportune time to discuss the logic of "Observational" investigation, summarize the major flaws in the EBV-MS studies, and bring to the readers' attention additional defects common to same.

First, in the previous study, the data base used had 238,371 individuals in which data was gathered from 1976 until 1999.[88] Blood was collected, and antibodies for several strains of the Epstein-Barr virus as well as the cytomegalovirus (CMV) were analyzed.

> The *main analyses* included only women with MS who provided blood samples *before* onset of the disease compared with their matched controls or with all controls combined.[89]

Ultimately, *only 18 cases* with blood collected *before* the onset of MS, were the "main" part of the study, which resulted in *claims* the MS group had significantly more antibodies to EBV than the non-MS controls, but not higher antibodies to CMV compared to the controls.

Yet the study's conclusion was made by using only 7.55e-5 of the cohort population. In others words, only .00000755 percent of the original data base of 238,371 was deemed sufficient for such a profound and far-reaching conclusion. We question whether samples that small are sufficient for any conclusion.

Second, even if there was a significant differences in EBV antibodies between the 18 women with MS and the 36 women in the control, while not stated explicitly, the major rational for claiming EBV "has a role in the etiology" of MS could not just be the above, but rather the fact that analysis of antibodies against the cytomegalovirus (CMV) showed no difference between the two groups.

That had to be the critical finding, since if there were significant differences for *that* virus, then stating there *is* a relationship between the EBV and MS would be untenable—as would findings that other viral antibodies also differentiated between an MS and non-MS group.

The CMV comparisons, therefore, can be seen as the "smoking gun," because a reasonable objection to the EBV results is that the MS subjects' immune system is busy fighting the various MS symptoms, such as inflammation, nerve damage, etc, leaving other battlegrounds without enough troops to defend their territory. After all, EBV has been demonstrated to be quite an adversary; a

[88] Page 3084.
[89] Page 3085.

powerful virus causing havoc in young boys and girls, and "mono" in adolescents, before they arrive at the 25-50 range in which MS is most prevalent. While in adults:

> EBV causes latent asymptomatic infection in most individuals . . . . has five programmes of gene usage . . . .the latency programme, in which few or no genes are expressed, *allows the virus to evade immune detection and persist at low levels in* >95*% of all adults*. . . . and has been associated with the development of a number of *cancers including Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin lymphoma, and immunoblastic lymphomas*.[90]

Accordingly, one would expect more EBV antibodies to be in the body wreaking havoc if the immune system was—as in MS—compromised by needing to be at many sites at one time. But EBV wouldn't be the only virus that benefited from a weakened immune system. Other viral antibodies, if they were assayed, should also come out significantly higher for MS subjects. Therefore since CMV did not do so, the study concludes the EBV must be associated with MS. What is curious is that *all four "Pro EBV-MS" studies reviewed used CMV as the "control" virus.* That seems too much of a "coincidence" to occur by chance. Is it known that CMV is a "mild" virus that will always show similar levels of antibodies in MS subjects and controls—are the decks stacked? Also, CMV is an HLA Type I virus and in the same *family* as EBV. Is it not possible the latter overshadows the former if both are in the same organism? Perhaps like an older brother, stronger, taller, and a better athlete who overshadows his younger sibling, could EBV possibly limit the prevalence of CMV? Stakeholders need to ask those questions!

Or perhaps like a sparkler on the 4th of July, could some other virus "cause" MS and then dissipate quickly, so there is no discernible trace of it by the time blood is analyzed decades later? Or perhaps this "unknown" virus, because of its chemical formula, deteriorates in the blood storage medium?

Rather, a true test of an EBV-MS relationship would be if 10 different viruses were assayed, and compared between the two groups. After all, humans are susceptible to almost 100 different viruses—See Appendix I—and 40 percent of humans have about 45 viruses floating in their body simultaneously.[91] If antibodies from 10 viruses, other than EBV—even 6-7 would do—also showed no difference between the two groups, the author would be the first to get on the EBV bandwagon as instrumental in MS, and lobby for a vaccine against it.

Finally, given that author's wife has MS, has been trying to cope with it for years, and find a "cure," it is extremely disappointing to read:

> In conclusion, our results, in conjunction with those of case-control studies, offer evidence that EBV infection *may* increase the risk of MS. *Because few individuals infected with EBV develop MS,*

[90] "Epstein–Barr virus infection is not a characteristic feature of multiple sclerosis brain" Wills, Simon. et al. *BRAIN; A Journal of Neurology*. 2009-132: Page 3319.
[91] www.viralzone.org

> *other cofactors are required.* These may include genetic predisposition and, perhaps, age at primary infection or infection with other microbes.[92]

If anything, the above statement is more *anti EBV-MS than pro!* Yet the abstract nonetheless states, "Our results support a role of EBV in the *etiology* of MS." The reader can come to his/her own conclusion, but given everything we pointed out, the researchers themselves seem to have doubts about their own study.

Before moving on, the author must repeat once more:

***Even if an association between EBV and MS can be found, studies that allude to the term <u>cause</u>, ignore the Number One, and most important rule of Epidemiological Studies. An association between two variables cannot prove cause and effect, and no Observational study should ever imply, let alone state, that it does*!**

[92] Page 3087.

Chapter 9

EBV-MS Studies Claiming a Relationship – Study IV

Study IV: *Primary Infection with the Epstein-Barr Virus and Risk of Multiple Sclerosis (2011)*[93]

Abstract
To determine whether multiple sclerosis (MS) risk increases following primary infection with the Epstein-Barr virus (EBV), we conducted a nested case-control study including 305 individuals who developed MS and 610 matched controls selected among the over 8 million active-duty military personnel with serum stored in the Department of Defense Serum Repository. Time of EBV infection was determined by measuring antibody titers in serial serum samples collected before MS onset among cases, and on matched dates among controls. Ten (3.3%) cases and 32 (5.2%) controls were initially EBV negative. . All of the 10 EBV-negative cases became EBV positive before MS onset ; in contrast, only 35.7 % (10) of the 28 controls with follow-up samples seroconverted (exact p value = 0.0008). We conclude that MS risk is extremely low among individuals not infected with EBV, but it increases sharply in the same individuals following EBV infection.

Study Population:
Active-duty US Army, Navy, and Marines personnel who have at least one serum sample in the ***Department of Defense Serum Repository (DoDSR)***, which stores approximately 46 million serum samples originally collected from over 8 million individuals for HIV testing. [11] [12]

Case and Control Ascertainment:
Cases were identified by searching the electronic databases of the ***Physical Disability Agencies [PDA]*** of the US Army and US Navy for the diagnostic code corresponding to MS reported between 1992 and 2004, and then reviewing hard copy medical records. Overall, 515 cases were reviewed, of which 315 had definite (n=237) or probable (n=78) MS according to previously described criteria [12] and had at least one pre-clinical serum sample, i.e. a sample collected prior to the development of neurological symptoms (date of MS onset), as attested from the medical record. For each case, we obtained up to three pre-clinical samples (the earliest and latest available, as well as a third sample collected between those two). [12] Two controls for each case were randomly selected from the DoDSR population, matched by branch of service, age, sex, race/ethnicity, and dates of blood collection, as previously reported. [12] Ten cases could not be matched, leaving 305 cases and 610 matched controls in the analyses.

Evaluation

This last study in Part III has a number of defects we pointed out in the previous three studies, and several unique problems not discussed earlier that need to be brought to the attention of stakeholders. At this juncture, we need only summarize several of the former, and discuss the latter in somewhat more detail. Again, any italics in the study proper can be assumed to be our own.

1. Again we are faced with a significant *biased* design. Controls should have been taken from the *Physical Disability Agencies* (PDA) data base, as were the MS subjects, not from the Defense Serum

[93] "Primary Infection with the Epstein-Barr Virus and Risk of Multiple Sclerosis," Lynn I. Levin, PhD, MPH, Kassandra L. Munger, ScD,2 Eilis J O'Reilly, ScD, Kerstin I Falk, PhD,4 and Alberto Ascherio, MD, DrPH2,3,5 Author manuscript; available in PMC 2011 Jun 1. *Ann Neurol*. 2010 Jun; 67(6): 824–830. doi: 10.1002/ana.21978 PMCID: PMC3089959-NIHMSID: NIHMS288261-PMID: 20517945

Repository data base. If controls were taken from the Physical Disabilities Database (PDA) and EBV antibodies still were "significantly" less than in the MS subjects, one would have more confidence in the conclusion. On the other hand, if high levels of EBV showed up in those controls, it could begin to put to bed the EBV-MS theory—indeed the whole viral theory—and use funds available for more productive research.

2. How do we know what criteria the army used to diagnose MS? They might have very different criteria than non-military neurologists. As in most cases, MS begins with a single symptom and army neurologists may not have had the time and/or expertise to provide the individual with the full range of tests such as MRIs, clinical scales, etc., which often take weeks if not months, that private neurologists would use for the general population. Considering their patients are charged with defending our country, it is reasonable to suppose army neurologists' diagnosis would be the condition most relevant to the symptom, to relieve their patients of their duties so as not to chance in time of crisis that these individuals' inability to perform optimally would risk their lives and that of their fellow soldiers.

3. We do not see how the data can possibly be unbiased:

> By design, the controls in this study provide an unbiased estimate of the distribution of exposure and covariates among the millions of individuals who comprise the source population of the cases, and the odds ratios are unbiased estimates of the corresponding rate ratios that would be obtained by testing for EBV positivity all the individuals in the source population. [13]
>
> *Results:* The main characteristics of cases and controls are shown in [Table 5.][94] The relatively high proportions of males (66%) and blacks (30%) among MS cases and their matched controls *reflect the demographic composition of the source population*.

TABLE 5
Characteristics of Cases and Controls (Study IV)

Demographics	**Cases (%)**	**Controls (%)**
Male	202 (66)	404 (66)
Female	103 (34)	206 (34)
White	173 (57)	350 (57)
Black	95 (30)	186 (30)
Hispanic	27 (9)	54 (9)
Other	10 (3)	20 (3)
Age (Mean)	28.2	N/A
Age (Range)	18-48	N/A

[94] Page 3. Data from the Study's Table 1 has been excerpted and reformatted in Table 5 for readability.

If we—conservatively—take an average of 150 MS cases per 100,000 in the U.S., other things being equal, in theory, the researchers should have found 12,000 MS cases using a data base of over 8 million personnel, (although given the preponderance of males in the army, that figure would be about 5,300.) What subjects *did* they choose and *why*? They don't say. Secondly, we remind the reader that MS really begins to make its appearance at 25 so the number of cases of army personnel in which the mean age was 28.2 is hardly reflective of the general population in which the median age is 37.5.

4. The fact the original laboratory assaying the blood samples closed after completing only a third of the analysis should be an extremely significant red flag.

> **Laboratory Analyses**
> Serum samples were sent to the laboratory ordered in triplets, each triplet including a case sample and the corresponding matched control samples in random order and without identification of case-control status. Blind quality control triplets . . .were randomly interspersed amongst the study samples to monitor the reproducibility of the assays. EBV serology was performed using indirect immunofluorescence (VCA) or anticomplement immunofluorescence (EBNA complex, EBNA-1, EBNA-2) for the detection of IgG antibodies. [5,14,16] IgG antibodies against cytomegalovirus (CMV), used here as a control herpes virus, were determined using an ELISA. [17] The serological assays for a first set of 83 cases and their 166 matched controls were performed at Virolab Inc., CA, USA. [5] Due to the closure of Virolab, the remaining samples (222 cases and 444 matched controls) were assayed at the Karolinska Institute in Stockholm, Sweden, under the supervision of one of the authors (KF); t*o reduce costs, antibodies to EBNA-1 and EBNA-2 were determined only in a subset of 166 cases and 332 matched controls.*[95]

First, why did the lab close? Perhaps there were problems with equipment, technique, or personnel, which would mean the assays were not reliable. Equally problematic, the fact the rest of the assays were done in Sweden, with differences in equipment, techniques, personnel, etc., given the extremely detailed process for a blood analysis is enough to invalidate the study, notwithstanding one of the authors "supervised" the assays. This, in addition to shipping very sensitive blood samples to Sweden, during which deterioration would be far more likely.

5. Three different EBV antibodies were used to determine if the individual was EBV-positive or EBV-negative. They were: VCA(IgG), EBNA-1, and EBNA-2. To reduce costs however, the EBNA-1 and EBNA-2 antibodies of only 166 subjects and 332 controls were assayed. Thus, 46 percent of the 305 MS subjects were not comparable to the 54 percent who had all three antibodies assayed. Eliminating 46 percent of subjects from the *full assay protocol* in the middle of any study, *we assert is equivalent to totally removing them from the study; what is left of their data should not be used*! As a

[95] Page 2.

result, the percentage of MS subjects who had the full assay protocol was now only .0000276 of the starting *point.* (222/8,000,000)

Removing that high percentage in the middle of any study, epidemiological or experimental, we deem totally unacceptable by any scientific standards and should also automatically disqualify the study! Any scientific study of magnitude—especially complicated with assays, different laboratories and serum samples stored for years—need to be very precise. Why were not the number of subjects limited to that which conformed to the budget? What controls and dependent variables were taken out of the study? Authors present no data as to what subjects were eliminated and the reasons for such. Indeed, this bears out our earlier criticism of having 2-4 controls for each MS subject as being unnecessarily complicated and increasing substantially the possibility for error. Had the study begun with a 1-1 subject-control ratio, it is likely the funds would not have run out and they could have completed the study. Even in the worst case, instead of 915 individuals, a 1:1 control ratio would have 610 subjects. As assays were completed on 498 individuals, only 18 percent of the original cohort would have been eliminated—not great, but a far cry from 46 percent!

> At baseline, 10 (3.3%) of the 305 MS cases and 32 (5.2%) of the 610 controls were EBV seronegative (p=0.2). At least one follow-up blood sample was available for all the EBV-seronegative cases and for 28 of the 32 seronegative controls. During the follow-up, all of the 10 initially seronegative cases became EBV positive before the onset of MS, while only 10 of the 28 controls seroconverted[96]. . . Because none of the EBV-negative individuals developed MS, the relative risk of MS following EBV infection cannot be directly estimated . . . . In addition, a statistically *non-significant relative risk* of 2.0 for MS was found for individuals who seroconverted during the follow-up as compared with individuals who were already EBV positive at baseline.
>
> *Discussion*
>
> In this large *prospective* investigation, we found a total absence of incident MS among individuals without detectable serum antibodies to EBV. About one third of these individuals seroconverted during the follow-up, and after seroconversion, manifested a rate of MS similar to that of individuals of the same age and sex who were already EBV positive at the study baseline.
>
> These findings suggest that EBV infection increases the risk of MS, but *several alternative interpretations need to be considered, including laboratory errors, reverse causation, and confounding*. . . . The occurrence of EBV infection is confirmed by the observation that in all cases the anti-VCA antibodies, undetectable in the initial sample, increased to titers of 320 and above before MS onset, and anti-EBNA complex or anti-EBNA-1 titers also became positive in all cases. The appearance of anti-EBV antibodies in previously seronegative individuals is a robust marker of primary EBV infection. *In contrast, seroconversion to CMV occurred at a similar rate in cases and controls, and was thus unrelated to MS risk*.
>
> The pathological process leading to MS starts before the clinical symptoms. [23] *The results of our study could therefore be explained if pre-clinical MS resulted in increased susceptibility to EBV infection*. While we cannot exclude this possibility, it should be noted that such an increase should

[96] The time period during which a specific antibody develops and becomes detectable in the blood. Before seroconversion, the antibody is absent. During seroconversion, the antibody is present but not yet detectable. Any time after seroconversion, the antibodies can be detected in the blood, indicating a prior or current infection. (Source: Wikipedia)

be selective for EBV, because the prevalence of CMV infection was not higher among MS cases than controls, and rather extreme to result in a 100% prevalence of EBV infection before MS onset, neither of which seems likely.

Finally, *a positive association between EBV infection and MS could be explained if both were affected by a common factor or confounder*. . . . Confounding by an infectious agent that is co-transmitted with EBV cannot be excluded . . . The most likely interpretation of the main results of our study, therefore, is that EBV infection itself increases the risk of MS.[97]

6. Finally, the authors state:

> Using only three parameters [viral capsid antigen (VCA) IgG, VCA IgM and EBV nuclear antigen (EBNA)-1 IgG], it is normally possible to distinguish acute from past infection: the presence of VCA IgM and VCA IgG without EBNA-1 IgG indicates acute infection, whereas the presence of VCA IgG and EBNA-1 IgG without VCA IgM is typical of past infection.[98]

But later they state a primary EBV infection can be determined by results of the VCA(IgG) assay alone. Thus, a "primary EBV infection was deemed to have occurred in these individuals if any of their subsequent samples became positive for anti-VCA antibodies, because these antibodies appear soon after infection and remain present indefinitely."[99] Are they correct? We don't believe so.[100] In any case, they designed the study and ran assays on EBNA-1 and EBNA-2 until their funding ran out. Why would that be necessary?

Finally, when all is said and done, they based their conclusion, "*The most likely interpretation of the main results of our study, therefore, is that EBV infection itself increases the risk of MS,*"[101] upon only 10 individuals who contracted MS after they became EBV positive—*or upon .00000125 of the starting population*!

Summary

There are far too many questions without answers to even remotely accept this study. Once again, according to our criteria in Chapter 5, this is not a *prospective* study, but a *retrospective* one. We discussed the manifestations of such an error in Study III. The reader can review our objections there. The researchers themselves, to their credit, point out several alternative reasons for the results; we have italicized them in the above excerpt. Regardless, we deem the study is unsound and its results cannot be taken at face value.

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[97] Page 6.
[98] Page 2.
[99] Page 2.
[100] Please see: "Serological diagnosis of Epstein-Barr virus infection: Problems and solutions," Massimo De Paschale and Pierangelo Clerici *World J Virol*. 2012 Feb 12; 1(1): 31–43. (Table 2, especially) Published online 2012 Feb 12. doi: 10.5501/wjv.v1.i1.31 PMCID: PMC3782265 PMID: 24175209
[101] Page 6.

Defects we pointed out include: removing 46 percent of subjects and controls; using subjects whose age is considerably younger than the average age one contracts MS; using a percentage of subjects whose gender is 400 percent opposite the MS population at large (starting with about 100 females, one would desire/expect 50 males in the MS subject group, as opposed to 200 (See Table V)); using a data base in which the prevalence of MS is only 5.68 percent of what might be expected. (i.e., at 150 MS cases per 100,000 in the U.S., on average, 12,000 subjects would be expected to have MS versus the 305 cases found; factoring the preponderance of males, that still results in 5,360 cases of MS).

Additional issues include using different laboratories in different countries with reasonable expectations that travel and other variables relating to the move could compromise the samples. (Were there no labs in the U.S. that could take over the analysis?) Interestingly, both the sponsor and the military specifically divorced themselves from the study's conclusions. We find that unusual:

> *The sponsor of this study had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. Nor did they participate in the decision to submit for publication.*[102]

> *The views expressed are those of the authors and should not be construed to represent the positions of the Department of the Army, Department of the Navy, or Department of Defense.*[103]

We will leave it to the reader to decide the significance of the above.

[102] Page 7.
[103] Page 7.

PART IV

Studies Anti EBV-MS

Chapter 10

Studies Showing No EBV-MS Relationship: Study V

Study V: *Epstein–Barr virus infection is not a characteristic feature of multiple sclerosis brain. (2009)*[104]

Abstract

Multiple sclerosis is an inflammatory demyelinating disease of the central nervous system (CNS) that is thought to be caused by a combination of genetic and environmental factors. To date, considerable evidence has associated Epstein–Barr virus (EBV) infection with disease development. However, it remains controversial whether EBV infects multiple sclerosis brain and contributes directly to CNS immunopathology. To assess [this], a large cohort of multiple sclerosis specimens containing white matter lesions with a heterogeneous B-Cell infiltrate and a second cohort of multiple sclerosis specimens (12 cases) that included B-Cell infiltration within the meninges and parenchymal B-Cell aggregates, were examined for EBV infection using multiple methodologies. . .

We report that EBV could not be detected in any of the multiple sclerosis specimens containing white matter lesions by any of the methods employed, yet EBV was readily detectable in multiple Epstein–Barr virus-positive control tissues including several CNS lymphomas. Furthermore, EBV was not detected in our second cohort of multiple sclerosis specimens by in situ hybridization.

. . . Our finding that CNS EBV infection was rare in multiple sclerosis brain indicates that EBV infection is unlikely to contribute directly to multiple sclerosis brain pathology in the vast majority of cases.

Evaluation

This is the first study we cite that found no relationship between EBV and MS. While there are any number of studies that discount that relationship as well—especially that EBV is the "cause" of MS—this study is particularly important for our purpose, not because its finding agrees with us, nor even because their methodology was well thought out and far superior to those of the earlier studies. Rather, explanations of why some of earlier EBV-MS studies might have found the results they did, confirm many of the criticisms we made by making exactly the same points!

We will let the study speak for itself, and when appropriate, detail the key points stated explicitly that are in common with what we stated earlier. As usual, italics or bold in the study proper are the author's.

1. By far, the most important point this study makes is what we stated again and again: an Observational Study finding a relationship between two variables A and B can *not* conclude A is the "cause" of B, since B can easily be the "cause" of A!

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[104] "Epstein–Barr virus infection is not a characteristic feature of multiple sclerosis brain," Simon N. Willis, Christine Stadelmann , Scott J. Rodig, Tyler Caron, Stefan Gattenloehner, Scott S. Mallozzi , Jill E. Roughan, Stefany E. Almendinger,,Megan M. Blewett, Wolfgang Brück. *Brain*, Volume 132, Issue 12, 1 December 2009, Pages 3318–3328, https://doi.org/10.1093/brain/awp200

Introduction

Multiple sclerosis is an inflammatory demyelinating disease of the central nervous system (CNS). . . . After decades of study, many causal factors have been implicated, including a large number of bacterial and viral pathogens While inconsistent results have left the involvement of many of these pathogens unresolved, considerable evidence has linked the human herpes Epstein–Barr virus (EBV) to disease development,

EBV is a human DNA herpes virus that predominantly infects B-Cells. It causes latent asymptomatic infection in most individuals and infectious mononucleosis in adolescents and young adults. EBV has five programmes of gene usage: one programme is used to produce the virus (lytic programme), while four other programmes are associated with latent infection in which no virus is produced. . . .

The latency programme, in which few or no genes are expressed, allows the virus to evade immune detection and persist at low levels in >95% of all adults. Although immunohistochemistry for EBV protein expression can reliably detect EBV infection in three of the four latency programmes *if performed correctly*, definitive evidence for the presence of EBV is based on the detection of either genomic EBV or the small, abundant, nuclear EBV encoded RNAs, which are expressed at high levels during all phases of latent infection. . . .

EBV has a number of properties that make it an attractive candidate as a potential causal factor of multiple sclerosis development, including its ubiquitous expression, its ability to cause latent infection, and its ability to undergo periodic reactivation. After decades of research, considerable evidence supporting a role for EBV in multiple sclerosis development has emerged primarily from sero-epidemiological and immunological studies. . . .

Despite these observations, it remains uncertain whether EBV infection is a causal factor in multiple sclerosis development or ***whether its disease association is a consequence of dysregulated immune function.***[105]

With regard to the former, it has been speculated that EBV could contribute to multiple sclerosis pathogenesis via an indirect effect on immune function; through molecular mimicry between EBV and CNS antigens; or alternatively by undergoing periodic re-activation within the CNS thus serving as a direct target of immune-mediated CNS demyelination.

However, in certain respects EBV infection in multiple sclerosis patients does not appear perturbed as no substantial increases have been observed in viral DNA load in the blood of multiple sclerosis patients relative to controls.[106]

2. Three of our criticisms included: (1) Study III used only .0000266 of the population for their study, (2) previous studies tested only one other non-EBV virus as a control and (3) Study IV used two different laboratories for blood analysis—let alone dropping 46 percent of the subjects for lack of funds. While this study is of a different type, there is much commonality in method that allows comparison. For example, the authors, commendably, used *four different and independent methods* to test for a positive EBV result—not relying totally on one method of analyzing the samples. This cannot

[105] Wills: Page 3319.
[106] Wills: Page 3319.

be over-emphasized, since most studies use one, or at most, two methods of testing for EBV, then somehow find significant differences by whatever statistical method they use.

Results

EBV was not detectable in multiple sclerosis lesions by ISH.[107]

To address whether EBV infection is a characteristic feature of multiple sclerosis brain, a total of 63 formalin-fixed multiple sclerosis tissue specimens (each containing white matter lesions) from 12 multiple sclerosis cases were initially examined for a CD20-positive B-Cell infiltrate, which allowed us to identify a subset of cases that had the potential to harbour EBV infection for further analysis. From this screen, a total of 23 specimens from 12 multiple sclerosis cases (adult and paediatric) were selected for further characterization. . . .

All 23 multiple sclerosis tissue specimens were negative for the EBV transcript EBER by *ISH* which is expressed at high levels during all phases of latent EBV infection. In contrast to the absence of EBER expression in these multiple sclerosis tissue specimens, *EBER was readily detected in several* ***control*** *tissues including multiple CNS lymphomas, a Hodgkin lymphoma isolated from lymph node and a post-transplant lymphoma isolated from lung*.

In addition to ISH, a subset of the multiple sclerosis specimens was also examined for the expression of several EBV latent proteins using immunohistochemistry. Consistent with our ISH data, all lesions were *negative* for LMP1, which is expressed during the growth and default programmes and also EBNA2, which is expressed during the growth programme. Conversely, both LMP1 and EBNA2 were *readily detectable in a Hodgkin lymphoma, and a post-transplant lymphoma, respectively.* Furthermore, ISH detected the house-keeping gene GAPDH in the majority of cells in both an EBV-positive CNS lymphoma and several multiple sclerosis lesions, confirming that ISH worked robustly on all formalin-fixed tissue specimens analyzed. . . .Conversely, both LMP1 and EBNA2 were readily detectable in a Hodgkin lymphoma and a post-transplant lymphoma, respectively.

EBV was not detectable in multiple sclerosis lesions by quantitative real-time PCR.[108]

The absence of detectable EBV as assessed by ISH and immunohistochemistry in our cohort of multiple sclerosis tissue specimens suggested that EBV infection was probably absent from multiple sclerosis brain. However, it remained possible that EBV infection was present at low levels that may have been missed through the examination of individual tissue sections using the above methodologies. . . . .[Thus] *we widened our search* [and] employed two highly sensitive real-time PCR methodologies to detect genomic EBV, or the abundant, EBV-encoded RNA called EBER1. Using the EBV-positive lymphoblastoid cell line IB4, which contains approximately four integrated copies of the EBV genome, we first demonstrated that our real-time PCR methodologies *could detect genomic EBV and EBER1 in a single EBV-positive cell . . . .*

Having confirmed the sensitivity and specificity of our EBV detection assays, we then serially sectioned 17 snap-frozen multiple sclerosis specimens from five multiple sclerosis cases, each with a B cell infiltrate confirmed by immunohistochemistry. . . . However, *consistent with our ISH data, genomic EBV or EBER1 were not detected.*

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[107] Wills: Page 3321.
[108] Wills: Page 3322.

EBV was largely absent from a second cohort of multiple sclerosis specimens that included B-Cell infiltration within the meninges and parenchymal B-Cell aggregates.[109]

Although EBV appeared to be largely absent from multiple sclerosis specimens containing white matter lesions, it remained possible that EBV resides almost exclusively in the recently described ectopic B-Cell follicles, located near or in the meninges . . . .

To address this possibility, 12 multiple sclerosis specimens from the same tissue bank as those used in the Serafini study were examined for EBV infection. . . . EBV was not detectable in any of the cases examined by ISH, yet was readily detectable in multiple control tissues including a CNS lymphoma. Next, snap-frozen multiple sclerosis specimens from the same cases were sectioned and examined for EBV using our real-time PCR methodologies. Largely consistent with our ISH data, EBV was rare in these samples, being detected in only 2 of the 12 cases examined . . . . Suggesting that while EBV infection could occasionally be detected, it was not a characteristic feature of multiple sclerosis brain. . . .

ISH revealed no EBER+ in any of the 23 multiple sclerosis specimens examined obtained from 12 autopsy cases, yet all specimens had a detectable CD20+ B-Cell infiltrate.

3. As we related, EBV is a powerful virus that 25 years after a blood sample, there are still traces of it. This, of course, is important for several reasons, including why it is still hanging around after MS is diagnosed. Yet the results were totally opposite of what we saw previously.

To summarize:

ISH revealed EBER+ cells were not present in multiple sclerosis brain.
EBV was not detectable in multiple sclerosis lesions by quantitative real-time PCR.
EBV was not detectable by quantitative real-time PCR in multiple sclerosis specimens with a confirmed B-Cell infiltrate.
EBV was largely absent from a second cohort of multiple sclerosis specimens that included B-Cell infiltration within the meninges and parenchymal B-Cell aggregates.

In short, this study examined EBV infection in MS specimens and controls using *multiple methodologies* including in s*itu hybridization, immunohistochemistry*, and two independent *real-time PCR methodologies* that measured both genomic EBV and an abundant EBV-associated non-polyadenylated RNA called EBER1. The two latter methodologies were capable of detecting two or fewer EBV infected B-Cells. Despite an exhaustive search with these highly sensitive methodologies:

We report that EBV was undetectable in all multiple sclerosis white matter lesions examined by all methodologies employed. Furthermore, EBV was not detected by ISH in our second cohort of multiple sclerosis specimens, despite the identification of parenchymal B-Cell aggregates and loose B-Cell infiltration within the meninges within a subset of the specimens examined. . . . EBV was not detectable in multiple sclerosis lesions by ISH . . . *Collectively, these results led us to conclude EBV infection was not a characteristic feature of multiple sclerosis brain***. . . .** [thus] indicat[ing] **that EBV is unlikely to contribute directly to multiple sclerosis brain pathology in the vast majority of cases**.[110]

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[109] Wills: Page 3323.
[110] Wills: Page 3320.

While the seroepidemiology studies have demonstrated a clear association between EBV infection and multiple sclerosis, *it must be stressed that caution should be taken in interpreting these results in terms of a* ***causal*** *relationship.* This is because it remains possible that the observed association is a consequence of host factors **that predispose individuals to both multiple sclerosis and infection with certain viruses such as EBV**. Furthermore, [while] many studies have reported increased antibody titres to a range of pathogens in multiple sclerosis patients including EBV, measles virus, rubella virus and Chlamydia pneumoniae . . . . the involvement of many of these pathogens has been called into question, as vaccination against measles, mumps and rubella has not altered the incidence of multiple sclerosis.[111]

In summary, despite an exhaustive search using multiple methodologies, we have shown that EBV appears largely absent from multiple sclerosis brain. While our findings do not exclude the notion that EBV may contribute to multiple sclerosis via an indirect effect on immune function or through molecular mimicry between EBV and CNS antigens, our results lead us to conclude that **EBV infection is unlikely to contribute directly to multiple sclerosis immunopathology in the vast majority of cases.**[112]

[111] Wills: Page 3326.
[112] Wills: Page 3327.

Chapter 11

Studies Finding No Relationship Between EBC and MS: Studies VI & VII

Study VI: *Possible Relations Between Epstein-Barr Virus Past Infection and Classic MS in Guilan, Iran* (2015)[113]

In the following two studies we cite the relevant text (leaving out tables and figures) with minimal comments and let them speak for itself. The reader can go to the source documents for additional information. Clearly, an EBV-MS connection, is controversial to say the least, and we simply wish to present studies on the "anti" side to level the playing field. Part V discusses in detail why a virus, EBV or otherwise, could ***not*** *be* ***a cause*** *of multiple sclerosis, let alone* ***the cause*****!**

1. Background

Classic multiple sclerosis (MS) is a chronic degenerative disease and demyelinating condition affecting mainly the central nervous system. . . . The etiology and pathogenesis of MS are unknown. . . . Epstein-Barr Virus (EBV) is a human DNA herpes virus infecting more than 90% of the world's population. . . . EBV is the etiological agent of infectious mononucleosis, [and] diverse malignancies such as Burkitt and Hodgkin lymphoma have been associated with EBV. More recently, a possible role for EBV has been suggested in chronic inflammatory/autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, and MS. . . .

In several studies, EBV seroprevalence rates were higher in adult and pediatric MS patients than in controls. Moreover, EBV antibody titers and EBV-specific T-Cells showed an increase in MS patients as compared to healthy individuals . However, there is still controversy as to whether EBV is a causative agent or an innocent bystander in the pathogenesis of MS.[114]

2. Objectives

We aimed to investigate whether the presence of a possible latent or active EBV infection in individuals with classic MS could play a role in the development of the disease. Classic MS is a debilitating disease that is more common after ages at which EBV is prevalent. If there is a causal relation between EBV past infection and classic MS, it may be possible to prevent the latter by controlling the former.[115]

3. Patients and Methods

This cross-sectional prospective study, conducted over an 8-month period between April 2012 and December 2012, included all clinical samples both from MS patients who were under the supervision of The MS Society of the Guilan Province, Northern Iran, and from healthy blood donors (age- and gender-matched with the patients) who voluntarily submitted specimens to Pars Medical Laboratory, Rasht, Iran. MS in the patients had been previously diagnosed via magnetic resonance imaging (MRI) and the Evoked Potential Test. . . . All the specimens were stored at -70°C until the experiment was performed. . . . Data consisted of age, gender, disease duration, number of crises, interferon intake duration, the Expanded Disability Status Scale (EDSS) score, MRI result, Evoked Potential Test result, and antiviral therapy type (if prescribed). . . .

46 subjects with classic MS along with 46 healthy controls were examined with the serological test for the presence of antibodies against EBV immunodominant antigens (EBNA-1, EBV-CA, and EBV-EA-D). . . . Serological tests were performed using commercial Enzyme-Linked Immunoassay

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[113] "Possible Relations Between Epstein-Barr Virus Past Infection and Classic MS in Guilan, Iran." Hamidreza Honarmand, Masoumeh Ahmadi, Jalali Moghadam, Hamidreza Hatamian, and Ali Roudbary *Jundishapur J Microbiol*. 2015 Jun; 8(6): e15985 Published online 2015 Jun 27. doi: 10.5812/jjm.15985v2 PMCID: PMC4548402--PMID: 26322199

[114] Honarmand: Page 2.

[115] Honarmand: Page 2.

(ELISA) assays (anti-EBNA-1 IgG, anti EBV-CA IgG, and anti EBV-Ea-D IgG kits). All the tests were performed by a laboratory technician and reported to the first author so as to blind the investigation. . . . Seropositivity to both anti-EBNA-1 IgG and anti-EBV-CA was interpreted as past infection and seropositivity to anti-EBV-EA-D was regarded as persistent infection.[116]

4. Results

The average age of the patients (n = 46) was 32.6 years with a min-max of 13 - 51. The age range of 42.8% of the patients was distributed between 28 and 38 years. The duration of the disease was more frequent between 2 and 9 years (76.3%). Most patients were women (71.4%). The Evoked Potential Test and MRI were positive in all the patients. Most patients (57.2%) had a history of 1 - 2 crises of the disease. . . . Seropositivity to anti-EBNA-1 . . . was not significant and did not indicate a causative association. . . . .There was no significant association between anti-EBV-EA-D IgG and the development of the disease. Seropositivity to both anti-EBNA-1 IgG and anti-EBV-CA indicating past infection did not yield a significant correlation with the development of MS. [117]

5. Discussion

Ruprecht reported that the notion of a persisting (possibly immunological) change caused during the acute phase of primary EBV infection and subsequently leading to permanently elevated MS risk appears compatible with several aspects of the association found between MS and EBV. Farrell found that the heightened immune response to EBV in MS is specifically related to EBNA-1 IgG, a marker of the latent phase of the virus. In the present study, we chose to screen seropositivity to EBNA-1 and EBV-CA because they are the most important indicators of an EBV past infection and also we chose serological investigation of EBV-EA-D because it is the main indicator of active infection. . . .

We did not find a significant association between seropositivity to EBNA-1 IgG and the development of classic MS, and nor did we detect a significant association between seropositivity to EBV-CA IgG and the development of MS. In addition, we found that seropositivity to both EBNA-1 and EBV-CA, which could be a potent indicator of persistent inactive (past) infection, was not significantly associated with the development of classic MS. Our finding is not consistent with that in the previous relevant studies.

The prevalence of MS is low, while the prevalence of EBV is very high in the area where we carried out the present study. Moreover, age at EBV infection acquisition is in early childhood (symptomless type) and mononucleosis is not a common type of EBV infection in the area. These are not in favor of the establishment of a potent association between EBV infection and MS. The low prevalence rate of MS indicates the low environmental load of the predisposing factors (other than infection) in the area. High spreads of EBV infection at lower ages have shown a *reverse association* with the development of MS in several studies.

As with all herpes viruses, EBV establishes a lifelong infection providing continuous stimulation to the immune system, and antibody titers to diagnostic EBV antigens in healthy subjects tend to remain constant over time. Furthermore, it is extremely unlikely that these data reflect an increase in EBV infection after the onset of MS because there is a conspicuous absence of recent EBV infection among individuals with MS.[118]

Conclusion

We did not find a significant difference in the immune response to EBNA-1, EBV-CA, and EBV-EA-D, the viral proteins associated with EBV, between the patient and control groups. These responses (IgG) were not significantly increased in the MS patients compared with the unaffected individuals.

We conclude that EBV past infection could not be a causative factor in the development of MS nor is it a protective factor against classic MS.

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[116] Honarmand: Page 2.
[117] Honarmand: Page 2.
[118] Honarmand: Page 4.

Study VII: Comparison of serum Epstein-Barr virus antibodies between patients with multiple sclerosis and healthy people in Sanandaj, Iran (2018)[119]

Introduction:

Multiple sclerosis (MS) is one of the chronic inflammatory diseases of the Nervous system. The cause of the disease has not yet been clearly identified. Environmental factors and infections, including the Epstein - Barr virus (EBV), are hypothesized to be the cause of the disease.

Our goal was to compare the serum antibody level against EBV in patients with MS and healthy people in Sanandaj, Iran.

Methods:

In this case-control study, 100 patients with MS who were registered in the MS Society of Sanandaj and 204 gender matched healthy blood donors from the Sanandaj Blood Transfusion Organization (control group) who signed an inform consent were studied from 2015 to 2016.

A 5 ml blood sample was obtained from all subjects and then after isolation of patients' sera, IgG antibodies against EBV-CA and EBNA-1 antigens were measured by ELISA method. Demographic data and the results of the tests were analyzed by SPSS software and Chi-squared test.

Results:

EBNA-1 antigen was found in 92% of patient group and 91% of control group (P= 0.959). Serum anti EBV positivity was significantly higher among women (p=0.012). The EBV-CA antigen was positive in 95% of the patient group and 90% of controls (p= 0.229). There was no significant gender difference for this test (p=0.115).

Conclusion:

There was no significant difference in the results of IgG antibodies against EBV in patients with MS and healthy controls in Sanandaj.

The conclusion speaks for itself. No need to comment.

One final word before moving on to Part V. As this paper has evolved, almost of equal importance as negating the EBV-MS theory is helping stakeholders become more knowledgeable about the immune system, and especially, research methodology: the type of problems and errors we have seen, that are too often made in observational studies, and the questions they need to ask before funding new studies.

[119] "Comparison of serum Epstein - Barr virus antibodies between patients with multiple sclerosis and healthy people in Sanandaj, Iran." Poorya-Foroutan-Pajoohian, Hooshmand Choubdarian. Yadollah Zarezadeh, Ashkan Faridi, Namamali Azadi, Mohammad Amin Boshagh *Int J BioMed Public Health*. 2018; 1(3):127-131 doi: 10.22631/ijbmph.2018.143846.1071 http://www.ijbmph.com

PART V

Conclusion and Recommendations

Chapter 12
Conclusions and Recommendations

Straight Shooting

John is walking in a forest when he comes to a clearing and spots a tree with an arrow dead center into a bulls-eye. On the other side, at least 100 feet away, is a decrepit old man who must be close to 100 years old with a bow in his hand. John goes to him and states incredulously, "Why that is unbelievably astounding shooting! I doubt there are more than 25 men in the world who could shoot that well from this distance, and all are under 40."

"Oh that is no big deal," the old man says. "My technique is a bit different than most."

"What do you mean?" asks John.

"Well," replies the old-man, "Most people draw the target first and then shoot the arrow. I shoot the arrow first, and then draw the target."

We cannot see the forest because of the trees. Intermolecular studies, whatever their value, by themselves are extremely unlikely to find the cause of a disease, especially one as heterogeneous as MS. Researchers need a new vision; the research itself, a new framework for discovering factors truly important to its "cause." And that research *must* include high level data! There is a reason for everything, and any purported "cause" must be consistent with known *demographics*—in the case of MS, close to a dozen. Accordingly, just as the old man shot the arrow first and then drew the target, finding more EBV antigens or antibodies for diseased subjects is much more likely to be the *result* of MS, when one considers they are in 90-95 percent of the population. This would be true even if the research was well designed and implemented, but as we shown, that is often not the case. Much of EBV-MS research is not well thought out, biases of one sort or another are rampant, and in many studies, controls are either non-existent or deficient.

Statistical Research Standards

In the months it took to learn the language of MS, the underlying physiology necessary to understand the studies reviewed, the logic and methodology of epidemiological research, the demographics, and enough details about the immune system to even attempt this review, the author never intended nor desires now to embarrass anyone personally, despite finding much EBV-MS research is seriously flawed and adds little if any information critical to an understanding of MS. Too often they elicit similar research equally suspect, aside from the fact that much of the EBV research is error-prone, and one cannot have any confidence in its conclusions.

For example, the author came across one study several weeks before this review was completed that is so striking, it is the epitome of why many EBV studies cannot be relied upon. The author had no plans to bring further studies in this concluding part, but he owes it to the stakeholders to make them aware of how using the wrong statistical measurement could totally and completely alter the conclusion from an EBV-MS "insignificant" verdict, to one of "significance" by the tiniest of margins.

This is a perfect example of the misuse of epidemiological research. Not to embarrass anyone associated with it, however, the author has removed all references to the study. In fact, because he was still able to find the study on Google using just five words in the original abstract, he has even removed the abstract and summarized its purpose, referring to the antigen tested, as ANTG-X, the measurement used, as STAT-X, and modified the number of subjects and controls slightly, so the study would not come up in a Google search. The author urges readers not to try to find the study. It would serve absolutely no purpose.

Study VIII:

Without concerning ourselves with methodology, subject recruitment, data collection details, or blood sample analysis, we focus simply and solely on the results of one comparison of EBV ANTG-X between MS patients and controls. We have rewritten it as follows:

> *This study used blood samples to compare an EBV ANTG-X of 136 MS subjects to 104 non-MS controls. All 136 MS subjects and 98 controls tested positive for the ANTG-X. Based upon STAT-X ($p = 0.0042$) the study concluded it was further evidence of EBV's relationship to MS.*

To the author, it is inconceivable that based upon these results, the study can conclude it was further evidence of EBV's relationship to MS. Common sense—not to mention our intuition—tells us the conclusion is totally unwarranted—*implausible* would be an understatement. We are asked to believe that when 98 out of 104 controls have the EBV antigen; that simply because no antigen was found in 6 subjects, one can conclude there is a relationship to MS—to be read and cited by other researchers, perpetuating the viral "cause" theory.

Yet statistically, the results are correct. Using what is known STAT-X, the difference between the two groups is indeed .0042, less than the .01 percent level typically used as the threshold for significance. What is not stated is that *if one MS subject was negative for ANTG-X the* results would no longer meet the significance level. *Are we to base conclusions of such magnitude on one blood sample test result for one subject*?

We cannot and must not take such studies at face value, and we do not intend to. Using a much more *meaningful* design and the well-established Chi-Squared Test for statistical comparison, we can easily see the results are a *statistical illusion*. Like most studies, Chi-Squared begins with NULL

hypothesis, meaning we assume there is no difference in two groups when comparing one or more variables. In this case, Chi-Squared is a far more appropriate test to determine whether the NULL hypothesis holds up. We will also be liberal by agreeing before-hand if the probability of the result is less than or equal to 5% (<= .05), we will conclude that the NULL hypothesis is *false*, and there *is* a relationship between EBV and MS. (Although, for the nth time, it tells us nothing about causality or what the relationship consists of.)

To demonstrate our assertion, we will use the well-established figure that 90 percent of adults have EBV antibodies, whether they have MS or not. (Indeed, recent data indicates as much as 95% of adults have EBV.)[120] Table 6 places that data where we can all visually see it. Thus, out of 136 MS subjects, 122 should test positive for EBV and out of 104 controls, 94 should test positive.

Table 6

Chi Squared for EBV between MS and Controls (Actual)

TYPE	Subjects	Expected EBV (90%)	Actual EBV
MS	136	122	136
Non-MS	104	94	98

Using the study data modified slightly, of which all 136 MS subjects tested positive, as did 98 of the controls, we plug the results into the well established formula for Chi-Squared:

$$\frac{\textbf{MS (Act – Expct)}^2}{\textbf{Actual}} + \frac{\textbf{Control (Act – Expct)}^2}{\textbf{Actual}} = \frac{\textbf{MS (136-122)}^2}{\textbf{136}} + \frac{\textbf{Control (98 -94)}^2}{\textbf{98}} = \frac{\textbf{196}}{\textbf{136}} + \frac{\textbf{16}}{\textbf{98}} = \textbf{1.44 + 0.16 =} \boxed{\textbf{1.60}}$$

Looking up 1.60 with One "Degree of Freedom (DF)"[121] on the Chi-Squared Table in Appendix III, we find it is at the 20th percentile or (>.20)—far above the (<.05) level which we agreed to accept as significant, indicating the NULL hypothesis is *true*—there is *no* relationship between EBV and MS,

But let us present another example (hypothetical) to show how Chi-Squared would conform to both common sense and our intuition if the data warranted it. Let us imagine that EBV was found in exactly 90 percent of the MS subjects, but in *only 40 percent* of the control group. Now, Table 7 would look as follows:

[120] See Page 58.
[121] Degrees of Freedom are one less than number of groups we are sampling.

Table 7

Chi Squared for EBV between MS and Controls (Hypothetical)

TYPE	Subjects	Expected EBV(90%)	Actual EBV
MS	136	122	122
Non-MS	104	94	40

Again, we plug the table data into the formula:

$$\underset{\text{Actual}}{\frac{(\text{Act}-\text{Expct})^2}{\text{Actual}}}_{\text{MS}} + \underset{\text{Actual}}{\frac{(\text{Act}-\text{Expct})^2}{\text{Actual}}}_{\text{Control}} = \frac{(122-122)^2}{122} + \frac{(40-94)^2}{40} = \frac{0}{120} + \frac{1681}{40} = 0.00 + 72.90 = \boxed{72.90}$$

This time we get a Chi-Squared value of 72.90, which with 1 DF, is seven times greater than the right most entry of Appendix III (10.828), which already corresponds to a probability of 1/10th of 1 percent or .001. Accordingly, we would be justified stating the probability of getting these results is far less than (< .001) and we would *reject* the NULL hypothesis, concluding there *is* some relationship between EBV and MS. **But never forget, any EBV study can at best show a relationship—*not* cause and effect.**

These results are reasonable, not opposed to our intuition and much more in line with good science. We believe this exercise is extremely important for stakeholders to be aware of, in order not to take every MS epidemiological study as gospel, unless, and until, *objective* experts in EPS review it and find it sound.

Editorial Bias

Arguably, the most profound issue in all research, virtually never discussed, and which the overwhelming majority of stakeholders have no awareness of is *Editorial Bias*! It is much more significant than one might imagine. In MS, we believe it is a major reason why progress is so slow. Simply put, it begins with the pressures of academia for its faculty to publish, publish, and publish. Accordingly many researchers will formulate studies that are non-controversial, don't make waves, follow "conventional wisdom," and in their area of expertise, design a study with a little "twist" to justify and fund the research. Another reason they are extremely reluctant to take on studies that go against the grain of established lore—such as EBV is not related to MS—is because editors of scientific journals have similar issues, somewhat different pressures, but when all is said and done, most are quite reluctant to *publish* studies that are controversial, particularly if they published several

earlier studies whose conclusion was the exact opposite. Stakeholders would do well to download a recent comprehensive review of epidemiological studies which noted:

> The scientific literature on MS is large and ever increasing; therefore, it is possible that some articles were still missed. ***Publication bias was a possibility we could not avoid; researchers may be less likely to complete and submit a "negative" study finding and Editors might be less likely to accept these papers.*** Many of the studies relied on prevalent cases of MS and thus could not collect information on the exposure prior to disease onset. As a result, it was possible that *some of the exposures under study may have actually occurred after disease onset or have been a consequence of the disease itself* or have been susceptible to recall bias.[122]

The purpose of review itself is also worth repeating*:*

> **Abstract**
> Multiple sclerosis (MS) is a chronic central nervous system disease with a highly heterogeneous course. . . . . Approximately 85% of patients present with relapsing-remitting MS (RRMS), while 10–15% present with primary progressive MS (PPMS). PPMS is associated with an older onset age, a different sex ratio, and a considerably more rapid disease progression relative to RRMS. We systematically reviewed the literature to identify modifiable risk factors that may be associated with these different clinical courses. We performed a search of six databases and integrated twenty observational studies into a descriptive review. . . . Despite the vast literature examining risk factors for the development of MS, relatively few studies reported findings by disease course. This review exposes a gap in our understanding of the risk factors associated with the onset of PPMS; our current knowledge being predominated by relapsing-onset MS.[123]

The author is under so such pressure. He is totally independent; not affiliated with any organization, has no need to be published in any journal, and this review is an attempt to provide information to stakeholders based upon the facts. (Although he dares state if he *were* in academia, he would not succumb to any pressures to design research that perpetuated what he believed was a "myth," but run studies that he thought would lead to progress.)

The Tisch MS Research Center

One of the major centers for MS research is the Tisch MS Research Center in New York City. Dr. Saud A Sadiq, Director/Chief Research Scientist is an internationally acknowledged expert in MS, receiving numerous awards for his research and clinical activities—my wife has gone to hear him speak numerous times—and the Center's views of the potential causes of MS are well worth considering. We excerpt the following from the Tisch website:

> At the Tisch MS Research Center, we firmly believe that discovery of the cause(s) of MS is a prerequisite first step in the quest for finding a cure. . . . another related and intriguing factor is to investigate the role played by the Epstein-Barr virus (EBV). Almost all people with MS, even in the

[122] "Risk Factors Associated with the Onset of Relapsing-Remitting and Primary Progressive Multiple Sclerosis: A Systematic Review" Kyla A. McKay, Vivian Kwan, Thomas Duggan, and Helen Tremlett *Biomed Res Int*. 2015; 2015: 817238. Published online 2015 Jan 31. doi: 10.1155/2015/817238 PMCID: PMC4329850 PMID: 25802867 Page 9.
See also: https://www.livescience.com/8365-dark-side-medical-research-widespread-bias-omissions.html
[123] McKay: Page 1.

> pediatric population have evidence of a prior infection with EBV. This may be important because after an infection with EBV, there is no cure and the virus remains in usually a latent form for life in the B-cells of an individual. . . . Although there is evidence that EBV may play a critical role in the development of MS, it is *clearly **NOT***[124] *the cause*! This is because about 90% of the general population has evidence of an infection by EBV by age 30, and the prevalence of MS is only about 1 in 800.[125]

Global Burden of Disease (GBD) Study

The *Global Burden of Disease* is a project of the *Institute for Health Metrics and Evaluation* (IHME), an independent population health research center at the University of Washington, that "provides rigorous and comparable measurement of the world's most important health problems and evaluates the strategies used to address them. IHME provides tools to quantify health loss from hundreds of diseases, injuries, and risk factors . . . and distills large amounts of complicated information into a suite of interactive data visualizations that allow people to make sense of the over 1 billion data points generated."[126]

Data is collected and analyzed by a consortium of more than 3,600 researchers in more than 145 countries, the data capture premature death and disability from more than 350 diseases and injuries in 195 countries, by age and sex, from 1990 to the present, allowing comparisons over time, across age groups, and among populations.

GBD recently reviewed the incidence of MS throughout the world, how it has evolved from 1990 to 2016, and analyzed over 80 factors thought to increase the risk of the disease, in what might be the most comprehensive review of MS ever, and is "must reading" for researchers, organizations, scientists and funders that specialize in MS.

As stated in the summary:

> The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) provides a systematic method of quantifying various effects of a given condition by demographic variables and geography. In this systematic analysis, we quantified the global burden of multiple sclerosis and its relationship with country development level.[127]

Besides their review of the incidence of MS throughout the world, and suggestions they make for future research, two findings are especially relevant here. First, they found that in Denmark, the

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[124] This is capitalized in the original text.
[125] https://www.tischms.org/discover
[126] See http://www.healthdata.org/about and http://www.healthdata.org/gbd/about
[127] "Global, regional, and national burden of multiple sclerosis 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016." *The Lancet* Volume 18, Issue 3, March 01, 2019, Mitchell T Wallin,William J Culpepper, et al. P.269

incidence of women with MS doubled from 1950 and 2009. An EBV-MS, or indeed, any viral theory would be hard pressed to account for same.

> Prevalence of multiple sclerosis differed substantially over the adult life of women and men. Rising multiple sclerosis morbidity among women in the later 20th century has been reported in studies of multiple sclerosis prevalence and incidence. An analysis of data from the Danish Multiple Sclerosis Registry showed that incidence in women doubled between 1950 and 2009, whereas increases among men have been more modest.[128]

Much more importantly, in assessing risk for MS of almost 85 variables, including EBV, only one—smoking—was deemed to be significant enough to be indicated as a potential ”cause.”

> *Risk Estimation*
>
> Relative risk data were pooled with a meta-analysis of cohort, case-control, and intervention studies. Risks and outcomes were paired, and for each we evaluated the evidence and judged whether the evidence fell into the categories of convincing or probable, as defined by the World Cancer Research Fund. From the prevalence and relative risk results, population-attributable fractions were estimated relative to the theoretical minimum risk exposure level. When we aggregated estimates for clusters of risks, such as metabolic or behavioral risks, we used a multiplicative function rather than simple addition, taking into account how much of each risk is mediated through another risk. The choice of covariates was based on any reported putative relationship with multiple sclerosis morbidity, but does not imply any causal relationships.
>
> *Smoking was the only environmental risk of 84 risks quantified in GBD 2016 that we judged to have sufficient evidence for a causal relationship with multiple sclerosis as an outcome. . . .* Criteria for inclusion of risks into GBD include the availability of sufficient evidence for a causal relationship between a risk and one or more disease or injury outcome; evidence to support generalisability of an effect size beyond the populations included in epidemiological studies; availability of sufficient data and methods to enable estimation of exposure levels by country.[129]

Conclusion

That MS is caused by a virus, EBV included, has had its peaks and valleys, *but ultimately the idea is untenable.* For one thing, in over seventy five years, there is no real evidence that a virus has been found that unequivocally can “cause” MS—regardless of claims to the contrary—nor is there any evidence how a virus would actually do so.

But that is the least of it. There are easily a half-a-dozen reasons to eliminate a virus as an etiological agent. First *virtually all viruses cause symptoms to appear in days or weeks.* Table 8 below shows common viruses and their incubation period.

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[128] Wallin: Page 283.
[129] Wallin: Page 271.

Table 8

Common Viral Diseases and their Incubation Period[130]

Disease	Incubation (Days)
Influenza	1-2
Common Cold	1-3
Bronchiolitis	3-5
Herpes	5-8
Measles	9-12
Smallpox	12-14
Chicken Pox	13-17
Mumps	16-20
Rubella	17-20
Hepatitis A	15-40
Mononucleosis	30-50

To believe a disease can appear decades after the virus infects the individual makes no sense. In the case of EBV, which is known to infect a majority of children around age five, many of whom do not have symptoms, the explanation that the virus lies dormant for 25-35 years, then becomes active and infects the individual at the age of 40 *causing MS is beyond implausible*.

Second, viruses typically are specific for one cell type (such as a cold virus that infects the upper respiratory tract), while in MS the range of issues from the optic neuritis, to spinal cord lesions; from drop-foot to numbness; from tingling to fatigue; from disoriented thinking to depression, is inconsistent with a viral manifestation.

Third relates to contagion. Many viruses are contagious, but MS is not. Indeed, EBV as a Herpes virus is extremely contagious as 90% - 95% of the population have it, and it is known to be passed by a mother to her child simply by a kiss. If EBV was the cause of MS, we would have a world–wide pandemic in which millions contracted the disease.

Then there is the whole area of *demographics*, which virtually rule out a virus from the get-go! Most known and widely accepted incidences of the disease could not explained by a viral cause.

- *Why are there significantly more cases in cold weather climates than in warm weather climates?*

[130] http://www.virology.ws/2014/10/08/the-incubation-period-of-a-viral-infection/

- *Why do women get MS 2-2.5 times as much as men*? Especially when repeated studies show men are significantly *more susceptible* to viruses than women—totally the opposite of the MS paradigm!

  Men die significantly more often from infectious diseases than women. For instance, men are 1.5 times more likely to die from tuberculosis, and twice as likely to develop Hodgkin's lymphoma following Epstein–Barr virus (EBV) infection. Men are also five times more likely to develop cancer after infection with human papillomavirus (HPV), than women.

  This is because women's immune systems mount a stronger response against foreign invaders, particularly viruses. While the male hormone testosterone tends to dampen immune responses, the female hormone oestrogen increases the number of immune cells and the intensity of their response. So women are able to recover more quickly from an infection.[131]

- *Why do the overwhelming majority of cases occur between 25-50*? If MS was caused by a virus, one would expect the older one gets, with the immune system not as robust, the more likelihood he/she would become infected. Indeed, senior citizens are constantly warned about various strains of influenza, and encouraged to take flu shots every winter, since the flu is much more dangerous to them than for, say, a 25 year old. Yet when it comes to MS, the older one becomes after 50, the *less* the likelihood of contracting it.

- W*hy do women have a remission during pregnancy*? According to the National MS Society:

  Before 1950, most women with MS were counseled to avoid pregnancy because of the belief that it might make their MS worse. Over the past 40 years, many studies have been done in hundreds of women with MS, and they have almost all reached the opposite conclusion: that pregnancy reduces the number of MS relapses, especially in the second and third trimesters.[132]

- W*hy has the prevalence of MS increased exponentially during the last 40-50 years, and the women-men ratio increased as well*?[133]

- Since the auto-immune theory became prevalent, many drugs suppress the immune system using the logic that a compromised immune system would reduce MS attacks or flare-ups, yet it also allows Epstein Barr—and other viruses and bacteria—to proliferate having nothing to do with MS *per se*.

- "Fever" is one of the first signs of a viral infection. *Why is it not a major symptom of MS*.

***When all is said and done, the evidence that MS is the result of a virus is unsustainable*!**

Recommendations

Since all the various and sundry theories of the cause of MS have, after all these years, accomplished little of significance, it is time to develop a brand new strategy. It is time to return to

[131] http://theconversation.com/man-flu-is-real-but-women-get-more-autoimmune-diseases-and-allergies-77248
[132] https://www.nationalmssociety.org/Living-Well-With-MS/Diet-Exercise-Healthy-Behaviors/Womens-Health/Pregnancy
[133] https://www.ncbi.nlm.nih.gov/pubmed/27682228

"GO," collect 200.00 and start again. Accordingly, below are a number of recommendations which have the potential to be beneficial, and allow progress to be made within a reasonable amount of time.

1. We should dispense with the viral theory of MS that has led nowhere and in the author's opinion is a waste of resources. For 75 years we have been trying to force a square into a round hole. It just hasn't worked.

2. Studies must show they are consistent with the *demographics* of MS. Otherwise they are waste of time and money, and only perpetuate more studies that do the same. It is like going through a maze, taking a wrong turn and either going around and around in circles or coming to a dead-end!

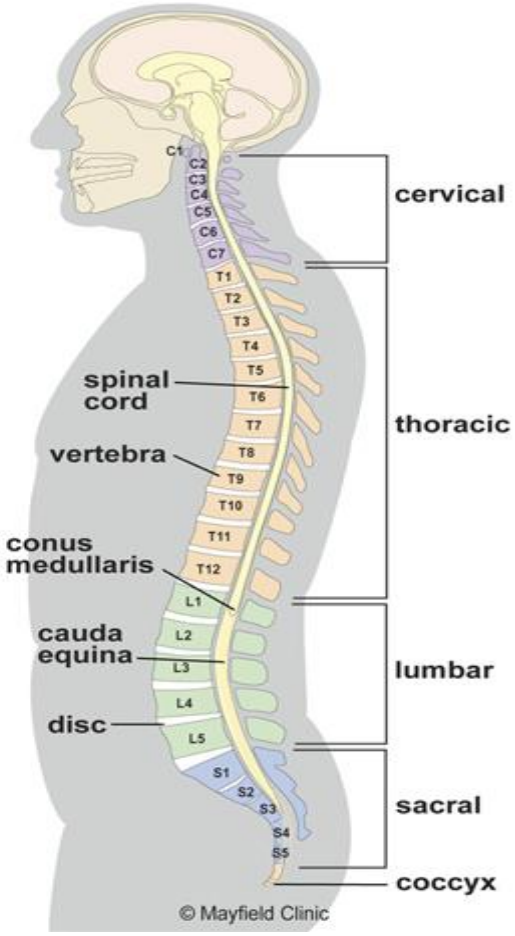
3. We should consider redefining MS into separate *diseases* based upon symptoms, as is done with cancer. Thus, there is breast, lung, colon, skin, and prostrate cancer, etc., all considered separate diseases, and treated differentially—which is the reason that significant progress has been made treating them. Just as we name cancers by their location, we should seriously consider that approach for MS. *Regardless, we assert Primary-Progressive MS (PPMS) is a separate disease having nothing to do with Relapsing Remitting (RRMS). Both should be researched separately as lumping them together has confounding virtually every MS study! Concentrating* research using this framework will exponentially increase the odds of discovering "causal" factors for each.

4. We should recognize that EDDS as a diagnostic tool is much too vague and use it sparingly in research. Spinal MRIs are far better method of learning about MS; we should use them to compare findings to symptoms. As the brain is much more complex, we should initially concentrate on the spinal cord. My wife, who has a problem with walking and "drop foot," has no idea if her spinal MRI show lesions in the vertebra responsible for that function. (See Figure 1.)

5. We should run an exploratory *cohort* study of *only* MS subjects who have *similar* if not identical motor symptoms, such as "drop foot," have them take MRIs to determine whether the lesions are in the expected area of the spinal cord, or vary all over the place. Once the results are in, it will be become evident which direction to take next.

Figure 1

Schematic of Spinal Cord[134]



6. We need to totally re-educate researchers regarding Observational studies so they do not make statements such as EBV is a cause of MS, when those studies are, at best, guides for future research—*and they just do not imply causes.* Most researchers are not epidemiologists, but PhDs, MDs, or immunologists who specialize in one area or another of disease. They seem ignorant, however, of the "science" of epidemiological research, particularly Case-Control and Cohort studies. The statements they make and the conclusions they come to are evidence of the above. We would advocate training programs for researchers; new studies should not be financed or approved unless there is a section such as "Architecture" with a clear statement of the type of study (i.e. cohort, nested case, prospective, retrospective), the reasons for it, and methodology, which would be submitted to an independent Epidemiological MS Review Board for approval. If applicable, they would make suggestions so the study would be more meaningful.

[134] From: https://mayfieldclinic.com/pe-anatspine.htm

7. Much of what we assert has already been echoed, as below. We believe it is sound advice. Undoubtedly, MS is an extremely complex disease. The heterogeneity of symptoms, its multiple manifestations, it various prognoses are highly variable from person to person. Some patients are already quite disabled by the outset of the disease, while others can live their lives somewhat normally, despite their symptoms. We basically agree with the following:

> Apart from its vast spectrum of symptoms this disease is of utter importance due to its immense socioeconomic impact. *The etiology of MS has been thoroughly studied yet not one single etiology has been defined. . . .*
>
> The main hypothesis is that there is no isolated factor as a cause for MS but a myriad of them acting together and in different ways on each individual, associated with genetic susceptibility. More studies are needed in an attempt to confirm this hypothesis and permit possible interventions that may minimize the effects of such interactions.[135]

To repeat, *far too many researchers cannot see the forest because of the trees!*

***But every problem has a solution!* The data is all there, waiting patiently for us to interpret it properly. *MS is crying out for you! It doesn't want people to suffer! Begin to think outside the box! And one more thing: Drug company reps, researchers, and MDs: Stop treating those with MS as specimens—as anonymous numbers in a chart! Don't tell me you don't do so. Remember my wife has MS, so I've seen it dozen of times!* (See Appendix IV.)**

[135] "Multiple sclerosis and herpesvirus interaction," *Arq. Neuro-Psiquiatr.* vol.71 no.9B São Paulo, Sept. 2013 Print version ISSN 0004-282X On-line version ISSN 1678-4227 http://dx.doi.org/10.1590/0004-282X20130160 Guilherme Sciascia do Olival, Bruna Mendonça Lima, Laura M. Sumita, Vitor Serafim, Maria Cristina Fink, Luis Henrique Nali, Camila Malta Romano, Rodrigo Barbosa Thomaz, Vitor Breseghello Cavenaghi, Charles Peter Tilbery, Augusto Cesar Penalva-de-Oliveira; http://www.scielo.br/scielo.php?pid=S0004-282X2013001000727&script=sci_arttext&tlng=pt

APPENDIX I

KNOWN HUMAN VIRUSES[136]

Virus	Genus, Family	Host	Transmission	Disease	genome	Proteome
Adeno-associated virus	Dependovirus, Parvoviridae	Human, vertebrates	Respiratory	None	Genome	Proteome
Aichi virus	Kobuvirus, Picornaviridae	Human	Fecal-oral	Gastroenteritis	Genome	Proteome
Australian bat lyssavirus	Lyssavirus, Rhabdoviridae	Human, bats	Zoonosis, animal bite	Fatal encephalitis	Genome	Proteome
BK polyomavirus	Polyomavirus, Polyomaviridae	Human	Respiratory fluids or urine	None	Genome	Proteome
Banna virus	Seadornavirus, Reoviridae	Human, cattle, pig, mosquitoes	Zoonosis, arthropod bite	Encephalitis	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	Proteome
Barmah forest virus	Alphavirus, Togaviridae	Human, marsupials, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain	Genome	Proteome
Bunyamwera virus	Orthobunyavirus, Bunyaviridae	Human, mosquitoes	Zoonosis, arthropod bite	Encephalitis	1, 2, 3	Proteome
Bunyavirus La Crosse	Orthobunyavirus, Bunyaviridae	Human, deer, mosquitoes, tamias	Zoonosis, arthropod bite	Encephalitis	1, 2, 3	Proteome
Bunyavirus snowshoe hare	Orthobunyavirus, Bunyaviridae	Human, rodents, mosquitoes	Zoonosis, arthropod bite	Encephalitis	Not available	Proteome
Cercopithecine herpesvirus	Lymphocryptovirus, Herpesviridae	Human, monkeys	Zoonosis, animal bite	Encephalitis	Genome	Proteome
Chandipura virus	Vesiculovirus, Rhabdoviridae	Human, sandflies	Zoonosis, athropod bite	Encephalitis	Not available	Proteome
Chikungunya virus	Alphavirus, Togaviridae	Human, monkeys, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain	Genome	Proteome
Cosavirus A	Cosavirus, Picornaviridae	Human	Fecal-oral (probable)	-	Genome	Proteome
Cowpox virus	Orthopoxvirus, Poxviridae	Human, mammals	Zoonosis, contact	None	Genome	Proteome
Coxsackievirus	Enterovirus, Picornaviridae	Human	Fecal-oral	Meningitis, myocarditis, paralysis	Genome	Proteome
Crimean-Congo hemorrhagic fever virus	Nairovirus, Bunyaviridae	Human, vertebrates, ticks	Zoonosis, arthropod bite	Hemorrhagic fever	1, 2, 3	Proteome
Dengue virus	Flavivirus, Flaviviridae	Human, mosquitoes	Zoonosis, arthropod bite	Hemorrhagic fever	Genome	Proteome
Dhori virus	Thogotovirus, Orthomyxoviridae	Human, ticks	Zoonosis, arthropod bite	Fever, encephalitis	Not available	Proteome
Dugbe virus	Nairovirus, Bunyaviridae	Human, ticks	Zoonosis, arthropod bite	Thrombocytopaenia	1, 2, 3	Proteome
Duvenhage virus	Lyssavirus, Rhabdoviridae	Human, mammals	Zoonosis, animal bite	Fatal encephalitis	Not available	Proteome
Eastern equine encephalitis virus	Alphavirus, Togaviridae	Human, birds, mosquitoes	Zoonosis, arthropod bite	Encephalitis	Genome	Proteome
Ebolavirus	Ebolavirus, Filoviridae	Human, monkeys, bats	Zoonosis, contact	Hemorrhagic fever	Genome	Proteome
Echovirus	Enterovirus, Picornaviridae	Human	Fecal-oral	Common cold	Genome	Proteome
Encephalomyocarditis virus	Cardiovirus, Picornaviridae	Human, mouse, rat, pig	Zoonosis	Encephalitis	Genome	Proteome
Epstein-Barr virus	Lymphocryptovirus, Herpesviridae	Human	Contact, saliva	Mononucleosis	Genome	Proteome

[136] Source: ViralZone:www.expasy.org/viralzone, SIB Swiss Institute of Bioinformatics)

Virus	Genus, Family	Host	Transmission	Disease	genome	Proteome
European bat lyssavirus	Lyssavirus, Rhabdovirus	Human, bats	Zoonosis, animal bite	Fatal encephalitis	Genome	Proteome
GB virus C/Hepatitis G virus	Pegivirus, Flaviviridae	Human	Blood, occasionally sexual	None	Genome	Proteome
Hantaan virus	Hantavirus, Bunyaviridae	Human, rodents	Zoonosis, urine, saliva	Renal or respiratory syndrome	1, 2, 3	Proteome
Hendra virus	Henipavirus, paramyxoviridae	Human, horse, bats	Zoonosis, animal bite	Encephalitis	Genome	Proteome
Hepatitis A virus	Hepatovirus, picornaviridae	Human	Fecal-oral	Hepatitis	Genome	Proteome
Hepatitis B virus	Orthohepadnavirus, Hepadnaviridae	Human, Chimpanzees	Sexual contact, blood	Hepatitis	Genome	Proteome
Hepatitis C virus	Hepacivirus, Flaviviridae	Human	Sexual, blood	Hepatitis	Genome	Proteome
Hepatitis E virus	Hepevirus, Unassigned	Human, pig, monkeys, some rodents, chicken	Zoonosis, food	Hepatitis	Genome	Proteome
Hepatitis delta virus	Deltavirus, Unassigned	Human	Sexual contact, blood	Hepatitis	Genome	Proteome
Horsepox virus	Orthopoxvirus, Poxviridae	Human, horses	Zoonosis, contact	None	Not available	Proteome
Human adenovirus	Mastadenovirus, Adenoviridae	Human	Respiratory, fecal-oral	Respiratory	Genome	Proteome
Human astrovirus	Mamastrovirus, Astroviridae	Human	Fecal-oral	Gastroenteritis	Genome	Proteome
Human coronavirus	Alphacoronavirus, Coronaviridae	Human	Respiratory	Respiratory	Genome	Proteome
Human cytomegalovirus	Cytomegalovirus, Herpesviridae	Human	Contact, urine, saliva	Mononucleosis, pneumonia	Genome	Proteome
Human enterovirus 68, 70	Enterovirus, Picornaviridae	Human	Fecal-oral	Diarrhea, neurological disorder	Genome	Proteome
Human herpesvirus 1	Simplexvirus, Herpesviridae	Human	Sexual contact, saliva	Skin lesions	Genome	Proteome
Human herpesvirus 2	Simplexvirus, Herpesviridae	Human	Sexual contact, saliva	Skin lesions	Genome	Proteome
Human herpesvirus 6	Roseolovirus, Herpesviridae	Human	Respiratory, contact	Skin lesions	Genome	Proteome
Human herpesvirus 7	Roseolovirus, Herpesviridae	Human	Respiratory, contact	Skin lesions	Genome	Proteome
Human herpesvirus 8	Rhadinovirus, Herpesviridae	Human	Sexual contact, saliva	Skin lymphoma	Genome	Proteome
Human immunodeficiency virus	Lentivirus, Retroviridae	Human	Sexual contact, blood	AIDS	Genome	Proteome
Human papillomavirus 1	Mupapillomavirus, Papillomaviridae	Human	Contact	Skin warts	Genome	Proteome
Human papillomavirus 2	Alphapapillomavirus, Papillomaviridae	Human	Contact	Skin warts	Genome	Proteome
Human papillomavirus 16,18	Alphapapillomavirus, Papillomaviridae	Human	Sexual	Genital warts, cervical cancer	Genome	Proteome
Human parainfluenza	Respirovirus, Paramyxoviridae	Human	Respiratory	Respiratory	Genome	Proteome
Human parvovirus B19	Erythrovirus, Parvoviridae	Human	Respiratory	Skin lesion	Genome	Proteome
Human respiratory syncytial virus	Orthopneumovirus, Pneumoviridae	Human	Respiratory	Respiratory	Genome	Proteome
Human rhinovirus	Enterovirus, Picornaviridae	Human	Respiratory	Respiratory	Genome	Proteome
Human SARS coronavirus	Betacoronavirus, Coronaviridae	Human, palm civet	Zoonosis	Respiratory	Genome	Proteome

Virus	Genus, Family	Host	Transmission	Disease	genome	Proteome
Human spumaretrovirus	Spumavirus, Retroviridae	Human	Contact, saliva	None	Not available	Proteome
Human T-lymphotropic virus	Deltaretrovirus, Retroviridae	Human	Sexual contact, maternal-neonatal	Leukemia	Genome	Proteome
Human torovirus	Torovirus, Coronaviridae	Human	Fecal-oral	Gastroenteritis	Not available	Not available
Influenza A virus	Influenzavirus A, Orthomyxoviridae	Human, birds, pigs	Respiratory or Zoonosis, animal contact	Flu	1, 2, 3, 4, 5, 6, 7 ,8	Proteome
Influenza B virus	Influenzavirus B, Orthomyxoviridae	Human	Respiratory	Flu	1, 2, 3, 4, 5, 6, 7, 8	Proteome
Influenza C virus	Influenzavirus C, Orthomyxoviridae	Human	Respiratory	Flu	1, 2, 3, 4, 5, 6, 7	Proteome
Isfahan virus	Vesiculovirus, Rhabdoviridae	Human, sandflies, gerbils	Zoonosis, arthropod bite	Undocumented, encephalitis?	Not available	Proteome
JC polyomavirus	Polyomavirus, Polyomaviridae	Human	Fecal-oral or urine	Encephalitis	Genome	Proteome
Japanese encephalitis virus	Flavivirus, Flaviviridae	Human, horses, birds, mosquitoes	Zoonosis, arthropod borne	Encephalitis	Genome	Proteome
Junin arenavirus	Arenavirus, Arenaviridae	Human, rodents	Zoonosis, fomite	Hemorrhagic fever	1, 2	Proteome
KI Polyomavirus	Polyomavirus, Polyomaviridae	Human	Fecal-oral or urine	Encephalitis	Genome	Proteome
Kunjin virus	Flavivirus, Flaviviridae	Human, horses, birds, mosquitoes	Zoonosis, arthropod borne	Encephalitis	Not available	Proteome
Lagos bat virus	Lyssavirus, Rhabdoviridae	Human, mammals	Zoonosis, animal bite	Fatal encephalitis	Not available	Proteome
Lake Victoria marburgvirus	Marburgvirus, Filoviridae	Human, monkeys, bats	Zoonosis, fomite	Hemorrhagic fever	Genome	Proteome
Langat virus	Flavivirus, Flaviviridae	Human, ticks	Zoonosis, arthropod borne	Encephalitis	Genome	Proteome
Lassa virus	Arenavirus, Arenaviridae	Human, rats	Zoonosis, fomites	Hemorrhagic fever	1, 2	Proteome
Lordsdale virus	Norovirus, Caliciviridae	Human	Fecal-oral	Gastroenteritis	Not available	Proteome
Louping ill virus	Flavivirus, Flaviviridae	Human, mammals, ticks	Zoonosis, arthropod bite	Encephalitis	Genome	Proteome
Lymphocytic choriomeningitis virus	Arenavirus, Arenaviridae	Human, rodents	Zoonosis, fomite	Encephalitis	1, 2	Proteome
Machupo virus	Arenavirus, Arenaviridae	Human, monkeys, mouse	Zoonosis, fomite	Encephalitis	1, 2	Proteome
Mayaro virus	Alphavirus, Togaviridae	Human, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain	Genome	Proteome
MERS coronavirus	Betacoronavirus, Coronaviridae	Human, Tomb bat	Zoonosis	Respiratory	Genome	Proteome
Measles virus	Morbilivirus, Paramyxoviridae	Human	Respiratory	Fever, rash	Genome	Proteome
Mengo encephalomyocarditis virus	Cardiovirus, Picornaviridae	Human, mouse, rabbit	Zoonosis	Encephalitis	Not available	Proteome
Merkel cell polyomavirus	Polyomavirus, Polyomaviridae	Human	-	Merkel cell carcinoma	Genome	Proteome
Mokola virus	Lyssavirus, Rhabdoviridae	Human, rodents, cat, dog shrew	Zoonosis, animal bite	Encephalitis	Genome	Proteome
Molluscum contagiosum virus	Molluscipoxvirus, Poxviridae	Human	Contact	Skin lesions	Genome	Proteome
Monkeypox virus	Orthopoxvirus, Poxviridae	Human, mouse, prairie dog	Zoonosis, contact	Skin lesions	Genome	Proteome
Mumps virus	Rubulavirus,	Human	Respiratory, saliva	Mumps	Genome	Proteome

Virus	Genus, Family	Host	Transmission	Disease	genome	Proteome
	Paramyxoviridae					
Murray valley encephalitis virus	Flavivirus, Flaviviridae	Human, mosquitoes	Zoonosis, arthropod bite	Encephalitis	Genome	Proteome
New York virus	Hantavirus, Bunyavirus	Human, mouse	Zoonosis, urine, saliva	Hemorrhagic fever	Not available	Not available
Nipah virus	Henipavirus, Paramyxoviridae	Human, bats	Zoonosis, animal bite	Encephalitis	Genome	Proteome
Norwalk virus	Norovirus, Caliciviridae	Human	Fecal-oral	Gastroenteritis	Genome	Proteome
O'nyong-nyong virus	Alphavirus, Togaviridae	Human, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain	Genome	Proteome
Orf virus	Parapoxvirus, Poxviridae	Human, mammals	Zoonosis, contact	Skin lesions	Genome	Proteome
Oropouche virus	Orthobunyavirus, Bunyaviridae	Human, wild animals(sloths)	Zoonosis, arthropod bite	Fever, joint pain	1, 2, 3	Proteome
Pichinde virus	Arenavirus, Arenaviridae	Human, rat, guinea pig	Zoonosis, fomite	Hemorrhagic fever	1, 2	Proteome
Poliovirus	Enterovirus, Picornaviridae	Human, mammals	Fecal-oral	Poliomyelitis	Genome	Proteome
Punta toro phlebovirus	Phlebovirus, Bunyaviridae	Human, sandflies	Zoonosis, arthropod bite	Hemorrhagic fever	Not available	Not available
Puumala virus	Hantavirus, Bunyavirus	Human, bank vole	Zoonosis, urine, saliva	Hemorrhagic fever	1, 2, 3	Proteome
Rabies virus	Lyssavirus, Rhabdoviridae	Human, mammals	Zoonosis, animal bite	Fatal encephalitis	Genome	Proteome
Rift valley fever virus	Phlebovirus, Bunyaviridae	Human, mammals, mosquitoes, sandflies	Zoonosis, arthropod bite	Hemorrhagic fever	1, 2, 3	Proteome
Rosavirus A	Rosavirus, Picornaviridae	Human			Genome	Proteome
Ross river virus	Alphavirus, Togaviridae	Human, mosquitoes, marsupials	Zoonosis, arthropod bite	Fever, joint pain	Genome	Proteome
Rotavirus A	Rotavirus, Reoviridae	Human	Fecal-oral	Gastroenteritis	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	Proteome
Rotavirus B	Rotavirus, Reoviridae	Human	Fecal-oral	Gastroenteritis	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	Proteome
Rotavirus C	Rotavirus, Reoviridae	Human	Fecal-oral	Gastroenteritis	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	Proteome
Rubella virus	Rubivirus, Togaviridae	Human	Respiratory	Rubella	Genome	Proteome
Sagiyama virus	Alphavirus, Togaviridae	Human, horse, pig, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain	Not available	Proteome
Salivirus A	Salivirus, Picornaviridae	Human		Gastroenteritis	Genome	Proteome
Sandfly fever sicilian virus	Phlebovirus, Bunyaviridae	Human, sandflies	Zoonosis, arthropod bite	Hemorrhagic fever	Not available	Not available
Sapporo virus	Sapovirus, Caliciviridae	Human	Fecal-oral	Gastroenteritis	Genome	Proteome
Semliki forest virus	Alphavirus, Togaviridae	Human, birds, hedgehog, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain	Genome	Proteome
Seoul virus	Hantavirus, Bunyavirus	Human, rats	Zoonosis, urine, saliva	Hemorrhagic fever	1, 2, 3	Proteome

Virus	Genus, Family	Host	Transmission	Disease	genome	Proteome
Simian foamy virus	Spumavirus, Retroviridae	Human, monkeys	Zoonosis, contact	None	Genome	Proteome
Simian virus 5	Rubulavirus, Paramyxoviridae	Human, dog	Zoonosis, contact	Undocumented	Not available	Proteome
Sindbis virus	Alphavirus, Togaviridae	Human, birds, mosquitoes	Zoonosis, arthropod bite	Pogosta_disease Fever, joint pain	Genome	Proteome
Southampton virus	Norovirus, Caliciviridae	Human	Fecal-oral	Gastroenteritis	Not available	Proteome
St. louis encephalitis virus	Flavivirus, Flaviviridae	Human, birds, mosquitoes	Zoonosis, arthropod bite	Encephalitis	Genome	Proteome
Tick-borne powassan virus	Flavivirus, Flaviviridae	Human, ticks	Zoonosis, arthropod bite	Encephalitis	Genome	Proteome
Torque teno virus	Alphatorquevirus, Anelloviridae	Human	Sexual, blood	None	Genome	Proteome
Toscana virus	Phlebovirus, Bunyaviridae	Human, mosquitoes	Zoonosis, arthropod bite	Hemorrhagic fever	1, 2, 3	Proteome
Uukuniemi virus	Phlebovirus, Bunyaviridae	Human, ticks	Zoonosis, arthropod bite	Hemorrhagic fever	1, 2, 3	Proteome
Vaccinia virus	Orthopoxvirus, Poxviridae	Human, mammals	Contact	None	Genome	Proteome
Varicella-zoster virus	Varicellovirus, Herpesviridae	Human	Respiratory, contact	Varicella	Genome	Proteome
Variola virus	Orthopoxvirus, Poxviridae	Human	Respiratory	Variola	Genome	Proteome
Venezuelan equine encephalitis virus	Alphavirus, Togaviridae	Human, rodents, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain	Genome	Proteome
Vesicular stomatitis virus	Vesiculovirus, Rhabdoviridae	Human, cattle, horse, pig, flies	Zoonosis, athropod bite	Encephalitis	Genome	Proteome
Western equine encephalitis virus	Alphavirus, Togaviridae	Human, vertebrates, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain	Genome	Proteome
WU polyomavirus	Polyomavirus, Polyomaviridae	Human	Respiratory fluids or urine	None	Genome	Proteome
West Nile virus	Flavivirus, Flaviviridae	Human, birds, ticks, mosquitoes	Zoonosis, arthropod bite	Encephalitis	Genome	Proteome
Yaba monkey tumor virus	Orthopoxvirus, Poxviridae	Human, monkeys	Zoonosis, contact	None	Genome	Proteome
Yaba-like disease virus	Orthopoxvirus, Poxviridae	Human, monkeys	Zoonosis, contact	None	Genome	Proteome
Yellow fever virus	Flavivirus, Flaviviridae	Human, monkeys, mosquitoes	Zoonosis, arthropod bite	Hemorrhagic fever	Genome	Proteome
Zika virus	Flavivirus, Flaviviridae	Human, monkeys, mosquitoes	Zoonosis, arthropod bite	Fever, joint pain, rash	Genome	Proteome

APPENDIX II

Baltimore System: Seven Categories of Human Viruses[137]

Herpesviridae
Simplexvirus
Varicellovirus
Cytomegalovirus
Roseolovirus
Lympho-cryptovirus
Rhadinovirus

Adenoviridae
Mastadenovirus
Papillomaviridae
Alpha-papillomavirus
Beta- papillomavirus
Chi- papillomavirus
Gamma-papillomavirus
Mupapillomavirus
Nupapillomavirus
Polyomaviridae
Alphapolyomavirus
Betapolyomavirus
Gamma-polyomavirus
Deltapolyomavirus

Poxviridae
Molluscipoxvirus
Orthopoxvirus
Parapoxvirus

Anelloviridae
Alphatorquevirus
Betatorquevirus
Gammatorquevirus

Circoviridae
Cyclovirus
Genomoviridae
Gemycircular-viruses
Gemykibivirus
Gemyvongvirus
Parvoviridae
Erythrovirus
Dependovirus
Bocavirus

Hepadnaviridae
Orthohepadnavirus
Retroviridae
Gammaretrovirus
Deltaretrovirus
Lentivirus
Simiispumavirus

(Picobirnaviridae?)
(Picobirnavirus?)
Reoviridae
Coltivirus
Rotavirus
Seavirusadorn

Coronaviridae
Alphacoronavirus
Betacoronavirus
Torovirus

Astroviridae
Mamastrovirus
Caliciviridae
Norovirus
Sapovirus

Flaviviridae
Flavivirus
Hepacivirus
Pegivirus

Hepeviridae
Orthohepevirus
Picornaviridae
Cardiovirus
Cosavirus
Enterovirus
Hepatovirus
Kobuvirus

Parechovirus
Rosavirus
Salivirus

Togaviridae
Alphavirus
Rubivirus

Filoviridae
Ebolavirus
Marburgvirus

Paramyxoviridae
Henipavirus
Morbilivirus
Respirovirus
Rubulavirus

Pneumoviridae
Metapneumovirus
Orthopneumovirus
Rhabdoviridae
Ledantevirus
Lyssavirus
Vesiculovirus
Arenaviridae

Mammarenavirus
Hantaviridae
Orthohantavirus
Nairoviridae
Orthonairovirus
Peribunyaviridae
Orthobunyavirus
Phenuiviridae
Phlebovirus
Orthomyxoviridae
Alphainfluenzavirus
Betainfluenzavirus
Gammainfluenzavirus
Quaranjavirus
Thogotovirus

Deltavirus

[137] From https://viralzone.expasy.org/656--(Source: ViralZone:www.expasy.org/viralzone, SIB Swiss Institute of Bioinformatics)

APPENDIX III

Values of the Chi-Squared Distribution

	Probability										
DF	**0.995**	**0.975**	**0.20**	**0.10**	**0.05**	**0.025**	**0.02**	**0.01**	**0.005**	**0.002**	**0.001**
1	0.0000393	0.000982	1.642	2.706	3.841	5.024	5.412	6.635	7.879	9.550	10.828
2	0.0100	0.0506	3.219	4.605	5.991	7.378	7.824	9.210	10.597	12.429	13.816
3	0.0717	0.216	4.642	6.251	7.815	9.348	9.837	11.345	12.838	14.796	16.266
4	0.207	0.484	5.989	7.779	9.488	11.143	11.668	13.277	14.860	16.924	18.467
5	0.412	0.831	7.289	9.236	11.070	12.833	13.388	15.086	16.750	18.907	20.515
6	0.676	1.237	8.558	10.645	12.592	14.449	15.033	16.812	18.548	20.791	22.458
7	0.989	1.690	9.803	12.017	14.067	16.013	16.622	18.475	20.278	22.601	24.322
8	1.344	2.180	11.030	13.362	15.507	17.535	18.168	20.090	21.955	24.352	26.124
9	1.735	2.700	12.242	14.684	16.919	19.023	19.679	21.666	23.589	26.056	27.877
10	2.156	3.247	13.442	15.987	18.307	20.483	21.161	23.209	25.188	27.722	29.588
11	2.603	3.816	14.631	17.275	19.675	21.920	22.618	24.725	26.757	29.354	31.264
12	3.074	4.404	15.812	18.549	21.026	23.337	24.054	26.217	28.300	30.957	32.909
13	3.565	5.009	16.985	19.812	22.362	24.736	25.472	27.688	29.819	32.535	34.528
14	4.075	5.629	18.151	21.064	23.685	26.119	26.873	29.141	31.319	34.091	36.123
15	4.601	6.262	19.311	22.307	24.996	27.488	28.259	30.578	32.801	35.628	37.697
16	5.142	6.908	20.465	23.542	26.296	28.845	29.633	32.000	34.267	37.146	39.252
17	5.697	7.564	21.615	24.769	27.587	30.191	30.995	33.409	35.718	38.648	40.790

APPENDIX IV

The Long and Winding Road

The long and winding road
That leads to the cure.
Will it ever disappear?
I've seen that road before.
It always leaves me here . . .
Leaves me insecure

Those long and sleepless nights
The fatigue and the pain.
I forget just who I am
It messes up my brain.
Why leave me lying here?
Let me know the way.

Many times I've been alone
And many times I cry for. . .
All of us who suffer most
And some of us who die . . .

But still I must go on
On that long, winding road. . .
Will it be inteferon
To keep the promise you all swore
“We are finally on the road
That leads to the cure.”

The long and winding road
That leads to the cure.
At times, my balance goes
And I wind up on the floor.
Please help me, someone, please…
I can’t get up anymore.

Many times I've been alone
And many times I cry for. . .
All of us who suffer most . . .
And some of us who die. . .

Still I try to keep my faith
On the long winding road. . .
We are all still lying here
From a long, long time ago.
Don't leave us waiting, anymore.
Lead us to the cure!

Yeah, Yeah, Yeah, Yeah.

(Based upon The Beatles "The Long and Winding Road.")

GLOSSARY[138]

Allele: A variation of a given gene. Most genes have two variations, but some cells and viruses have 20 or more and produce different strains of the original. The Epstein-Barr virus has over a dozen alleles.

Antibody: Also known as an immunoglobulin. It is a large, Y-shaped protein produced mainly by Plasma Cells of the immune system which recognizes and neutralizes *pathogens* such as bacteria and viruses. The antibody recognizes a unique molecule of the pathogen, called an *antigen.* Each tip of the "Y" of an antibody contains a *paratope* (analogous to a socket) that is specific for one particular *epitope* (analogous to a plug) on the antigen, allowing the two structures to bind together with precision.

Antigen: A receptor found on the cell membrane of pathogens that stimulate production of and/or are recognized by antibodies. Sometimes antigens reside on the host itself as in an autoimmune disease. Antigens are "targeted" by antibodies. Antibodies produced by the immune system proliferate and match each antigen it comes in contact with.

Axon: A long, slender projection of a nerve cell, or neuron in vertebrates, that typically conducts electrical impulses away from the cell body. Axons transmit information to different neurons, muscles, and glands by both chemical and electrical means. Axons are surrounded by a fatty layer called *myelin* that protects them and helps in the conduction of the nerve impulse. One of the major signs of MS is the breaking-up of the myelin in various parts of the Central Nervous System which interferes with conduction of the nerve impulse.

Baltimore Classification System: A viral classification system that groups viruses into families, depending on their type of genome (i.e. DNA, RNA, single-stranded, double-stranded) and their method of replication.

B-Cell: A white blood cell of the lymphocyte subtype, which is a mainstay of the immune system. They function in the *humoral* immunity component of the specific immune system by secreting antibodies. B-Cells, unlike the other two classes of lymphocytes, *T-Cells* and *Natural Killer Cells*, express B-Cell receptors (BCRs) on their cell membrane. BCRs allow the B-Cell to bind to a specific antigen, against which it will initiate an antibody response. Some B-Cells can also engulf pathogens and present them on their surface. These are known as Professional *Antigen Presenting Cells.*

Capsid: A viral protein structure in which the genetic material of a virus is stored. The capsid is a "shield" that protects the viral nucleic acids from getting degraded by host enzymes or other types of pesticides or pestilences. It also functions to attach the *virion* to its host, and enable the virion to penetrate the host's cell membrane. Many copies of a single viral protein or a number of different viral proteins make up the capsid, and each of these viral proteins are coded for by one gene from the viral genome.

[138] The glossary is mainly, but not totally, excerpts from Wikipedia. The intention was to define each entry in the least technical language possible.

Case-Control Studies (CSS): *Case-control* studies define two groups at the *start*, one with the disease and one without (the control group). Researchers then look *backwards* to a specific *exposure(s)* or *risk factor(s)* to assess whether they may have contributed to the disease, based upon a statistically significant difference between the two groups. Unlike cohort studies, CSS are *a*lways *retrospective* because the outcome has already occurred. CSS have specific advantages compared to other study designs. They are comparatively short-term and inexpensive. Since they start with subjects having the disease (rather than starting with a population free of disease and waiting to see who develops it), it is possible to enroll a sufficient number of patients with a *rare disease.*

Cerebral Spinal Fluid (CSF): A clear, colorless body fluid found in the brain and spinal cord. A sample of CSF can be taken via lumbar puncture. This can reveal the intracranial pressure, as well as indicate diseases, including infections of the brain.

Chromosome: Thread-like molecules that carry hereditary information for everything from height to eye color. They are made of protein and one molecule of DNA, which contains an organism's genetic instructions, passed down from parents. Specifically, the information is contained in *genes* which number 50 to 500 for human chromosomes. In humans, chromosomes are arranged in pairs within the nucleus of a cell. Humans have 22 pairs and an additional pair of sex chromosomes for a total of 46. The sex chromosomes are referred to as X and Y, with the mother providing the X chromosome and the father the Y chromosome, which combined determines the sex of their offspring.

Cohort Studies: A *cohort* is a group of individuals who share a common characteristic or experience, such as being a certain age, living in a certain area, treated with a certain drug or vaccine, or being exposed to one or more pollutants. They are then followed for a defined period of time which could be years or even decades to see if the exposure, characteristic, or experience results in some *outcome* (i.e. disease).

Cluster Studies: Cluster Studies are a common technique for statistical data analysis in a number of disciplines. In general, they involve comparing the observed number of cases with the number expected, based upon size, age and other demographics of a population. They also can be used to investigate an unexpectedly high prevalence of a disease in a location, by time of year, or because of an unusual event in a defined area, such as a state, city, town or even neighborhood.

Legionaries Disease (a form of very virulent pneumonia) in Philadelphia during the American Legion’s annual three-day convention in 1976 is one example of cluster analysis which found that the Bellevue-Stratford Hotel’s cooling tower for air conditioning bred the bacteria.

Dependent Variable: In medical studies, it refers to the occurrence, or the improvement of a disease or condition based upon one or more factors. Each factor studied is known as the *independent variable*.

Ecologic Studies: *Ecologic Studies* evaluate the relationship between one or more environmental agents and disease in a specific population, such as city dwellers, rural areas, a county, or even a community. Average measures of exposure of some variable, and disease frequency are obtained for each aggregate, and the analyst tries to determine whether or not high levels of the variable are associated with high disease rates. Often the information about disease and exposure is abstracted from published statistics, and therefore does not require expensive or time consuming data collection.

Expanded Disability Status Scale (EDSS): A functional test to assess the disability of an MS patient, scaled from 0 to10 in .5 intervals. The higher the score, the higher the level of disability.

Epstein–Barr Virus (EBV): EBV is one of eight known human virus types in the *herpes* family, and is one of the most *common.* It is best known as the cause of *infectious mononucleosis*. It is also associated with various non-malignant, premalignant, and malignant lymphoproliferative diseases such as Burkitt's lymphoma, Hodgkin's lymphoma, and non-lymphoid malignancies such as gastric cancer and conditions associated with human immunodeficiency such as central nervous system lymphomas
Infection with EBV occurs by the oral transfer of saliva and genital secretions. Most people become infected with EBV and gain adaptive immunity. In the United States, about half of all five-year-old children and about 90% of adults have evidence of previous infection. Childhood infections usually cause no symptoms or are indistinguishable from other mild, brief illnesses. When infection with EBV occurs during adolescence, it can cause infectious mononucleosis up to 50 percent of the time. Once EBV's initial infection is brought under control, EBV latency persists in the B-Cells for the rest of the individual's life.

Epidemiological Studies (EPS): Epidemiological Studies measure *outcomes* (diseases) in a population related in one or more ways that is thought to be associated with one or more variables, and/or seek to understand how often diseases occur in different populations and the factor(s) that might give a *clue* as to the reasons. Information gathered is used to plan and/or evaluate strategies to reduce/prevent the illness, and to guide the management of patients in whom disease has already developed. Like clinical findings and laboratory tests, such as MRIs and blood samples, the epidemiology of a disease is an integral part of its basic description.

Epithelial Cells: One of the four basic types of animal tissue, along with connective tissue, muscle tissue and nervous tissue. Epithelial tissues line the outer surfaces of organs and blood vessels throughout the body, as well as the inner surfaces of cavities in many internal organs. An example is the epidermis, the outermost layer of the skin.

All glands are made up of epithelial cells. Functions of epithelial cells include secretion, selective absorption, protection, transcellular transport, and sensing. Epithelial layers contain no blood vessels, so they must receive nourishment via diffusion of substances from the underlying connective tissue, through the basement membrane.

Epitope: The part of an antigen that is recognized by the immune system, specifically by antibodies, B-Cells, or T-Cells. The epitope is the specific piece of the antigen to which an antibody binds. The part of an antibody that binds to the epitope is called a *paratope*.

Gene: A gene is a region of DNA that encodes function. A chromosome consists of a long strand of DNA containing many genes. A human chromosome can have up to 500 million base pairs of DNA with thousands of genes.

During gene expression, the DNA is first copied into RNA. The RNA can be directly functional or be the intermediate template for a protein that performs a function. The transmission of genes to an organism's offspring is the basis of the inheritance of phenotypic traits. Genes can acquire mutations in their sequencing, leading to different variants, known as alleles.

Genome: The genetic material of an organism. It consists of DNA (or RNA in RNA viruses).

Glial Cells: Surround CNS neurons and provide support for and insulation between them. Glial cells are the most abundant cell types in the Central Nervous System. There are several types of glial cells, including *oligodendrocytes*, *astrocyte*s, *ependymal cells, Schwann cells,*

Human Leukocyte Antigen (HLA) System/Complex: is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface proteins are responsible for the regulation of the immune system. HLA genes are highly polymorphic, which means that they have many different alleles, allowing them to fine-tune the specific immune system. The proteins encoded by certain genes are known as antigens, as a result of their historic discovery as factors in organ transplants.

The proteins encoded by HLAs are those on the outer part of body cells that are (in effect) unique to that person. The immune system uses the HLAs to differentiate self cells and non-self cells. Any cell displaying a person's HLA type belongs to that person and, therefore, is not an invader.

When a foreign pathogen enters the body, specific cells called antigen-presenting cells (APCs) engulf the pathogen through a process called phagocytosis. Proteins from the pathogen are digested into small pieces and loaded onto HLA antigen. They are then displayed by the antigen-presenting cells to CD4+ helper T-Cells, which then produce a variety of effects to eliminate them.

Humoral Immunity: That part of the immune system in which macromolecules, such as antibodies, are found in body (extracellular) fluid that protect the organism from foreign invaders (i.e. pathogens). Also known as *antibody-mediated immunity*.

Interferon (IFN): Refers to a group of "signaling" proteins released by host cells in response to the presence of certain viruses. The virus-infected cell will release interferon causing nearby cells to heighten their anti-viral defenses. IFNs belong to a class of proteins known as *cytokines*, molecules used for communication between cells to trigger the protective defenses of the immune system. IFNs have various other functions as well: they activate immune cells, such as natural killer cells and macrophages, and increase host defenses by up-regulating antigen presentation.

Interferon drugs for treatment of MS include Rebif and Avonex.

Leukocytes: Another name for *White Blood Cells*

Locus (plural *loci*): Refers to the position on a chromosome, especially associated with genes. Each human chromosome contains many genes; approximately 20,000 total. The ordered list of loci known for a particular *genome* is called a gene map.

Lymph Nodes: Groups of "terminals" located in all over your body— about 600 in humans—that store several cells involved in the immune system. All lymphatic vessels drain into the blood.

Lymphatic System: A part of the vascular system and an important part of the immune system, comprising a large network of lymphatic vessels that carry a clear fluid called lymph towards the heart.

A major function is that of defense in the immune system. Lymph is very similar to blood plasma: it contains lymphocytes. It also contains waste products and cellular debris together with bacteria and proteins. Lymphocytes are concentrated in the lymph nodes.

Lymphocyte: A type of white blood cell in a vertebrate's immune system. Lymphocytes include Natural Killer Cells, T-Cells, and B-Cells.

Lysis: Refers to the breaking down of the membrane of a cell, often by viral, enzymic, or osmotic mechanisms that compromise its integrity. Cytolysis occurs when a cell bursts due to an osmotic imbalance that has caused excess water to move into the cell.

Macrophage: Macrophages are large white blood cell of the immune system, that engulfs and digests cellular debris, foreign substances, microbes, cancer cells, and anything else that does not have the type of proteins specific to healthy body cells on its surface in a process called phagocytosis. These large phagocytes are found in essentially all tissues, where they patrol for potential pathogens by amoeboid movement.

Macrophages play an important anti-inflammatory role and can decrease immune reactions through the release of cytokines. Macrophages can also increase inflammation. Those that encourage inflammation are called M1 macrophages, whereas those that decrease inflammation and encourage tissue repair are called M2 macrophages.

Major Histocompatibility Complex (MHC): A set of cell surface proteins essential for the acquired immune system to recognize foreign molecules in vertebrates, which in turn determines histocompatibility. The main function of MHC molecules is to bind to antigens derived from pathogens and display them on the cell surface for recognition by the appropriate T-cells. MHC molecules mediate interactions of leukocytes, which are immune cells, with other leukocytes or with body cells. The MHC determines compatibility of donors for organ transplant, as well as one's susceptibility to an autoimmune disease via crossreacting immunization. The human MHC is also called the HLA (Human Leukocyte Antigen) complex.

Mole: The mole is the unit of measurement for amount of substance in the International System of Units (SI). It is defined as the amount of a chemical substance that contains exactly 6.02214076×1023 (Avogadro's constant) constitutive particles, e.g., atoms, molecules, ions or electrons.

Natural Killer Cell (NK): A type of cytotoxic lymphocyte critical to the non-specific immune system. NK cells provide rapid responses to viral-infected cells, acting at around 3 days after infection, and respond to tumor formation. Typically, immune cells detect major histocompatibility complex (MHC) presented on infected cell surfaces, triggering cytokine release, causing lysis. NK cells are unique, however, as they have the ability to recognize stressed cells in the absence of antibodies and MHC, allowing for a much faster immune reaction. They do not require activation to kill cells that are missing "self" markers of MHC class 1. This role is especially important because harmful cells that are missing MHC-I markers cannot be detected and destroyed by other immune cells, such as T lymphocyte cells.

Neurotransmitters: Proteins that are instrumental in propagating nerve conduction from one neuron cell to another via chemical means. They can be divided into four categories:

Amino Acids: Examples: glutamate; gamma-aminobutyric acid [GABA]
Peptides: Example: endorphin
Monoamines: Examples: serotonin; histamine; dopamine; epinephrine
Other: Example: acetycholine

Nested Case Control Studies: (NCSS) A subtype of Case Control Studies, in which the investigator does not use the *entire* population as the control, but a *representative sample*.

Oligodendrocytes: Cells that provide support and insulation to axons in the Central Nervous System equivalent to the function performed by Schwann cells in the peripheral nervous system. Oligodendrocytes do this by creating the myelin sheath, which is 80% lipid and 20% protein. A single oligodendrocyte can extend its processes to 50 axons, Schwann cells, on the other hand, can wrap around only one axon. Each oligodendrocyte forms one segment of myelin for several adjacent axons.

Paratope: Also called an antigen-binding site, is a part of an antibody which recognizes and binds to an antigen. Each arm of the Y shaped antibody is tipped with a *paratope*, which is a set of complementary determining regions. The part of the antigen to which the paratope binds is called an *epitope*.

Pathogen: The generic term used for foreign cells that can cause the body harm, such as bacteria, viruses, fungi and parasites.

Phagocyte: *(Also known as Antigen Presenting Cells)* A white blood cell (Leukocyte) of the non-specific immune system that has two receptors that hook on to pathogens and wrap around it and engulf it. It then "digests it," but removes key proteins and attaches them to other proteins called of MHC Type II, and places them on its surface.

Placebo: Relates to the positive effect that occurs if an individual *believes* he is getting medicine, even when not. Thus, in studies testing the effects of a drug, one group is given what seems to be the drug, but is in effect getting only a water pill. Then the two groups are compared statistically to determine if the drug was beneficial to the group actually receiving it.

Plasma Cells: Also called Plasma B-Cells, or *Effector* B-Cells, are white blood cells that secrete large volumes of antibodies. They are transported by the blood plasma and the lymphatic system. Plasma cells originate in the bone marrow; B-Cells differentiate into plasma cells that produce antibody molecules closely modeled after the receptors of the precursor B-Cell. Once released into the blood and lymph, they bind to the target *antigen* and initiate its neutralization or destruction.

Prospective Study: Identifies subjects who were exposed to a variable—or variables—that is thought to be associated with a certain disease and followed over a given period of time.

Primary-Progressive MS (PPMS): Defined up to now, as a type of MS in which the initial symptoms tend to be mild and gradually worsen over time. There are no distinct flare-ups or periods of remission. About 10-15 percent of people with MS are diagnosed with PPMS. *We assert it is a separate disease and should not be considerted a subset of Multiple Sclerosis!*

Progressive-Relapsing MS (PRMS): A subtype of MS with steadily worsening neurologic function from the beginning with occasional relapses. New standards based on advances in the understanding of the disease process have placed PRMS into the PPMS category.

Relapsing-Remitting MS (RRMS): A subtype of MS in which symptoms appear suddenly and can be severe. The disease can then go into remission for months or years. Between "flare-ups," the disease tends not to progress or progresses relatively slowly, and symptoms may be gone. 85 percent of newly diagnosed patients fall into this category

Retrospective Study: Examines diseases (outcomes) that have already occurred, and looks backwards to factors (exposures) that they believe were related to those outcomes.
Retrovirus: A type of RNA virus that inserts a copy of its genome into the DNA of a host's cell, changing the genome. Such viruses are specifically classified as single-stranded positive-sense RNA viruses.

Once inside the host cell's cytoplasm, it produces DNA from its RNA genome, the reverse of the usual pattern, thus retro. The host cell then treats the viral DNA as part of its own make-up, transcribing and translating the viral genes along with the cell's own genes, producing the proteins required to assemble new copies of the virus. It is difficult to detect the virus until it has infected the host. At that point, the infection can persist indefinitely.

Schwann Cells: Are the principal glia of the Peripheral Nervous System (PNS). Glial cells function to support neurons. There are the two types of Schwann cells: myelinating and nonmyelinating. Myelinating Schwann cells wrap around axons of motor and sensory neurons to form the myelin sheath. They are also involved in many important aspects of peripheral nerve functions—the conduction of nervous impulses along axons, nerve development and regeneration, modulation of neuromuscular synaptic activity, and presentation of antigens to T-lymphocytes.

Secondary-Progressive MS (SPMS): A subtype of MS in which the disease tends to progress more steadily. This can happen with or without relapses. A number of those with RRMS may transition to SPMS at some point in the course of their disease.

Serotype (Serovar): A distinct variation within a species of bacteria or virus or among immune cells of different individuals. These microorganisms, viruses, or cells are classified together based on their cell surface antigens, allowing the epidemiologic classification of organisms to the sub-species level.

Steroid: A biologically active organic compound with four rings arranged in a specific molecular configuration. Steroids have two principal biological functions: as important components of cell membranes which alter membrane fluidity; and as signaling molecules. Hundreds of steroids are found in plants, animals and fungi.

Significance: Relates to the result in a study between two or more groups. The raw data of each group are run though statistical tests appropriate to the study which calculates the probability that the results only occurred by chance. By convention, most studies use either .01 or .05 as their standard. That is, if the probability of the results was less than (<.01), the study would conclude whatever variable(s) was being tested had an effect that did not occur by chance.

Titer: Measures how much antibody an organism has produced that recognizes a particular epitope.

T-Cell: A type of lymphocyte that plays a central role in cell-mediated immunity. T-Cells can be distinguished from other lymphocytes, such as B-Cells and Natural Killer Cells, by the presence of a T-cell receptor on the cell surface. Several subsets of T-Cells each have a distinct function. The majority of human T-Cells rearrange their alpha and beta chains on the cell receptor and are part of the specific immune system. Types of T-Cells include:
Memory-T-Cells
Helper T-Cells

Cytotoxic T-Cells attack cells that have infiltrated other cells, as opposed to free floating in the fluid. When T-Cells split, each daughter cell has a different receptor. Both B and T-Cells when mature migrate to *lymph nodes* where they await until called for "duty."

Virus: A virus is a small infectious agent that can only replicate inside the cells of an organism. They can infect all types of life forms, from animals and plants to microorganisms, even bacteria. Grouped according to whether they are structural proteins, nonstructural proteins, regulatory, or accessory proteins, they depend on their host's cell's metabolism for energy, enzymes, and precursors, in order to reproduce. While not inside an infected cell, viruses exist in the form of independent particles. These viral particles, are known as *virion*s, and consist of either (1) DNA or RNA, long molecules that carry genetic information; (2) a protein coat, called the *capsid*, which surrounds and protects the genetic material, and in some cases (3) an envelope of lipids that surrounds the protein coat.

THE END