Journal of Academic Inquiry and Knowledge Volume 2024 (4) 70-80
ISBN 979-8-9888908-2-9

The Limitations and Drawbacks of Using Experimental Autoimmune Encephalomyelitis to Study Multiple Sclerosis

Bryan Kim

Los Angeles, CA, USA

The Limitations and Drawbacks of Using Experimental Autoimmune Encephalomyelitis to Study Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE) is the most widely accepted animal model for multiple sclerosis (MS) (Ucar et al., 2024). MS is a common, chronic, autoimmune disease that affects the brain and central nervous system (CNS), which leads to demyelination, causing disability through loss of motor function (Melamed et al., 2022). MS is not yet curable, but great strides are being made to develop better treatments and to find a cure. EAE is vital in these efforts (Glatigny & Bettelli, 2018). However, while EAE has been pivotal in researching MS, the model has limitations, such as being unable to produce some symptoms of human MS (Glatigny & Bettelli, 2018; Voskuhl & MacKenzie-Graham, 2022). This paper demonstrates how various EAE types are used to research MS, and the animal model's limitations and drawbacks.

Background

MS is a chronic, inflammatory, demyelinating autoimmune disease of the CNS (Rossi & Constantin, 2016). The most common type of MS is relapsing-remitting MS (RRMS) (Rossi & Constantin, 2016), making up 85% of cases (Francis et al., 2023). RRMS occurs when symptoms

worsen for days or weeks, followed by weeks to months of recovery (Francis et al., 2023). RRMS typically leads to another form of MS known as secondary progressive MS (SPMS) over the years (Dobson & Giovannoni, 2018; Rossi & Constantin, 2016). SPMS is a period of disease progression following the relapsing-remitting stage (Francis et al., 2023). In contrast, primary progressive MS (PPMS) is characterized by steady progression without relapse (Francis et al., 2023). The final type of MS is progressive-relapsing MS (PRMS), which is distinguished by the progression of the disease with some relapses or acute worsening of the condition.

Despite extensive studies, the exact etiology of MS is unknown (Bjelobaba et al., 2018; Bittner et al., 2014). Some studies suggest an association between the development of MS and environmental and genetic factors (Bjelobaba et al., 2018). These factors may be the Epstein-Barr virus (EBV), sunshine (UVB) exposure, vitamin D, and smoking (Alfredsson & Olsson, 2019; Dobson & Giovannoni, 2018; Melamed, Palmer, & Fonken, 2022). MS typically presents in young adults aged 20-40 (Melamed et al., 2022) and is almost three times as prevalent in women (Bjelobaba et al., 2018). While the cause of MS is unknown, research using EAE tends to back the hypothesis that MS is an autoimmune response in the CNS (Glatigny & Bettelli, 2018).

Since its initial discovery over 90 years ago, EAE has broadened our understanding of MS (Steinman et al., 2023). The first name for the experimental disease was acute disseminated encephalomyelitis (ADEM). This term is still used today as the disease is seen in humans. In 1947 and 1948, the disease was labeled experimental 'allergic' encephalomyelitis. In 1978, the name experimental 'autoimmune' encephalomyelitis was born, which is what EAE stands for (Steinman et al., 2023). EAE is typically induced by injecting a myelin protein alongside an injection of pertussis toxin on the day of immunization and two days post-immunization, and a mixture of complete Freund’s adjuvant as the protein alone would not be enough to stimulate an immune response (Steinman et al., 2023; Bjelobaba et al., 2018). Immunization primes myelin-

specific T-cells. The activated cells then mature and multiply. Once grown and multiplied, they reach the CNS and reactivate, causing demyelination (Bjelobaba et al., 2018). This is known as *active EAE*, the induction of EAE through immunization (Burrows et al., 2019; Diebold et al., 2023).

EAE can also be induced by transferring encephalitogenic T-cells from an animal with EAE to one without it, typically a mouse. This proves that MS is a T-cell-mediated disease (Glatigny & Bettelli, 2018). Transfer of encephalitogenic T-cells to cause EAE is called passive EAE (Burrows et al., 2019). EAE is excellent for studying MS as it mimics many of the same symptoms, such as demyelination, inflammatory responses, axonopathy, CNS penetration, and neuronal loss (Diebold et al., 2023; Bjelobaba et al., 2018; Kanellopouloss, 2015). Over the years, several animal models have been created to target specific symptoms because one model cannot reproduce all symptoms (Melamed et al., 2022). Several models are necessary to mimic the various symptoms and types of MS.

Guiding Questions

1. What are the types of EAE, and how are they used to research MS?
2. What are the limitations and drawbacks of EAE

Types of EAE

Myelin Oligodendrocyte Glycoprotein (MOG) 35-55 Induced EAE

Myelin Oligodendrocyte Glycoprotein (MOG) is a myelin protein comprising an extremely minuscule portion of membrane proteins, about 0.01%-0.05% (Glatigny & Bettelli, 2018). MOG-specific T-cells are a type of myelin-specific T-cell and follow the cycle mentioned previously. The MOG-specific T-cells get primed, mature, multiply, and spread to the CNS, causing demyelination (Bjelobaba et al., 2018). For example, scientists will induce EAE in C57BL/6 (B6) mice, one of the most common animals used in EAE studies (Burrows et al.,

2019), and immunize them with MOG to study the chronic form of MS, primary progressive MS (Melamed et al., 2022). This is one way scientists have researched and discovered more about MS pathogenesis (Bittner et al., 2014).

A chronic, non-remitting EAE can be produced by injecting between 50 and 300 µg (µg = one-millionth of a gram) of MOG 35-55 into the B6 mice (Steinman et al., 2023; Burrows et al., 2019; Carvo-Barreiro et al., 2020). When 300 µg of MOG was injected, symptoms were observed to start between 11 and 15 days after induction and did not see recovery for at least four months. In the same study, an injection of 50 µg saw a relapsing-remitting EAE course with less myelin loss and total damage (Burrows et al., 2019). On the other hand, Bittner and colleagues (2014) did a study in which they injected 200 µg of MOG into the mice. They saw symptoms arise between 10 and 20 days (Bittner et al., 2014). A different study conducted by Yan et al. (2022) was done to see how different treatments affected the mean clinical score of the mice. This study found that Fingolimod, a current treatment for MS, given at 0.5 milligrams per kilogram (mpk), worked best. Along with Fingolimod, the effects of BTKI-PRN2246 (PRN2246) were examined, but almost the same results were achieved as those of the control group. Researchers used medications Secukinumab and Telitacicept to treat hIL17A/hIL17F mice immunized with MOG 35-55. Of the C57BL/6 mice, Fingolimod worked much better than the PRN2246. Between the hIL17A/hIL17F mice, Telitacicept showed a more aggressive effect than the vehicle control, and the Secukinumab saw significantly better conditions than both (Yan et al., 2022).

Scientists use another strain of mice, non-obese diabetic (NOD) mice with MOG-induced EAE(Glatigny & Bettelli, 2018; Melamed et al., 2022; Miyamura et al., 2019). When EAE is induced in NOD mice, a disease course similar to that of SPMS is observed. This can help study the pathogenesis of SPMS (Glatigny & Bettelli, 2018; Melamed et al., 2022). While many

scientists agree that inducing MOG into NOD mice produces a disease course similar to that of SPMS, others disagree. Baker et al. (2019) claim that MOG-induced NOD mice produce a relapsing disease course that is not reflective of chronic, progressive MS. However, it should be noted that Baker and colleagues did not run an experiment using MOG-induced NOD mice. These studies indicate that MOG-induced EAE can be valuable for researching and finding MS treatment breakthroughs.

Proteolipid Protein (PLP) 139-151 Induced EAE

Proteolipid Protein (PLP) is a vital transmembrane protein in the CNS that is responsible for CNS myelin compaction (Glatigny & Bettelli, 2018). PLP was the first myelin component found to be encephalitogenic; however, it was initially thought to be contaminated with myelin basic protein (MBP). Only in the 1980s was a purified form of PLP found to be encephalitogenic (Glatigny & Bettelli, 2018). Induction of PLP in SJL/J mice produces a disease course that represents RRMS (Glatigny & Bettelli, 2018; Melamed et al., 2022; Voskuhl & MacKenzie-Graham, 2022; Wang et al., 2017; Ucar et al., 2024; Diebold et al., 2023). PLP-induced EAE can be administered actively or passively. Active EAE is achieved with immunization of CNS peptides; in this situation, PLP, or one of its immunodominant epitopes, is present in susceptible strains of mice. Passive EAE can be induced through the adoptive transfer of encephalitogenic T-cells (Burrows et al., 2019; Glatigny & Betelli, 2018).

PLP is typically induced in SJL/J mice but Ucar et al. (2024) caused it in BALB/c (BalbC) mice. While SJL/J and BALB/c mice are different strains, it should be noted that a relapsing-remitting EAE disease course was observed in the BALB/c mice, similar to the SJL/J mice (Ucar et al., 2024). This study observed the effects of Carbenoxolone (CBX), a hemichannel blocker typically used to treat inflammation and ulceration of the gastroesophageal tract, on fibrosis and amelioration (Davis, 2019). Of the four groups of BalbC mice, three were

injected with 100 µg of PLP, and one was used as a control. Of the three groups injected, only two were treated with CBX. One group had the CBX injected intracerebroventricularly (CBX ICV), and another had the CBX injected intraperitoneally (CBX IP). After letting the experiment run for 20 days, the CBX IP group yielded the best results, and the CBX ICV group did almost the same (Ucar et al., 2024). The multiple models of EAE, as shown from the reviewed studies, are great for researching different types of MS. However, despite the many successes with EAE, the model has limitations.

Limitations and Drawbacks

No animal model can perfectly replicate all of the effects of MS (Glatigny & Bettelli, 2018; Voskuhl & MacKenzie-Graham, 2022) and EAE is no exception. For example, in human MS, lesions are formed in the brain and the spinal cord. However, when EAE is induced in mice, lesions are found mainly in the spinal cord but are lacking in the brain. (Melamed, Palmer, & Fonken, 2022). Further, human MS typically activates B-cells and produces MOG-specific antibodies, which MOG 35-55 fails to do effectively with EAE (Voskuhl & MacKenzie-Graham, 2022). Other limitations are present when looking at the mode of induction. When immunizing and inducing EAE into mice, the process typically also includes an adjuvant that changes how the immune system reacts to the disease (Bittner et al., 2014). This can be a problem as humans do not get MS through adjuvant induction.

Despite much research into EAE, finding a working treatment in humans has been exceptionally difficult (Hart et al., 2015; Baker et al., 2019). This might be partially due to differences in the genetic makeup of rodents and humans. This causes treatments that work on mice not to work when applied to humans (Melamed et al., 2022). Also, there is the problem of high research expenses. The National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORT) (2024) showed that between 2016 and 2023, almost one billion

dollars has gone into MS research (National Institutes of Health, 2024). These drawbacks make the goal of the study difficult to achieve.

Discussion

Experimental autoimmune encephalomyelitis (EAE) has facilitated the understanding of multiple sclerosis (MS) pathogenesis through its ability to mimic several key aspects of the disease, such as demyelination, inflammatory responses, and neuronal loss (Diebold et al., 2023; Bjelobaba et al.; 2018, Kanellopoulos, 2015). It should be noted that all myelin proteins are injected alongside a mixture of complete Freund's adjuvant (CFA), as the protein alone would not elicit an autoimmune response (Steinman et al., 2023; Bjelobaba et al., 2018). Myelin oligodendrocyte glycoprotein (MOG) 35-55 induction studies have shown a disease course that can be compared to primary-progressive MS (PPMS) (Melamed et al., 2022). Bittner et al. (2014) found that symptoms of EAE began to arise after 10 to 20 days when 200 µg of MOG was injected into C57BL/6 (B6) mice (Bittner et al., 2014). Burrows and colleagues (2019) injected 300 µg and 50 µg of MOG into B6 mice. They saw symptoms break out between 11 and 15 days in the mice with 300 µg of MOG. They also noticed that 50 µg of MOG induced a relapsing-remitting disease course when MOG induction typically causes a chronic progressive disease course (Burrows et al., 2019; Melamed et al., 2022; Diebold et al., 2023). Studies show that a disease course similar to secondary-progressive MS (SPMS) is observed when non-obese diabetic (NOD) mice are used in MOG-induced EAE studies (Glatigny & Bettelli, 2018; Melamed et al., 2022). However, some researchers claim a relapsing disease course is produced (Baker et al., 2019).

Proteolipid protein (PLP) induced EAE studies have shown a disease course representing relapsing-remitting MS (RRMS) in SJL/J mice. Ucar and colleagues (2024) injected three groups of BALB/c (BalbC) mice with 100 µL of PLP emulsified in 100 µL of complete Freund's

adjuvant (CFA). They treated two of those groups with carbenoxolone (CBX), a gastroesophageal ulcer and inflammatory treatment (Davis, 2019). One group was treated with CBX intracerebroventricularly (CBX ICV), and the other was treated intraperitoneally (CBX IP). After 20 days, this study showed the best results from the CBX IP group; however, the CBX ICV injection did almost the same. While the type of mouse was different, the disease took the same course (Ucar et al., 2024).

Animal models will always have shortcomings, as each cannot reproduce all disease symptoms, such as MS (Glatigny & Bettelli, 2018; Voskuhl & MacKenzie-Graham, 2022). For example, Melamed, Palmer, and Fonken (2022) found that lesions formed mainly in the spinal cord with few lesions in the brain. This is a problem because humans with MS typically form lesions in the brain and the spinal cord (Melamed et al., 2022). In MS, B-cell activity and MOG-specific antibody production are prominent. When examining EAE, the immune systems of animals cannot efficiently activate B-cells and produce MOG-specific antibodies (Voskuhl & MacKenzie-Graham, 2022). On top of the roadblocks during experimentation, applying the findings to building a treatment or a cure has been difficult (Hart et al., 2015; Baker et al., 2019). This can be due to the different genetic makeup of rodents and humans (Melamed et al., 2022).

Conclusion

EAE is critical to MS research. EAE has helped scientists develop several treatments for MS patients, relieving some of the disease's discomfort. EAE continues to provide information about the pathogenesis and physiopathology of MS, which deepens our understanding of the disease. If new information is continuously being discovered, a cure may be seen in the foreseeable future. Although the EAE study has produced many benefits, the frequent testing on mice may be unethical. As unfortunate as it is, mice are the best animal models for MS research, so their use will likely continue. EAE has been essential in MS research and is the best chance of finding a

cure.

References

Alfredsson, L., & Olsson, T. (2018). Lifestyle and environmental factors in multiple sclerosis. Cold Spring Harbor Perspectives in Medicine, 9(4), a028944.

Baker, D., Nutma, E., O'Shea, H., Cooke, A., Orian, J. M., & Amor, S. (2019). Autoimmune encephalomyelitis in NOD mice is not initially a progressive multiple sclerosis model. Annals of Clinical and Translational Neurology, 6(8), 1362–1372.

Bittner, S., Afzali, A. M., Wiendl, H., & Meuth, S. G. (2014). Myelin Oligodendrocyte Glycoprotein (MOG₃₅₋₅₅) Induced Experimental Autoimmune Encephalomyelitis (EAE) in C57BL/6 Mice. Journal of Visualized Experiments, 86.

Bjelobaba, I., Begovic-Kupresanin, V., Pekovic, S., & Lavrnja, I. (2018). Animal models of multiple sclerosis: Focus on experimental autoimmune encephalomyelitis. Journal of Neuroscience Research, 96(6), 1021–1042.

Burrows, D. J., McGown, A., Jain, S. A., De Felice, M., Ramesh, T. M., Sharrack, B., & Majid, A. (2018). Animal models of multiple sclerosis: From rodents to zebrafish. Multiple Sclerosis, 25(3), 306–324.

Calvo-Barreiro, L., Eixarch, H., Ponce-Alonso, M., Castillo, M., Lebrón-Galán, R., Mestre, L., Guaza, C., Clemente, D., Del Campo, R., Montalban, X., & Espejo, C. (2020). A commercial probiotic induces tolerogenic and reduces pathogenic responses in experimental autoimmune encephalomyelitis. Cells, 9(4), 906.

Davis, M. P. (2019). PC-FACS. Journal of Pain and Symptom Management, 57(1), 176–182.

Diebold, M., Fehrenbacher, L., Frosch, M., & Prinz, M. (2023). How myeloid cells shape experimental autoimmune encephalomyelitis: At the crossroads of outside-in immunity. European Journal of Immunology, 53(10).

Dobson, R., & Giovannoni, G. (2018). Multiple sclerosis – a review. European Journal of Neurology, 26(1), 27–40.

Francis, K. R. S., Govindhan, E., Pavithra, J., Yuvaraj, K., & Muralidharan, P. (2023). A comprehensive review on multiple sclerosis: It's etiology, symptoms, epidemiology and current therapeutic approaches. International Journal of Science and Research Archive, 8(2), 462–474.

Glatigny, S., & Bettelli, E. (2018). Experimental autoimmune encephalomyelitis (EAE) as Animal Models of Multiple sclerosis (MS). Cold Spring Harbor Perspectives in Medicine, 8(11), a028977.

Hart, B. A. '., Van Kooyk, Y., Geurts, J. J. G., & Gran, B. (2015). The primate autoimmune encephalomyelitis model; a bridge between mouse and man. Annals of Clinical and Translational Neurology, 2(5), 581–593.

Kanellopoulos, J. (2015). Experimental autoimmune encephalomyelitis. Biomedical Journal, 38(3), 181.

Melamed, E., Palmer, J. L., & Fonken, C. (2022). Advantages and limitations of experimental autoimmune encephalomyelitis in breaking down the role of the gut microbiome in multiple sclerosis. Frontiers in Molecular Neuroscience, 15.

Miyamura, S., Matsuo, N., Nagayasu, K., Shirakawa, H., & Kaneko, S. (2019). Myelin oligodendrocyte glycoprotein 35-55 (MOG 35-55)-induced experimental autoimmune encephalomyelitis: a model of chronic multiple sclerosis. BIO-PROTOCOL, 9(24).

National Institutions of Health (2024). *Estimates of Funding for Various Research, Condition, and Disease Categories (RCDC).*

Peplow, P., & Martinez, B. (2020). MicroRNAs as disease progression biomarkers and therapeutic targets in experimental autoimmune encephalomyelitis model of multiple sclerosis. Neural Regeneration Research/Neural Regeneration Research, 15(10), 1831.

Rossi, B., & Constantin, G. (2016). Live imaging of immune responses in experimental models of multiple sclerosis. Frontiers in Immunology, 7.

Steinman, L., Patarca, R., & Haseltine, W. (2023). Experimental encephalomyelitis at age 90, still relevant and elucidating how viruses trigger disease. The Journal of Experimental Medicine, 220(2).

Ucar, E. A., Ozkan, E., Shomalizadeh, N., Sekerdağ-Kilic, E., Akpunar, F., Sapanci, S., Kesibi, J., Ozler, C., Bilgez, A. S., & Gursoy-Ozdemir, Y. (2024). Carbenoxolone mitigates extensive fibrosis formation in PLP-induced EAE model and multiple sclerosis serum-exposed pericyte culture. Frontiers in Cellular Neuroscience, 18.

Voskuhl, R. R., & MacKenzie-Graham, A. (2022). Chronic experimental autoimmune encephalomyelitis is an excellent model to study neuroaxonal degeneration in multiple sclerosis. Frontiers in Molecular Neuroscience, 15.

Wang, L., Winnewisser, J., Federle, C., Jessberger, G., Nave, K., Werner, H. B., Kyewski, B., Klein, L., & Hinterberger, M. (2017). Epitope-Specific tolerance modes differentially specify susceptibility to proteolipid Protein-Induced experimental autoimmune encephalomyelitis. Frontiers in Immunology, 8.

Yan, Y., Zhao, Q., Huang, Y., Yang, J. Y., Zou, J., Ao, C., Chai, X., Tang, R., & Yang, W. (2022). Experimental autoimmune encephalomyelitis animal models induced by different myelin antigens exhibit differential pharmacologic responses to Anti-Inflammatory drugs. Journal of Immunological Sciences, 6(1), 18–24.