Peer-reviewed version available at *J. Clin. Med.* **2018**, *7*, 494; doi:10.3390/jcm7120494

Review

The short and long-term effects of pregnancy on multiple sclerosis and experimental autoimmune encephalomyelitis

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Abstract: The role of pregnancy in multiple sclerosis (MS) is of importance because many patients with MS are young women in the childbearing age who require information to inform their reproductive decisions. Pregnancy is now well-known to be associated with fewer relapses of MS and reduced activity of autoimmune encephalomyelitis (EAE). However, in women with multiple sclerosis, this benefit is not always sufficient to protect against a rebound of disease activity if disease modulating therapy is ceased for pregnancy. There is reason to be concerned that use of assisted reproductive therapies can be associated with relapses of MS. It is thought that the beneficial effects of pregnancy are due to the pregnancy-associated changes in the maternal immune system. There is some evidence of this in human studies and studies of EAE. There is also evidence that having been pregnant leads to better long-term outcome of MS. The mechanism for this is not fully understood but it could result from epigenetic changes resulting from pregnancy or parenthood. Further studies of the mechanisms of the beneficial effects of pregnancy could provide information that might be used to produce new therapies. **Keywords:** multiple sclerosis; pregnancy; experimental autoimmune encephalomyelitis; epigenetics

1. Introduction

Multiple sclerosis is a common inflammatory disease of the central nervous system (CNS) [1, 2]. The characteristic pathological features are inflammation and demyelination that occur in plaques, usually around a vein. There is also axonal degeneration, and as disease progresses there is microglial activation in so-called normal appearing white matter. The finding of lymphocytic infiltrates suggests that this is an immune mediated disease. Genetic studies show that the risk of MS is associated with genes of the immune system, most notably the genes of the HLA region [3-5], but also other genes of the adaptive and innate immune

systems. A number of CNS antigens have been proposed as the target of the immune attack [6, 7]. Lymphocytes (CD4+ and CD8+) and B cells and antibodies are thought to participate in MS pathology and are thought to be activated or expanded in the peripheral immune system. The success of therapies directed against T cells and B cells supports this. In the peripheral blood of patients with MS, there is evidence of immune activation. This includes increased levels of T cells reactive with CNS antigens, and increased activated T cells [8, 9]. Recently it has also been found that the gut microbiota influences the development of MS, possibly through immune effects [10].

Experimental autoimmune encephalomyelitis (EAE) can be induced by injection of CNS antigens and adjuvant or by adoptive transfer of T cells reactive to CNS antigens [11]. The similarity between EAE and MS was noted in 1946 [12] and EAE is now used as a model of multiple sclerosis (MS), although it differs from MS in needing to be induced, rather than occurring spontaneously. Modern forms of EAE came about with the introduction of adjuvants [13] Usually EAE is induced with single purified antigens, most commonly myelin basic protein (MBP), myelin proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), although a wide range of CNS antigens can be used [14]. In all forms of EAE the pathological appearances include some degree of inflammation and demyelination.

The clinical features of EAE have been well-studied in rodents, and the pathological features and clinical course vary with the antigen and animal that is used. EAE induced with MBP (MBP-EAE) in the Lewis rat has inflammation and demyelination of the CNS mainly in the meninges, subpial region and nerve roots [15]. MBP-EAE is usually monophasic, but low

dose immune suppression can change the clinical course into a chronic relapsing form of disease [16]. EAE induced in Lewis rats with myelin proteolipid protein (PLP) (PLP-EAE) shows more widespread CNS demyelination than MBP-EAE [17-19]. EAE can be induced with PLP in SJL mice to produce a relapsing disease [20-22]. EAE induced with myelin oligodendrocyte glycoprotein (MOG) (MOG-EAE) in a variety of mouse strains including C57/BL6 mice is a chronic disease [23]. The pathology of most types of EAE is characterized by microglial activation and infiltration with T cells and macrophages [14, 24, 25]. PCR and gene expression studies indicate upregulation of immune related genes and down-regulation of many central nervous system genes in MBP-EAE [26, 27]. Microarray studies of EAE induced with MOG in the spinal cords of mice show increased expression of immune-related molecules [28] and of rats showing upregulation of immune related molecules and downregulation of cholesterol synthesis [29].

The ability to transfer MBP-EAE [30] and PLP-EAE [31] with lymphocytes cells indicates that these cells are primarily responsible for these types of EAE. Disease is largely caused by Th17 cells [32, 33] and is suppressed by regulatory T cells (Treg) [34]. In contrast, MOG-EAE is largely antibody mediated [35]. Acute EAE is a monophasic disease and animals do not have subsequent attacks, even if re-inoculated. The tolerance that occurs after acute EAE can be broken by mild immune suppression, suggesting that this is active tolerance [36]. It is now thought likely that regulatory T cells play a role in the active control of EAE [34, 37]. It is also known that changes in the gut microbiota can also influence the development of EAE [10].

Pregnancy involves physiological changes in the mother, including elevation of cardiac output, increased basal metabolic rate, increased lipid levels and weight gain [38-41]. In pregnancy there are changes in the levels of hormones such as estriol, progesterone, prolactin, early pregnancy factor (EPF), alpha-fetoprotein [42] and leptin [43], and elevated levels of growth factors such as insulin-like growth factor (IGF) [44]. After delivery, there is a rapid decline in the levels of pregnancy hormones and maternal physiology rapidly returns to normal, although during lactation the levels of prolactin and oxytocin are elevated [45]. It is thought that the changes in maternal physiology are mediated through the effects of the pregnancy hormones. In pregnancy, the mother plays host to the fetus, which contains foreign antigens. Immune changes in pregnancy tend to suppress the maternal immune system, and this is thought to be important in preventing rejection of the fetus [46-48]. During pregnancy, there are increased Th2-related and reduced Th1- and Th17-related activities, correlating with improvement of Th1/Th17-mediated autoimmune diseases and worsening of Th2-mediated allergic diseases [49, 50]. In pregnant women there are increased levels of circulating regulatory T cells (Treg) cells [51, 52]. Other evidence of increased Treg activity in pregnancy comes from findings of increased foxp3 expression and increased functional suppression in pregnant and estrogen treated rats [53]. The increased level of Treg cells in pregnancy is thought to be due to the effects on the immune system of estradiol [54]. The changes have been reported to include a shift to Th2 immune responses and a shift to greater humoral than cell mediated immunity [55]. It is generally thought that this occurs to lessen the possibility of immunological rejection of the foetus by the mother, although this is complex, with both changes at the placenta and systemically [56, 57]. It is thought that the

changes in the maternal immune system in pregnancy are a response to endocrine changes in the mother [58, 59].

In the first part of the 20th century, MS was thought to be worse in pregnancy [60] [61]. This idea was re-inforced by several influential case reports [62-64] and it was considered that pregnancy was a precipitant of MS [65]. However, a study by Tillman in 1950 cast doubt in the previously held view that pregnancy was not advisable in MS [61] and instead found that relapses of MS occurred after delivery. After, this further studies indicated that MS was better in pregnancy and relapses occurred post-partum. The issue of pregnancy and MS is currently of great interest and there have been a number of previous reviews of pregnancy in MS [66-71]. This review will provide an update on the data concerning the short and long term effect of pregnancy in MS. It will also include a review of the effects of pregnancy in EAE. This has not been previously reviewed and could provide evidence of possible underlying mechanisms that are relevant to MS.

2. **Multiple sclerosis and EAE during pregnancy**

Current evidence demonstrates that there are fewer relapses of MS during pregnancy, especially in the later part of pregnancy [72-78]. A systematic review of 22 papers confirms the findings of reduced relapses in pregnancy [79]. However, it is worth noting that the risk of relapse in pregnancy is not zero, and recent studies in the era when patients are on immunomodulatory therapies have shown a greater rate of relapses in pregnancy. This was greatest in women who had previously been on active treatment with long washout periods and could represent a rebound of disease [80]. There have also been reports of severe relapses during pregnancy after withdrawal of fingolimod [81]

The reduction in relapses during pregnancy is thought to be due to the immunological changes of pregnancy. The sex hormones, which are present in higher levels during pregnancy, are known to influence the immune system and also the cells of the nervous system [82]. There have been studies of the immune changes in multiple sclerosis patients during pregnancy. The mechanism by which pregnancy protects against MS relapses is thought to be related to changes in the maternal immune system, with changes in cytokines and T cells in pregnant compared to non-pregnant MS patients [83]. This is not thought to be due to increased Treg cells in pregnancy [84]. However, another study found that the frequency of interferon -γ producing T cells declined during pregnancy in women with MS [85]. A decline in interferon- γ was confirmed in a further study which also showed an increase in natural killer cells, a Th1-Th2 shift but no changes in Treg cells [86]. A conflicting results was found in another study that showed an increase in $CD4+CD25^{hi}$ cells during pregnancy in patients with MS [52]. An increase in natural killer cells during pregnancy in MS was confirmed in s study that also showed an increase in NK T cells [87]. There are changes in the expression of inflammation related genes during pregnancy in patients with multiple sclerosis [88].

There have been studies of the effects of pregnancy in EAE, which provide further information about the possible immune mechanisms of the beneficial effects of pregnancy in autoimmune demyelinating disease. Pregnancy suppressed EAE in guinea pigs and rats [89]. Lewis rats inoculated with guinea pig spinal cord and adjuvant during the second or third weeks of pregnancy were protected against the development of disease, although rats

inoculated in the first week had less protection [90]. Induction of MBP-EAE in Lewis rats during pregnancy also leads to reduced severity of disease [91]. SJL/J mice inoculated with PLP and adjuvant in the late part of pregnancy were protected against disease [92]. In PLP-induced EAE in SJL/J mice, that when immunization occurs during pregnancy, mice show a reduction in the incidence of EAE as well as a decrease in clinical severity, while mice immunized during the postpartum period exhibit more severe disease [93]. In C57/BL6 mice, EAE induced with the MOG 35-55 was less severe when mice were inoculated in late pregnancy (McLain et al. 2007). In DA rats, EAE induced with bovine brain homogenate was less severe when disease was induced in pregnancy although there were relapses post-partum [94]. There is also a report that adoptive transfer EAE was less severe in mice in mid-pregnancy but not in late pregnancy or the post-partum period [95]. There have also been studies of the effects of becoming pregnant after the onset of EAE. In SJL mice inoculated with PLP139-151 and in C57/BL6 mice inoculated with MOG 35-55, induction of pregnancy led to clinical improvement but the pathological lesions persisted [96].

There have been attempts to transfer the protective effects of pregnancy. In rats, offspring from rats with EAE in pregnancy show transient protection from EAE induced with GPSC, and rats that are suckled by mothers that have EAE in pregnancy also acquired protection [97]. Exosomes from pregnant and non-pregnant mice can suppress EAE. However, exosomes are more abundant in pregnancy and it is suggested that exosomes contribute to protection during pregnancy [98]. There is alteration of cytokine production in the spinal cord of pregnant rats with EAE compared to non-pregnant rats, and serum from pregnant rats

suppressed lymphocyte proliferation in response to antigen and to phytohaemagglutinin, but not to concanvalin A, possibly indicating Treg activity [91].

There are no differences in lymphocyte proliferation or expression of activation markers when immunization occurred during pregnancy as compared with the non-pregnant controls [93]. Mice immunized during pregnancy produced less TNF-α and IL-17, and showed an increased number of IL-10-secreting cells within the CD11b+, CD11c+, CD19+, and CD4+/CD25+ Treg populations. No differences were noted in the production of IFN-γ, IL-2, IL-4, and IL-5. These results suggest that when an antigen is introduced during pregnancy, an immunoregulatory rather than an immunosuppressive or Th2 environment predominates. The glucocorticoid T cell receptor is necessary for the protective effect of pregnancy in MOG-EAE [99].

There have been studies that show that EAE can be suppressed by treatment with estrogen to produce levels similar to those found in pregnancy. This has been associated with changes in the gut microbiota [100]and with changes in regulatory B cells [101].

3. **Post-partum relapses**

The studies that showed fewer relapses in pregnancy also showed an increased frequency of relapses in the post-partum period [72-75, 79, 102-104]. A selection of studies is shown in Table 1. Post-partum relapses are important because they lead to disability [105]. However, post-partum relapses do not occur in all patients, and there have been attempts to predict post-partum relapses using clinical and immunological approaches. The frequency of relapses before pregnancy is the best predictor of relapse after pregnancy [74, 106, 107]. One study of 298 full term pregnancies found that breast feeding did not influence the frequency of

relapses [107]. Relapses are nor predicted by epidural anaesthesia or caesarean section [108]. There have been studies suggesting that breast-feeding is protective against relapses [109]. However, later studies found that breast-feeding did not suppress disease [110] or influence the frequency of relapses [107] [76]. One study suggests that post-partum relapses correlate with increased levels of IL-8 in the first trimester [111].

There have been some clinical studies of methods to prevent post-partum relapses. In a retrospective analysis of study of patients who were followed in a database, 20 subjects who were given monthly intravenous methylpredisolone 1g for six months post-partum had a lower annualized relapse rate for the first three months post-partum than those who had no treatment [112]. There have been suggestions that IVIG could be useful in preventing post-partum relapses [113-115] but a meta-analysis has failed to confirm this [116].

4. **Relapses with ART pregnancies**

Increasing numbers of pregnancies now occur with assisted reproduction technology (ART). This generally involves the use of hormone therapy to induce ovulation or to assist in implantation. Such hormones can include Gonadotrophin releasing hormone (GnRH) agonists, Follicle stimulating hormone, luteinizing hormone, human chorionic gonadotrophin and progesterone. In some patients Gonadotrophin releasing hormone antagonists are also used. When high doses are used, women can experience ovarian hyperstimulation syndrome. Women can be exposed to these hormones when ovulation is induced or when embryos are implanted. It is also the case that when patients are trying to become pregnant, they often cease immunomodulatory therapy for MS.

Relapses of multiple sclerosis have been reported to occur after pregnancies after ART [117-119]. A study from Argentina showed the disease activity was increased 9-fold [120]. A study from France confirmed this [121]. In one study, the annualized relapse rate was increased in 6 women after cycles of ART compared to the previous period. These women had been treated with GnRH agonists and some had also been treated with FSH and Clomiphene [117]. The authors of this study found that two women who were treated with GnRH antagonists did not have increased relapses and suggested that agonists of GnRH are more likely to casue relapses that GnRH antigonists. In this respect, it is worth noting that GnRH has immune stimulatory effects [122]

However, another group also showed that there was an increased relapse rate after ART, but this occurred in women treated with GnRH agonists and GnRH antagonists, and suggested that relapses could be due to either to cessation of immune therapy, or to stress, which has been suggested to trigger relapses of MS, as well as to the possible effects of the hormones used in ART [118, 119].

5. **Use of immunomodulatory medications in pregnancy**

Whether to use of immunomodulatory therapies during pregnancy is a common clinical issue and a concern for women with MS. The rate of pregnancy in women with MS may be increasing, as shown in a study in the USA [123], so this topic is of importance. It is recommended that most of the medications are avoided during conception, pregnancy and breast-feeding. This is in part because of suggestions of toxicity from animal studies but also because of a lack of evidence of safety of use in humans. A recent study showed that

few women in the USA use immunomodulatory therapies during pregnancy or the puerperium [77].

However, although pregnancy is protective against relapses, the rate of relapse is not zero. Furthermore, in more recent studies there have been reports of relapses during pregnancy, possibly due to a rebound effect after ceasing effective therapy. This has led to a re-consideration of the possible use of medications during pregnancy, to protect the health of the mother. There are detailed reviews of these effects [70, 71]. However, because of the risk of pregnancy and post-partum relapses, there have been attempts to find medications that can be used during pregnancy. There is evidence that glatiramer acetate is safe in pregnancy and during breast feeding [124]. There have also been reports of the use of natalizumab throughout pregnancy in patients with active disease [125, 126]. The main adverse effect is an alteration of the white cell count of the newborn. In the future, it is likely that MS patients, particularly those with active disease, will be offered some type of therapy during pregnancy. Although the medications are not recommended during pregnancy, many pregnancies are unplanned, and exposures to disease modifying therapies have occurred. There are registries that are attempting to collect data about the outcomes of pregnancy exposure. If a large number of exposed pregnancies were found to have the same outcomes as unexposed pregnancies, the recommendations regarding use in pregnancy could be re-considered.

6. **Effects of pregnancy on risk of developing MS**

Given the immune effects of pregnancy, it has been questioned whether pregnancy influences the development of MS. In a study from Denmark, parents of both sexes had a lower risk of MS than childless persons [127]. There is a possibility that this is due to reverse causality

and that even before diagnosis of MS there could be some changes (either biological or behavioural) that lead to decreased fertility compared to healthy subjects. This has been recently described as a multiple sclerosis "prodrome" [128-130]. Possibly healthy people are more likely to become parents than those with poor health.

7. **Long term effects of pregnancy on the clinical course of MS**

The long-term effects of parity on MS is less certain. Some earlier studies suggested that parity is associated with better long-term outcome [69, 131-133]. A retrospective study from Turkey suggested that parity had a beneficial effect on transition from relapsing to secondary progressive MS [134]. However, this could arise because women with more severe MS might decide not to have children or to have fewer children.

However, there is accumulating evidence that parity is beneficial. Ponsonby and colleagues examined the association between past pregnancy, offspring number, and risk of a first episode of demyelination [135]. The study demonstrated that higher parity was associated with reduced risk of a first episode of clinical demyelination and the results were consistent with a cumulative beneficial effect of pregnancy. In another study using the MSBase registry, where patients were matched according to clinical characteristics, pregnancy was 4.5 times more potent than first-line disease modifying therapies (interferon-beta, glatiramer acetate) in preventing long-term disability accrual, and any time spent pregnant (including induced and spontaneous abortions) was beneficial [136]. Other evidence of a benign effect of parity on MS is the findings that having been pregnant does not increase the risk of secondary progression of disease [137] and that women with children with different partners

do not have greater risk of disability as might be expected to occur if the immune response to paternal genes was harmful [138]

For parity to have a long-term effect on MS, it would be expected that there would be permanent changes that persist after the woman is no longer pregnant and hormone levels have returned to normal. This could lead to accumulation of changes with successive pregnancies, due to the cumulative effects of pregnancy hormones, or to the effects of the experience of parenthood. One possible mechanism could be epigenetics, with changes in DNA methylation, leading to changes in gene expression. It is known that hormones such as estrogen can cause epigenetic changes [139, 140]. It is also known that life experiences, such as stress and emotional well-being can lead to epigenetic changes [141, 142] . This could lead to cumulative epigenetic changes with parity and parenthood. If this is the mechanism by which prior pregnancies leads to better outcome of MS, it would be important to know which genes are involved.

8. **Conclusion**

The effects of MS on pregnancy and the effects of pregnancy on MS are common issues of concern for people with MS. Knowledge of this field is advancing and it is possible to provide information to address these concerns. It is now clear that pregnancy is not harmful, that there can be short term and possible long term beneficial effects. However despite the beneficial effects of pregnancy there has been an understanding that in many women, treatment with pregnancy could be justified, and there is an increased emphasis on obtaining data that could provide more information about the risks and benefit of such treatment.

The mechanisms by which pregnancy reduces relapses and by which parity leads to a beneficial effect on outcome are still not fully understood. Further work in this area would be a great value, because understanding the mechanisms of the beneficial effects of pregnancy might lead to strategies to improve outcome of disease.

Conflicts of Interest: "The authors declares no conflict of interest."

References

1. Reich, D.S., Lucchinetti, C.F. Calabresi, P.A., *Multiple Sclerosis.* N Engl J Med, 2018. **378**: p. 169-180. DOI: 10.1056/NEJMra1401483.

2. Thompson, A.J., et al., *Multiple sclerosis.* Lancet, 2018. **391**: p. 1622-1636. DOI: 10.1016/s0140-6736(18)30481-1.

3. Patsopoulos, N.A., et al., *Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects.* PLoS Genet, 2013. **9**: p. e1003926. DOI: 10.1371/journal.pgen.1003926.

4. Beecham, A.H., et al., *Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis.* Nat. Genet, 2013. **45**: p. 1353-1360. DOI: 10.1038/ng.2770

5. Sawcer, S., et al., *Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis.* Nature, 2011. **476**: p. 214-219.

6. Hohlfeld, R., et al., *The search for the target antigens of multiple sclerosis, part 1: autoreactive CD4+ T lymphocytes as pathogenic effectors and therapeutic targets.* Lancet Neurol, 2016. **15**: p. 198-209. DOI: 10.1016/s1474-4422(15)00334-8.

7. Hohlfeld, R., et al., *The search for the target antigens of multiple sclerosis, part 2: CD8+ T cells, B cells, and antibodies in the focus of reverse-translational research.* Lancet Neurol, 2016. **15**: p. 317-31. DOI: 10.1016/s1474-4422(15)00313-0.

8. Khoury, S.J., et al., *Changes in activated t cells in the blood correlate with disease activity in multiple sclerosis.* Archives of Neurology, 2000. **57**: p. 1183-1189. DOI: 10.1001/archneur.57.8.1183.

9. Kaskow, B.J. Baecher-Allan, C., *Effector T Cells in Multiple Sclerosis.* Cold Spring Harb Perspect Med, 2018. **8**. DOI: 10.1101/cshperspect.a029025.

10. Chu, F., et al., *Gut Microbiota in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis: Current Applications and Future Perspectives.* Mediators Inflamm, 2018. **2018**: p. 8168717. DOI: 10.1155/2018/8168717.

11. van der Star, B.J., et al., *In vitro and in vivo models of multiple sclerosis.* CNS. Neurol. Disord. Drug Targets, 2012. **11**: p. 570-588.

12. Kabat, E.A., Wolf, A. Bezer, A.E., *THE RAPID PRODUCTION OF ACUTE DISSEMINATED ENCEPHALOMYELITIS IN RHESUS MONKEYS BY INJECTION OF HETEROLOGOUS AND HOMOLOGOUS BRAIN TISSUE WITH ADJUVANTS.* J Exp. Med, 1947. **85**: p. 117-130.

13. Rivers, T.M., Sprunt, D.M. Berry, G.P., *Observations on attempts to produce acute disseminated encephalomyelitis in monkeys.* J. Exp. Med, 1933. **58**: p. 39-53.

14. Gold, R., Linington, C. Lassmann, H., *Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research.* Brain, 2006. **129**: p. 1953-1971.

15. Pender, M.P., *The pathophysiology of myelin basic protein-induced acute experimental allergic encephalomyelitis in the Lewis rat.* J. Neurol. Sci, 1988. **86**: p. 277-289.

16. McCombe, P.A., Harness, J. Pender, M.P., *Effects of cyclosporin A treatment on clinical course and inflammatory cell apoptosis in experimental autoimmune encephalomyelitis induced in Lewis rats by inoculation with myelin basic protein.* J. Neuroimmunol, 1999. **97**: p. 60-69.

17. Greer, J.M., et al., *Immunogenic and encephalitogenic epitope clusters of myelin proteolipid protein.* J Immunol, 1996. **156**: p. 371-379.

18. Chalk, J.B., et al., *Clinical and histological findings in proteolipid-protein- induced experimental autoimmune encephalomyelitis (EAE) in the Lewis rat. Distribution of demyelination differs from that in EAE induced by other antigens.* J. Neurol. Sci, 1994. **123**: p. 154-161.

19. Chalk, J.B., McCombe, P.A. Pender, M.P., *Conduction abnormailites are restricted to the central nervous system in experimental autoimmune encephalomyelitis induced by*

inoculation with proteolipid protein but not myelin basic protein. Brain, 1994. **117**: p. 975-986.

20. Lu, E., et al., *Disease-modifying drugs for multiple sclerosis in pregnancy: a systematic review.* Neurology, 2012. **79**: p. 1130-1135.

21. Kuchroo, V.K., et al., *Induction of experimental allergic encephalomyelitis by myelin proteolipid-protein-specific T cell clones and synthetic peptides.* Pathobiology, 1991. **59**: p. 305-312.

22. Greer, J.M., et al., *Identification and characterization of a second encephalitogenic determinant of myelin proteolipid protein (residues 178-191) for SJL mice.* J. Immunol, 1992. **149**: p. 783-788.

23. Schluesener, H.J., et al., *A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease.* J. Immunol, 1987. **139**: p. 4016-4021.

24. McCombe, P.A., et al., *Expression of CD45RC and Ia antigen in the spinal cord in acute experimental allergic encephalomyelitis: an immunocytochemical and flow cytometric study.* J. Neurol. Sci, 1992. **113**: p. 177-186.

25. McCombe, P.A., de Jersey, J. Pender, M.P., *Inflammatory cells, microglia and MHC class II antigen-positive cells in the spinal cord of Lewis rats with acute and chronic relapsing experimental autoimmune encephalomyelitis.* J. Neuroimmunol, 1994. **51**: p. 153-167.

26. McCombe, P.A., Nickson, I. Pender, M.P., *Cytokine expression by inflammatory cells obtained from the spinal cords of Lewis rats with experimental autoimmune encephalomyelitis induced by inoculation with myelin basic protein and adjuvants.* J. Neuroimmunol, 1998. **88**: p. 30-38.

27. Inglis, H.R., Greer, J.M. McCombe, P.A., *Gene expression in the spinal cord in female lewis rats with experimental autoimmune encephalomyelitis induced with myelin basic protein.* PLoS. ONE, 2012. **7**: p. e48555.

28. Ibrahim, S.M., et al., *Gene expression profiling of the nervous system in murine experimental autoimmune encephalomyelitis.* Brain, 2001. **124**: p. 1927-1938.

29. Mueller, A.M., et al., *Novel role for SLPI in MOG-induced EAE revealed by spinal cord expression analysis.* J Neuroinflammation, 2008. **5**: p. 20.

30. Mokhtarian, F., McFarlin, D.E. Raine, C.S., *Adoptive transfer of myelin basic protein-sensitized T cells produces chronic relapsing demyelinating disease in mice.* Nature, 1984. **309**: p. 356-358.

31. van der Veen, R.C., et al., *The adoptive transfer of chronic relapsing experimental allergic encephalomyelitis with lymph node cells sensitized to myelin proteolipid protein.* J. Neuroimmunol, 1989. **21**: p. 183-191.

32. Zepp, J., Wu, L. Li, X., *IL-17 receptor signaling and T helper 17-mediated autoimmune demyelinating disease.* Trends Immunol, 2011. **32**: p. 232-239.

33. Rostami, A. Ciric, B., *Role of Th17 cells in the pathogenesis of CNS inflammatory demyelination.* J Neurol. Sci, 2013. **333**: p. 76-87.

34. O'Connor, R.A. Anderton, S.M., *Foxp3+ regulatory T cells in the control of experimental CNS autoimmune disease.* J. Neuroimmunol, 2008. **193**: p. 1-11.

35. Slavin, A., et al., *Induction of a multiple sclerosis-like disease in mice with an immunodominant epitope of myelin oligodendrocyte glycoprotein.* Autoimmunity, 1998. **28**: p. 109-120.

36. Harness, J., Pender, M.P. McCombe, P.A., *Cyclosporin A treatment modulates cytokine mRNA expression by inflammatory cells extracted from the spinal cord of rats with experimental autoimmune encephalomyelitis induced by inoculation with myelin basic protein.* J. Neurol. Sci, 2001. **187**: p. 7-16.

37. Puentes, F., et al., *Active suppression induced by repetitive self-epitopes Protects against EAE development.* PLoS. ONE, 2013. **8**: p. e64888.

38. Granger, J.P., *Maternal and fetal adaptations during pregnancy: lessons in regulatory and integrative physiology.* Am. J. Physiol Regul. Integr. Comp Physiol, 2002. **283**: p. R1289-R1292.

39. Lain, K.Y. Catalano, P.M., *Metabolic changes in pregnancy.* Clin. Obstet. Gynecol, 2007. **50**: p. 938-948.

40. Forsum, E. Lof, M., *Energy metabolism during human pregnancy.* Annu. Rev. Nutr, 2007. **27**: p. 277-292.

41. Tan, E.K. Tan, E.L., *Alterations in physiology and anatomy during pregnancy.* Best. Pract. Res. Clin. Obstet. Gynaecol, 2013.

42. Brunton, P.J. Russell, J.A., *Endocrine induced changes in brain function during pregnancy.* Brain Res, 2010. **1364**: p. 198-215.

43. Molvarec, A., et al., *Serum leptin levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in normal pregnancy and preeclampsia.* Reprod. Biol. Endocrinol, 2011. **9**: p. 124.

44. Lauszus, F.F., et al., *Increased serum IGF-I during pregnancy is associated with progression of diabetic retinopathy.* Diabetes, 2003. **52**: p. 852-856.

45. Uvnas-Moberg, K., et al., *Oxytocin and prolactin levels in breast-feeding women. Correlation with milk yield and duration of breast-feeding.* Acta Obstet. Gynecol. Scand, 1990. **69**: p. 301-306.

46. Munoz-Suano, A., Hamilton, A.B. Betz, A.G., *Gimme shelter: the immune system during pregnancy.* Immunol. Rev, 2011. **241**: p. 20-38.

47. Veenstra van Nieuwenhoven, A.L., Heineman, M.J. Faas, M.M., *The immunology of successful pregnancy.* Hum. Reprod. Update, 2003. **9**: p. 347-357.

48. Yip, L., McCluskey, J. Sinclair, R., *Immunological aspects of pregnancy.* Clin. Dermatol, 2006. **24**: p. 84-87.

49. Doria, A., et al., *Th2 immune deviation induced by pregnancy: the two faces of autoimmune rheumatic diseases.* Reprod. Toxicol, 2006. **22**: p. 234-241.

50. Saito, S., et al., *Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy.* Am. J. Reprod. Immunol, 2010. **63**: p. 601-610.

51. Somerset, D.A., et al., *Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset.* Immunology, 2004. **112**: p. 38-43.

52. Sanchez-Ramon, S., et al., *Pregnancy-induced expansion of regulatory T-lymphocytes may mediate protection to multiple sclerosis activity.* Immunol. Lett, 2005. **96**: p. 195-201.

53. Polanczyk, M.J., et al., *Enhanced FoxP3 expression and Treg cell function in pregnant and estrogen-treated mice.* J. Neuroimmunol, 2005. **170**: p. 85-92.

54. Tai, P., et al., *Induction of regulatory T cells by physiological level estrogen.* J. Cell Physiol, 2008. **214**: p. 456-464.

55. Jamieson, D.J., Theiler, R.N. Rasmussen, S.A., *Emerging infections and pregnancy.* Emerg Infect Dis, 2006. **12**: p. 1638-43. DOI: 10.3201/eid1211.060152.

56. Mor, G. Cardenas, I., *The immune system in pregnancy: a unique complexity.* Am J Reprod Immunol, 2010. **63**: p. 425-33. DOI: 10.1111/j.1600-0897.2010.00836.x.

57. Racicot, K., et al., *Understanding the complexity of the immune system during pregnancy.* Am J Reprod Immunol, 2014. **72**: p. 107-16. DOI: 10.1111/aji.12289.

58. Polese, B., et al., *The Endocrine Milieu and CD4 T-Lymphocyte Polarization during Pregnancy.* Front Endocrinol (Lausanne), 2014. **5**: p. 106. DOI: 10.3389/fendo.2014.00106.

59. Schumacher, A., Costa, S.D. Zenclussen, A.C., *Endocrine factors modulating immune responses in pregnancy.* Front Immunol, 2014. **5**: p. 196. DOI: 10.3389/fimmu.2014.00196.

60. Birk, K., et al., *The clinical course of multiple sclerosis during pregnancy and the puerperium.* Arch. Neurol, 1990. **47**: p. 738-742.

61. TILLMAN, A.J., *The effect of pregnancy on multiple sclerosis and its management.* Res. Publ. Assoc. Res. Nerv. Ment. Dis, 1950. **28**: p. 548-582.

62. Peckham, C.H., *Multiple sclerosis complicating pregnancy.* New York State Medical Journal, 1945. **45**: p. 618-622.

63. Birner, I.M., *Pregnancy and multiple sclerosis: Case report.* New Your State Journal of Medicine, 1945. **45**: p. 634-635.

64. Douglass, L.H. Jorgensen, C.L., *Pregnancy and n multiple sclerosis.* American Journal of Obstetrics and Gynecology, 1948. **55**: p. 332-336.

65. Denny-Brown, D., *Multiple sclerosis; the clinical problem.* Am. J. Med, 1952. **12**: p. 501-509.

66. McCombe, P.A. Greer, J.M., *Female reproductive issues in multiple sclerosis.* Mult. Scler, 2012. **19**: p. 392-402.

67. Tsui, A. Lee, M.A., *Multiple sclerosis and pregnancy.* Curr. Opin. Obstet. Gynecol, 2011. **23**: p. 435-439.

68. Langer-Gould, A. Beaber, B.E., *Effects of pregnancy and breastfeeding on the multiple sclerosis disease course.* Clin. Immunol, 2013.

69. D'Hooghe, M.B., D'Hooghe, T. De, K.J., *Female gender and reproductive factors affecting risk, relapses and progression in multiple sclerosis.* Gynecol. Obstet. Invest, 2013. **75**: p. 73-84.

70. Coyle, P.K., *Management of women with multiple sclerosis through pregnancy and after childbirth.* Ther Adv Neurol Disord, 2016. **9**: p. 198-210. DOI: 10.1177/1756285616631897.

71. Lu, E., et al., *Safety of disease-modifying drugs for multiple sclerosis in pregnancy: current challenges and future considerations for effective pharmacovigilance.* Expert. Rev. Neurother, 2013. **13**: p. 251-261.

72. Confavreux, C., et al., *Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group.* N. Engl. J. Med, 1998. **339**: p. 285-291.

73. De Las Heras, V., et al., *Pregnancy in multiple sclerosis patients treated with immunomodulators prior to or during part of the pregnancy: a descriptive study in the Spanish population.* Mult. Scler, 2007. **13**: p. 981-984.

74. Fernandez, L.N., et al., *Epidemiological characteristics of pregnancy, delivery, and birth outcome in women with multiple sclerosis in Argentina (EMEMAR study).* Mult. Scler, 2009. **15**: p. 555-562.

75. Finkelsztejn, A., et al., *The Brazilian database on pregnancy in multiple sclerosis.* Clin. Neurol. Neurosurg, 2010. **113**: p. 277-280.

76. Jesus-Ribeiro, J., et al., *Pregnancy in Multiple Sclerosis: A Portuguese cohort study.* Mult Scler Relat Disord, 2017. **17**: p. 63-68. DOI: 10.1016/j.msard.2017.07.002.

77. Houtchens, M.K., Edwards, N.C. Phillips, A.L., *Relapses and disease-modifying drug treatment in pregnancy and live birth in US women with MS.* Neurology, 2018. DOI: 10.1212/wnl.0000000000006382.

78. Fares, J., et al., *Pregnancy outcomes in Lebanese women with multiple sclerosis (the LeMS study): a prospective multicentre study.* BMJ Open, 2016. **6**: p. e011210. DOI: 10.1136/bmjopen-2016-011210.

79. Finkelsztejn, A., et al., *What can we really tell women with multiple sclerosis regarding pregnancy? A systematic review and meta-analysis of the literature.* BJOG, 2011. **118**: p. 790-797.

80. Alroughani, R., et al., *Relapse occurrence in women with multiple sclerosis during pregnancy in the new treatment era.* Neurology, 2018. **90**: p. e840-e846. DOI: 10.1212/wnl.0000000000005065.

81. Novi, G., et al., *Dramatic rebounds of MS during pregnancy following fingolimod withdrawal.* Neurol Neuroimmunol Neuroinflamm, 2017. **4**: p. e377. DOI: 10.1212/nxi.0000000000000377.

82. Ysrraelit, M.C. Correale, J., *Impact of sex hormones on immune function and MS development.* Immunology, 2018. DOI: 10.1111/imm.13004.

83. Gilmore, W., et al., *Preliminary studies of cytokine secretion patterns associated with pregnancy in MS patients.* J Neurol Sci, 2004. **224**: p. 69-76. DOI: 10.1016/j.jns.2004.06.011.

84. Neuteboom, R.F., et al., *Pregnancy-induced fluctuations in functional T-cell subsets in multiple sclerosis patients.* Mult. Scler, 2010. **16**: p. 1073-1078.

85. Langer-Gould, A., et al., *Interferon-gamma-producing T cells, pregnancy, and postpartum relapses of multiple sclerosis.* Arch Neurol, 2010. **67**: p. 51-7. DOI: 10.1001/archneurol.2009.304.

86. Airas, L., et al., *Immunoregulatory factors in multiple sclerosis patients during and after pregnancy: relevance of natural killer cells.* Clin. Exp. Immunol, 2008. **151**: p. 235-243.

87. de Andrés, C., et al., *Activation of Blood CD3+CD56+CD8+ T Cells during Pregnancy and Multiple Sclerosis.* Frontiers in Immunology, 2017. **8**. DOI: 10.3389/fimmu.2017.00196.

88. Gilli, F., et al., *Learning from nature: pregnancy changes the expression of inflammation-related genes in patients with multiple sclerosis.* PLoS. ONE, 2010. **5**: p. e8962.

89. Keith, A.B., *Effect of pregnancy on experimental allergic encephalomyelitis in guinea pigs and rats.* J. Neurol. Sci, 1978. **38**: p. 317-326.

90. Mertin, L.A. Rumjanek, V.M., *Pregnancy and the susceptibility of Lewis rats to experimental allergic encephalomyelitis.* J. Neurol. Sci, 1985. **68**: p. 15-24.

91. Harness, J. McCombe, P.A., *The effects of pregnancy on myelin basic protein-induced experimental autoimmune encephalomyelitis in Lewis rats: suppression of clinical disease, modulation of cytokine expression in the spinal cord inflammatory infiltrate and suppression of lymphocyte proliferation by pregnancy sera.* Am. J. Reprod. Immunol, 2001. **46**: p. 405-412.

92. Langer-Gould, A., et al., *Late pregnancy suppresses relapses in experimental autoimmune encephalomyelitis: evidence for a suppressive pregnancy-related serum factor.* J. Immunol, 2002. **169**: p. 1084-1091.

93. McClain, M.A., et al., *Pregnancy suppresses experimental autoimmune encephalomyelitis through immunoregulatory cytokine production.* J. Immunol, 2007. **179**: p. 8146-8152.

94. Barac-Latas, V., Muhvic, D. Radosevic-Stabic, B., *The influence of pregnancy on development and course of chronic relapsing experimental autoimmune encephalomyelitis in rats: implications for multiple sclerosis.* Coll. Antropol, 2010. **34 Suppl 1**: p. 267-271.

95. Song, F., et al., *Pregnancy modulates adoptively transferred experimental autoimmune encephalomyelitis (EAE).* FASEB Journal, 2008. **22**: p. 853.16.

96. Gatson, N.N., et al., *Induction of pregnancy during established EAE halts progression of CNS autoimmune injury via pregnancy-specific serum factors.* J. Neuroimmunol, 2011. **230**: p. 105-113.

97. Brenner, T., et al., *Experimental allergic encephalomyelitis: passive transfer of resistance during lactation.* J. Neuroimmunol, 1986. **12**: p. 317-327.

98. Williams, J.L., et al., *Serum exosomes in pregnancy-associated immune modulation and neuroprotection during CNS autoimmunity.* Clin. Immunol, 2013. **149**: p. 236-243.

99. Engler, J.B., et al., *Glucocorticoid receptor in T cells mediates protection from autoimmunity in pregnancy.* Proc Natl Acad Sci U S A, 2017. **114**: p. E181-e190. DOI: 10.1073/pnas.1617115114.

100. Benedek, G., et al., *Estrogen protection against EAE modulates the microbiota and mucosal-associated regulatory cells.* J Neuroimmunol, 2017. **310**: p. 51-59. DOI: 10.1016/j.jneuroim.2017.06.007.

101. Benedek, G., et al., *Novel feedback loop between M2 macrophages/microglia and regulatory B cells in estrogen-protected EAE mice.* J Neuroimmunol, 2017. **305**: p. 59-67. DOI: 10.1016/j.jneuroim.2016.12.018.

102. Jalkanen, A., Alanen, A. Airas, L., *Pregnancy outcome in women with multiple sclerosis: results from a prospective nationwide study in Finland.* Mult. Scler, 2010. **16**: p. 950-955.

103. Roullet, E., et al., *Pregnancy and multiple sclerosis: a longitudinal study of 125 remittent patients.* J. Neurol. Neurosurg. Psychiatry, 1993. **56**: p. 1062-1065.

104. Worthington, J., et al., *Pregnancy and multiple sclerosis--a 3-year prospective study.* J Neurol, 1994. **241**: p. 228-233.

105. Portaccio, E., et al., *Postpartum relapses increase the risk of disability progression in multiple sclerosis: the role of disease modifying drugs.* J. Neurol. Neurosurg. Psychiatry, 2014. **85**: p. 845-850.

106. Vukusic, S., et al., *Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse.* Brain, 2004. **127**: p. 1353-1360.

107. Portaccio, E., et al., *Breastfeeding is not related to postpartum relapses in multiple sclerosis.* Neurology, 2011. **77**: p. 145-150.

108. Pasto, L., et al., *Epidural analgesia and cesarean delivery in multiple sclerosis post-partum relapses: the Italian cohort study.* BMC Neurol, 2012. **12**: p. 165. DOI: 10.1186/1471-2377-12-165.

109. Langer-Gould, A., et al., *Exclusive breastfeeding and the risk of postpartum relapses in women with multiple sclerosis.* Arch. Neurol, 2009. **66**: p. 958-963.

110. Airas, L., et al., *Breast-feeding, postpartum and prepregnancy disease activity in multiple sclerosis.* Neurology, 2010. **75**: p. 474-476.

111. Neuteboom, R.F., et al., *First trimester interleukin 8 levels are associated with postpartum relapse in multiple sclerosis.* Mult. Scler, 2009. **15**: p. 1356-1358.

112. de Seze, J., et al., *Intravenous corticosteroids in the postpartum period for reduction of acute exacerbations in multiple sclerosis.* Mult. Scler, 2004. **10**: p. 596-597.

113. Achiron, A., et al., *Effect of intravenous immunoglobulin treatment on pregnancy and postpartum-related relapses in multiple sclerosis.* J. Neurol, 2004. **251**: p. 1133-1137.

114. Haas, J. Hommes, O.R., *A dose comparison study of IVIG in postpartum relapsing-remitting multiple sclerosis.* Mult. Scler, 2007. **13**: p. 900-908.

115. Hellwig, K., et al., *Immunomodulation and postpartum relapses in patients with multiple sclerosis.* Ther. Adv. Neurol. Disord, 2009. **2**: p. 7-11.

116. Rosa, G.R., et al., *There is no benefit in the use of postnatal intravenous immunoglobulin for the prevention of relapses of multiple sclerosis: findings from a systematic review and meta-analysis.* Arq Neuropsiquiatr, 2018. **76**: p. 361-366. DOI: 10.1590/0004-282x20180041.

117. Laplaud, D.A., et al., *Increase in multiple sclerosis relapse rate following in vitro fertilization.* Neurology, 2006. **66**: p. 1280-1281.

118. Hellwig, K., et al., *Increased MS relapse rate during assisted reproduction technique.* J. Neurol, 2008. **255**: p. 592-593.

119. Hellwig, K., et al., *Increase in relapse rate during assisted reproduction technique in patients with multiple sclerosis.* Eur. Neurol, 2009. **61**: p. 65-68.

120. Correale, J., Farez, M.F. Ysrraelit, M.C., *Increase in multiple sclerosis activity after assisted reproduction technology.* Ann. Neurol, 2012. **72**: p. 682-694.

121. Michel, L., et al., *Increased risk of multiple sclerosis relapse after in vitro fertilisation.* J Neurol. Neurosurg. Psychiatry, 2012. **83**: p. 796-802.

122. Chen, A., et al., *The neuropeptides GnRH-II and GnRH-I are produced by human T cells and trigger laminin receptor gene expression, adhesion, chemotaxis and homing to specific organs.* Nat. Med, 2002. **8**: p. 1421-1426.

123. Houtchens, M.K., et al., *Pregnancy rates and outcomes in women with and without MS in the United States.* Neurology, 2018. DOI: 10.1212/wnl.0000000000006384.

124. Sandberg-Wollheim, M., et al., *Pregnancy Outcomes from the Branded Glatiramer Acetate Pregnancy Database.* Int J MS Care, 2018. **20**: p. 9-14. DOI: 10.7224/1537-2073.2016-079.

125. Hellwig, K., Haghikia, A. Gold, R., *Pregnancy and natalizumab: results of an observational study in 35 accidental pregnancies during natalizumab treatment.* Mult. Scler, 2011. **17**: p. 958-963.

126. Hoevenaren, I.A., et al., *Delivery of healthy babies after natalizumab use for multiple sclerosis: a report of two cases.* Acta Neurol. Scand, 2011. **123**: p. 430-433.

127. Nielsen, N.M., et al., *Reproductive history and risk of multiple sclerosis.* Epidemiology, 2011. **22**: p. 546-552.

128. Disanto, G., et al., *Prodromal symptoms of multiple sclerosis in primary care.* Ann Neurol, 2018. **83**: p. 1162-1173. DOI: 10.1002/ana.25247.

129. Hogg, T., et al., *Mining healthcare data for markers of the multiple sclerosis prodrome.* Mult Scler Relat Disord, 2018. **25**: p. 232-240. DOI: 10.1016/j.msard.2018.08.007.

130. Wijnands, J.M.A., et al., *Health-care use before a first demyelinating event suggestive of a multiple sclerosis prodrome: a matched cohort study.* Lancet Neurol, 2017. **16**: p. 445-451. DOI: 10.1016/s1474-4422(17)30076-5.

131. Runmarker, B. Andersen, O., *Pregnancy is associated with a lower risk of onset and a better prognosis in multiple sclerosis.* Brain, 1995. **118**: p. 253-261.

132. D'Hooghe M, B., Nagels, G. Uitdehaag, B.M., *Long-term effects of childbirth in MS.* J Neurol Neurosurg Psychiatry, 2010. **81**: p. 38-41. DOI: 10.1136/jnnp.2008.163816.

133. Verdru, P., et al., *Pregnancy and multiple sclerosis: the influence on long term disability.* Clin Neurol Neurosurg, 1994. **96**: p. 38-41.

134. Altintas, A., et al., *Pregnancy Data in a Turkish Multiple Sclerosis Population.* Eur Neurol, 2015. **74**: p. 296-302. DOI: 10.1159/000441450.

135. Ponsonby, A.L., et al., *Offspring number, pregnancy, and risk of a first clinical demyelinating event: the AusImmune Study.* Neurology, 2012. **78**: p. 867-74. DOI: 10.1212/WNL.0b013e31824c4648.

136. Jokubaitis, V.G., et al., *Predictors of long-term disability accrual in relapse-onset multiple sclerosis.* Ann Neurol, 2016. **80**: p. 89-100. DOI: 10.1002/ana.24682.

137. Koch, M., et al., *Parity and secondary progression in multiple sclerosis.* J. Neurol. Neurosurg. Psychiatry, 2009. **80**: p. 676-678.

138. Basso, O., et al., *Multiple sclerosis in women having children by multiple partners. A population-based study in Denmark.* Mult. Scler, 2004. **10**: p. 621-625.

139. Thompson, E.E., et al., *Global DNA methylation changes spanning puberty are near predicted estrogen-responsive genes and enriched for genes involved in endocrine and immune processes.* Clin Epigenetics, 2018. **10**: p. 62. DOI: 10.1186/s13148-018-0491-2.

140. Yang, Y.M., et al., *Estrogen-dependent epigenetic regulation of soluble epoxide hydrolase via DNA methylation.* Proc Natl Acad Sci U S A, 2018. **115**: p. 613-618. DOI: 10.1073/pnas.1716016115.

141. Pfeiffer, J.R., Mutesa, L. Uddin, M., *Traumatic Stress Epigenetics.* Curr Behav Neurosci Rep, 2018. **5**: p. 81-93. DOI: 10.1007/s40473-018-0143-z.

142. Gapp, K., et al., *Potential of Environmental Enrichment to Prevent Transgenerational Effects of Paternal Trauma.* Neuropsychopharmacology, 2016. **41**: p. 2749-58. DOI: 10.1038/npp.2016.87.