An Explanation of the Gender Disparity in Multiple Sclerosis: Investigating the Genetic and Hormonal Bases for Increased Frequency of MS in Women

Francesca Marini

BBB 030: The Neurobiology of Brain Disorders

Dr. Dichter

7 December 2017

Introduction

Multiple sclerosis (MS) is the most common demyelinating disease which affects the central nervous system (CNS), and it has long been known that this autoimmune attack on the body's own myelinated neurons affects women at least twice as frequently as men, with some current estimates bringing the ratio to as high as 4:1 (4). With this knowledge, an obvious avenue for researchers to pursue has been the biological differences between men and women, whether genetic or hormonal, which might help to explain such a gender disparity in epidemiology and, consequently, provide further evidence for the etiology of MS and lead to possible new treatment methods. The earliest research on gender differences in MS strictly focused on hormones, whereas more current research has attempted to identify genes expressed more frequently in women than men which could possibly hold the key to this autoimmune disease. While observational studies of the genetic differences in MS manifestation focus predominately on humans, the majority of experiments discussed in this paper utilize the induction of experimental autoimmune encephalomyelitis (EAE) in either mice or rats as a model for MS (5). Recent research into the gender disparity in the manifestation of MS has led to promising laboratory and clinical findings regarding the S1P receptors on CNS blood vessels, which in turn indicate possible future therapies while still leaving certain leading research questions ambiguous.

The Hormonal Basis of the Gender Disparity

Early investigations of the fact that MS affects women much more frequently than men focused on hormones and initially existed simply in the observational stages. The Pregnancy in Multiple Sclerosis (PRIMS) study took place in Europe in the 1990's and observed 254 women who had been diagnosed with MS prior to becoming pregnant; the goal of this study was to see if pregnancy itself really did have a protective effect against MS attacks in women and if the post-partem period truly was marked by an increase in attacks, as was commonly noticed by physicians and women with MS in the past. Based on detailed observations and statistical analysis, it was determined that "compared with the prepregnancy year, in which the mean rate of relapse was 0.7 ± 0.9 per woman per year, the relapse rates in the first and second trimesters of pregnancy were slightly lower, and that during the third trimester was substantially lower (0.2 ± 1.0 relapse per pregnancy per year). After delivery, the rate during the first three months was higher than that before pregnancy, but in the second, third, and fourth three-month periods it was similar to the rates before pregnancy." While PRIMS did find an association between pregnancy and rate of MS attacks, it did not find any relationship between pregnancy and a change in severity of MS symptom progression. In addition, the study found that women who breast-fed their babies had a much lower chance of an MS attack during the post-partem period (3).

Based on the PRIMS results, the authors hypothesized that somehow the biological changes of pregnancy in MS patients caused a "shift away from cell-mediated immunity toward increased humoral

immunity. The fetal-placental unit secretes cytokines such as interleukin-10 that down-regulate the production of other cytokines mediating cellular immunity…explaining the tolerance of the fetus by the mother. In contrast, delivery might be associated with an inversion of this cytokine balance and could be regarded…as a graft-rejection process." The above proposed process could provide insight into the behaviors of T-cell-mediated autoimmune diseases (like MS and rheumatoid arthritis), which get better during pregnancy and worse post-partem, as well as the behaviors of B-cell-mediated diseases (like lupus) which behave conversely (3).

A slightly more recent investigation into hormonal effects on the development of MS was conducted by Rhonda R. Voskuhl and Karen Palaszynski at the UCLA Department of Neurology which utilized EAE as a model for MS in SJL mice (which is a strain of mouse in which susceptibility is increased in females with a decrease in disease activity during pregnancy, similar to the manifestation of MS in human women). To test the hypothesis that hormonal differences between men and women (and those between pregnant and non-pregnant women) influence susceptibility to MS, the EAE model in SJL mice was used to observe disease behavior in both castrated males and females as well as to determine the effects of using exogenous sex hormones as treatment for MS, on the basis that sex hormones do influence the manifestation of the disease. In addressing the variations in EAE behavior during pregnancy, the authors looked to hormones present during late pregnancy (progesterone, estradiol, and estriol) and found that estriol has a substantial protective effect in the SJL mouse model compared to placebo or other estrogens, a finding which is consistent with observations of MS during pregnancy, since estriol is only present during pregnancy (while progesterone and estradiol are both present in nonpregnant females). Significantly, the level of estriol which inhibited EAE attacks matched the approximate levels of the hormone which occur during pregnancy. An immunological analysis demonstrates that increased estriol levels are correlated with a change in immune response away from the T-cells active in MS; this change is hypothesized to exist from an evolutionary basis as a safeguard against the mother's body rejecting the fetus, and it also explains reduction in MS activity during pregnancy. Therefore, the presence of estriol during pregnancy is protective against EAE, and presumably MS (7).

In addition, this same study investigated the effects of male and female sex hormones on the manifestation of EAE in SJL mice, finding that male sex hormones are protective against EAE by measuring disease behavior in castrated males and females (versus controls with gonads intact). Results demonstrated that castrated males experienced a frequency of EAE which approximated that of females. Furthermore, females treated with testosterone also benefitted from the protective effects of dihydrotestosterone (DHT). When testing to see if female sex hormones were disease promoting, there was no measured difference in disease manifestation between females which had received ovarectomies and controls. These results led to the conclusion that testosterone is protective while the female sex hormones which occur normally during a

menstrual cycle minimally influence disease presentation. Interestingly, comparative immune analyses of male and female SJL mice with EAE demonstrated that "gender played a role in the generation of encephalitogenic T cells. Disease severity was greater when T cells derived from females as compared with males were transferred [in the induction phase of creating the EAE models]…This indicated that T cells harvested after immunization of females were inherently more encephalitogenic than those harvested after immunization of males" (7). This finding alludes to underlying genetic factors which differ between males and females which could contribute to the high female to male ratio in the development of MS.

The Genetic Basis of the Gender Disparity

The more recent investigations of possible genetic causes for unequal rates of MS in men and women have primarily focused on identifying and testing candidate genes; in 2014, a study led by Lillian Cruz-Orengo and Brian P. Daniels at the Washington University School of Medicine identified a connection between an increase in expression of sphingosine-1-phosphate receptor 2 (S1PR2) (which occurs much more frequently in females) and susceptibility of the blood-brain barrier (BBB) to immune cells which are the agents of destruction in MS in humans and EAE in the SJL mouse models. Cruz-Orengo and Daniels initially "hypothesized that sexual dimorphism in this MS model arises from sexually dimorphic and CNS-region specific expression of genes that regulate BBB permeability and leukocyte entry." The gene for S1PR2 was particularly identified because of its role in vascular permeability; "S1PR2 belongs to a family of G protein-coupled receptors expressed by the cells of the vascular, immune, and nervous systems," and this particular receptor is closely involved in disruptions in permeability of vessels. When testing S1PR2, levels present in the CNS of SJL mice were measured, noting that females expressed the receptor much more than males, and that when given sodium fluorescein, the mice which expressed the receptor most highly experienced the most BBB permeability to fluorescein. Interestingly, this permeability was not noticed in the spinal cord, implying that while S1PR2 is a probable link to understanding the expression of these demyelinating diseases, it is probably not the only contributing factor in play (4).

Utilizing both SJL mice (which show a sex based difference in disease development) and B6 mice (which, as wild-type controls, do not demonstrate a sex based difference in disease development) with EAE as models, the authors then proceeded to measure BBB permeability, noting the highest permeability in female SJL mice (in which receptor expression was not affected by either ovarectomy or estrogen exposure). This behavior of S1PR2 expression in the models was also found to be consistent with the behavior of sexually dimorphic CNS autoimmunity in humans (based on in vitro measurements as well as postmortem biopsies). In addition, the EAE models of disease which experienced less lesions and demyelination were associated with a less-than-average expression of S1PR2 (found through genetic and pharmacological knockouts of the S1PR2 gene). Ultimately, this study and its results are particularly significant to MS

research because it was "the first to identify a sexually dimorphic regulator of the BBB. The identification of sexually dimorphic changes in mRNA levels [for the coding of S1PR2] within different CNS regions was not due to direct effects of sex steroids on transcriptional proteins." This finding was established by testing for a correlation between MS and either the Y chromosome or exposure to estradiol (4), (though the study did not investigate whether expression was affected by estriol, which has previously been found to be protective and would be an interesting avenue of future study). While this rules out direct hormonal influences on S1PR2 expression, it does not rule out some form of indirect hormonal effect, possibly through the biological changes of sexual maturation. It is likely that S1PR2, in particular, is involved in some fashion in MS pathology and this newfound knowledge could be used to develop future therapies.

Noting that S1PR2 overexpression could be explanatory for some, but not all, MS symptoms (demyelinating lesions in the cerebellum and cortex but not the spinal cord), a more recent study completed in 2016 at the Washington University School of Medicine by Liu, Jin, Yue, et al., found a correlation between expression of sphingosine-1-phosphate receptor 1 (S1PR1) and increased development of lesions in the spinal cords of EAE rat models (with no development of lesions in the brain), identifying a possible inciting cause of the remaining, unexplained MS symptoms via a related G protein-coupled receptor that affects vascular permeability. This study involved microPET imaging of EAE rats to measure frequency of spinal cord lesions, followed by immunohistochemical staining to ascertain that S1PR1 expression levels were, in fact, significantly above average, as expected. Not only were the incidence of spinal cord lesions in EAE and control rats compared, but the study also compared the permeability of vessels in the spinal cord and the abdominal aorta (utilizing non-CNS vessels as controls), and also found increased permeability in the CNS vessels only, a finding consistent with MS pathology of CNS immune response (but no abnormal immune in other physiological locales). "Quantitative analysis showed 24.6 % increase in tracer uptake [indicative of vessel permeability] in the EAE rat lumbar spinal cord, compared with the sham group…The increased percentage was 37.0 % when directly using lumbar spinal cord SUV [standardized uptake value] values for comparison." Additionally, treatment of EAE rats with fingolimod (a current MS drug) found that the medicine "downregulated activated microglial production of pro-inflammatory cytokines [and]…upregulated microglial production of brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) and promoted the neuroprotective effects of microglia…[while also significantly decreasing] the infiltration of IL-17-producing T cells into the CNS," (6) strong evidence that the findings connecting sphingosine-1-phosphate receptors and EAE pathology do, in fact, present as a model for MS mechanisms.

Possible Therapeutic Advances in MS Treatment

With new advances in identifying the expression of vascular receptors which affect MS symptom development, therapies targeting such receptors' behaviors are a next natural area for research. The 2014 Washington University study which identified S1PR2 expression as correlated with CNS immune susceptibility provided a recommendation that researchers develop and test drugs which target specific S1P receptors, which may be more effective and have less side effects than the current drug of choice, fingolimod, "which targets several S1PRs but not S1PR2. As S1PR2 also maintains the germinal center B cells, its blockade is unlikely to lead to the lymphopenia observed with FTY720" (fingolimod), or some of the fatal side effects associated with the current drug (4). Following this line of reasoning, in 2016, the combined phase 2 and 3 clinical trial called RADIANCE, which took place in Europe and the United States and compared the effects of ozanimod (a selective sphingosine-1-phosphate receptor (S1PR) modulator) with those of fingolimod (a nonspecific S1PR modulator which affects S1PR1,3,4,5), was completed. Particularly, the interactions between fingolimod and the S1PR3 is associated with many of the severely adverse side effects of the medicine, and since ozanimod specifically targets S1PR1 alone, it was hypothesized to at least match the effects of fingolimod but not produce the same side effects. The clinical trials demonstrated that ozanimod did, in fact, significantly reduce the activity of lesions in patients with relapsing remitting MS (RRMS), while its most serious side effects, were medically minor (a few patients reported nasopharyngitis, headaches, or urinary tract infections), with "no increased incidence of atrioventricular block or sinus pause" (which were the initial possible side effect concerns identified pre-trial because of the targeting of S1PR1). The effects of ozanimod were so positive during the phase 2 trial that the FDA actually approved phase 3 to begin early, before the actual conclusion of phase 2. An additional phase 3 trial called SUNBEAM was performed, resulting in similar findings. Therefore, ozanimod is undergoing further trials and is currently making progress on its way toward FDA approval as an MS treatment; the above trials discussed showed few negative effects in the short term, though long term testing of ozanimod for side effects still remains to be performed. Nevertheless, the results of ozanimod efficacy from RADIANCE and SUNBEAM are promising for the future of targeted MS therapies (2).

Conclusion

Initial research into why women are much more likely to develop MS than men chiefly focused on hormonal differences between the sexes. Pregnancy studies found that estriol, which is released in a woman's body only during pregnancy, has a protective effect against MS, while other female sex hormones do not influence the disease manifestation or progression. Studies comparing male and female sex hormones' effects on MS found testosterone to be protective against MS. Later studies identified gene loci associated with receptors which influence CNS vascular permeability to immune cells, leading to the investigation of

the effects of overexpression of S1PR2, which is sexually dimorphic (but not a sex-linked gene). S1PR2 expression is not directly dictated by sex hormones. This means that sex hormones could possibly still provide some residual, indirect effect on the body simply from having once been circulating in the body in particular quantities for a time (though this is unlikely). A more probable explanation is that some other unknown factor which differs between men and women affects the expression of S1PR2. This leaves the question of how testosterone can have a protective effect against MS somewhat ambiguous. S1PR2 overexpression, which predominately occurs in susceptible females, is likely a chief (if not causal) factor in the development of MS lesions in the brain, while S1PR1 is likely a contributing factor in the development of MS lesions in the spinal cord. Ozanimod, a drug which specifically targets S1PR1, has performed well in clinical trials without the adverse effects of the currently prescribed drug, fingolimod. A future therapeutic advance could occur in the development of a selective S1PR modulator that targets not only S1PR1 but also S1PR2. Ultimately, though S1PR2 expression was not found to be influenced by sex hormones, there is a known effect by sex hormones on MS development, which still remains inexplicable; meanwhile, the cause of S1PR2 expression's sexually dimorphic nature remains to be understood. Possibly, the hormonal and genetic factors contribute separately to the uneven gender ratio of MS, though it seems unlikely that there could be absolutely no connection between the two factors. The investigation of the gender differences in occurrence of MS has led to a much vaster understanding of MS mechanisms and a wider range of hypotheses beyond the basic hormonal hypothesis; this newly acquired knowledge surrounding this disease's mechanisms may also present useful avenues of research for other autoimmune disorders with large gender differences. More research into the possible connections (or lack thereof) between the hormonal and genetic factors discussed above could help to consolidate a theory of MS's pathology and epidemiology. In addition, as gene therapy becomes more advanced and applicable in medicine in the future, there is the possibility of developing a gene therapy designed to target specific S1P receptors and change their level of expression in the body. The identification of S1PR1 and S1PR2 as the receptors immediately involved in MS pathogenesis is useful in explaining biological mechanisms of symptoms and in the development of possible therapies, the puzzling question still remains of whether the overexpression of these receptors is a cause or an effect of MS itself. Therefore, though significant advances have been made in understanding the behavior the demyelinating disease multiple sclerosis, leading to the possibilities of highly effective, new, targeted treatments, MS ultimately remains, in many ways, a puzzling medical phenomenon which requires intensive further research to unearth its underlying causes.

Bibliography

1. Bordon Y. 2014. A breakthrough to explain sex bias?. *Nat. Rev. Immunol.* 15:355
2. Cohen JA, Arnold DL, Comi G, et al. 2016. Safety and efficacy of the selective sphingosine 1-phosphate receptor modulator ozanimod in relapsing multiple sclerosis (RADIANCE): a randomized, placebo-controlled, phase 2 trial. *Lancet Neurol.* 15:373-381
3. Confavreux C, Hutchinson M, Hours MM, et al. 1998. Rate of pregnancy-related relapse in multiple sclerosis. *N. Engl. J. Med.* 339:285-291
4. Cruz-Orengo L, Daniels BP, Dorsey D, et al. 2014. Enhanced sphingosine-1-phosphate receptor 2 expression underlies female CNS autoimmunity susceptibility. *J. Clin. Investig.* 124(6):2571-2584
5. De Jager PL, Hafler DA. 2007. New therapeutic approaches for multiple sclerosis. *Annu. Rev. Med*. 58:417-32
6. Liu H, Jin H, Yue X, et al. 2016. PET imaging study of S1PR1 expression in a rat model of multiple sclerosis. *Mol. Imaging Biol.* 18:724-732
7. Voskuhl RR, Palaszynski K. 2001. Sex hormones in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *Neuroscientist*. 7(3):258-270