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Review Article

Role of estrogen in the treatment of multiple sclerosis: novel insights regarding its mechanism of action

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ABSTRACT

Multiple sclerosis (MS) is a long-term illness predominantly affecting young adults, characterized by chronic inflammation and neurodegeneration as well as autoimmune responses. It causes various neurological symptoms, including vision problems, motor dysfunction, and cognitive impairment. MS arises from a complex interplay between genetic susceptibility and environmental influences, leading to an aberrant immune response against myelin. Microglial activation and the production of inflammatory mediators, such as cytokines and reactive oxygen species (ROS), contribute to myelin damage and axonal loss. MS occurs more frequently in females than males. This fact draws attention to the hormonal role of estrogen in the pathology and disease progression. Besides the role of estrogen in regulating the menstrual cycle and pregnancy, it binds to specific receptors and activates intracellular signaling pathways. It generally shifts T-helper lymphocytes (Th)-1type cellular immunity into Th-2 humoral immunity with the help of regulatory T cells (T-reg). Additionally, estrogen halts the production of inflammatory markers such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). Recent studies have explored the potential therapeutic, neuroprotective, and immunomodulatory roles of estrogen in MS. Estriol, a specific form of estrogen, has shown promise in reducing relapse rates in patients with relapsing-remitting MS (RRMS). Clinical trials have demonstrated that estradiol, when used alongside standard MS therapies, can significantly reduce relapse frequency and improve neurological function. Understanding the intricate relationship between estrogen and MS pathogenesis can yield crucial insights for developing novel, innovative therapeutic approaches. By targeting estrogen-related pathways, researchers aim to develop protective and therapeutic agents to combat MS progression.

Keywords: *Multiple sclerosis; Inflammation; Oxidative stress; Estrogen; TNF-α.*

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1. Background and Disease Overview

Multiple Sclerosis (MS) is considered a heterogeneous inflammatory, immune-mediated disorder that constitutes the most prevalent acquired chronic condition leading to significant physical disability, particularly among young adults **[1]**.

Epidemiological research has greatly contributed to clarifying the nature and interaction of environmental and genetic risk factors for MS, highlighting a significant rise in the number of people developing MS, especially in the Middle East, in the last few decades **[2]**.

Women are more prone to develop MS than men, exhibiting a higher prevalence ratio that reaches 2-3 times **[3]**. The global median prevalence of MS has risen lately to reach 35.9/100,000 [1]. In Egypt, the prevalence is increasing, reaching approximately 25/100,000 recorded from different centers **[4]**.

While the exact cause of MS remains unknown, it is thought to result from a combination of genetic factors and environmental triggers, such as poor diet, vitamin D deficiency, obesity, smoking, and infections, can result in an abnormal immune response that damages the nervous system as a result of acute then chronic inflammation **[5, 6]**.

The main distinctive features of MS are the demyelination and degeneration of the central nervous system (CNS) neurons. This results in the formation of a de-myelinated plaque due to myelin loss and the formation of astrocytic scars, and consequently causes axonal loss, which is believed to be the major determinant of long-term disability **[7]**.

It was also suggested that there are humoral and cellular immune mechanisms involved in MS pathology in addition to primary oligodendrocytic degeneration and apoptosis **[8]**.

1.1. Diagnosis of MS

1.1.1. Clinical manifestations

Patients with MS usually experience a range of deficits, including visual and sensory dysfunction, limb weakness, ataxia, spasticity, bladder, and bowel problems. Initially, the symptoms might improve, but gradually lead to a progressive physical disability over time **[9]**.

The disease course is mainly divided into three distinct subtypes:

- The most common subtype pattern of MS is relapsing-remitting MS (RRMS), affecting about 85% of patients. It is marked by consequent periods of neuron de-myelination followed by re-myelination, where the relapse period is defined as the appearance of new or worsening neurological symptoms with a duration of more than 24 hours after a period of improvement or complete absence of symptoms. These relapses are a hallmark of MS.
- Primary progressive MS (PPMS): In this type, symptoms progressively deteriorate from the onset of the disease without periods of recovery.
- Secondary progressive MS (SPMS): This type often begins as RRMS but later progresses to a steady decline in function, with or without occasional relapses **[10]**.

1.1.2. Diagnostic tests

The diagnosis of MS relies on the medical history of the patient and neurological evaluations utilizing different imaging methods like lumbar puncture and magnetic resonance imaging (MRI) for analyzing cerebrospinal fluid (CSF). MRI is used to detect any scars in the brain and damage in the CNS. CSF analysis is used for the determination of myelin basic protein and immunoglobulin G. In addition, evoked potentials, analysis of blood samples, and examination of eyes are helpful as well **[11]**.

1.2. Pathogenesis of MS

As mentioned, the precise cause of MS is unidentified, but it is believed that the main primary mechanisms that play a role in the

pathogenesis of MS are the immune-mediated mechanism (both innate and adaptive) and the inflammatory-mediated mechanism, which are interlinked in the progression of the disease course **[12]**.

1.2.1. Inflammatory process

It is assumed that the auto-reactive T-helper lymphocytes play the most important role in the demyelination of the CNS **[13]**. These Th-1 cells, specifically $(CD)4^+$ T-cells, are activated peripherally and then cross the blood-brain barrier (BBB) to enter the CNS. Once inside the CNS, they recognize their specific antigens on the proteins of the myelin sheath on neurons, and this is a crucial process in MS pathogenesis **[14]**. Then, this initiates a chronic inflammatory cascade which eventually damages the axons by their de-myelinating effect with the help of macrophages **[15]**.

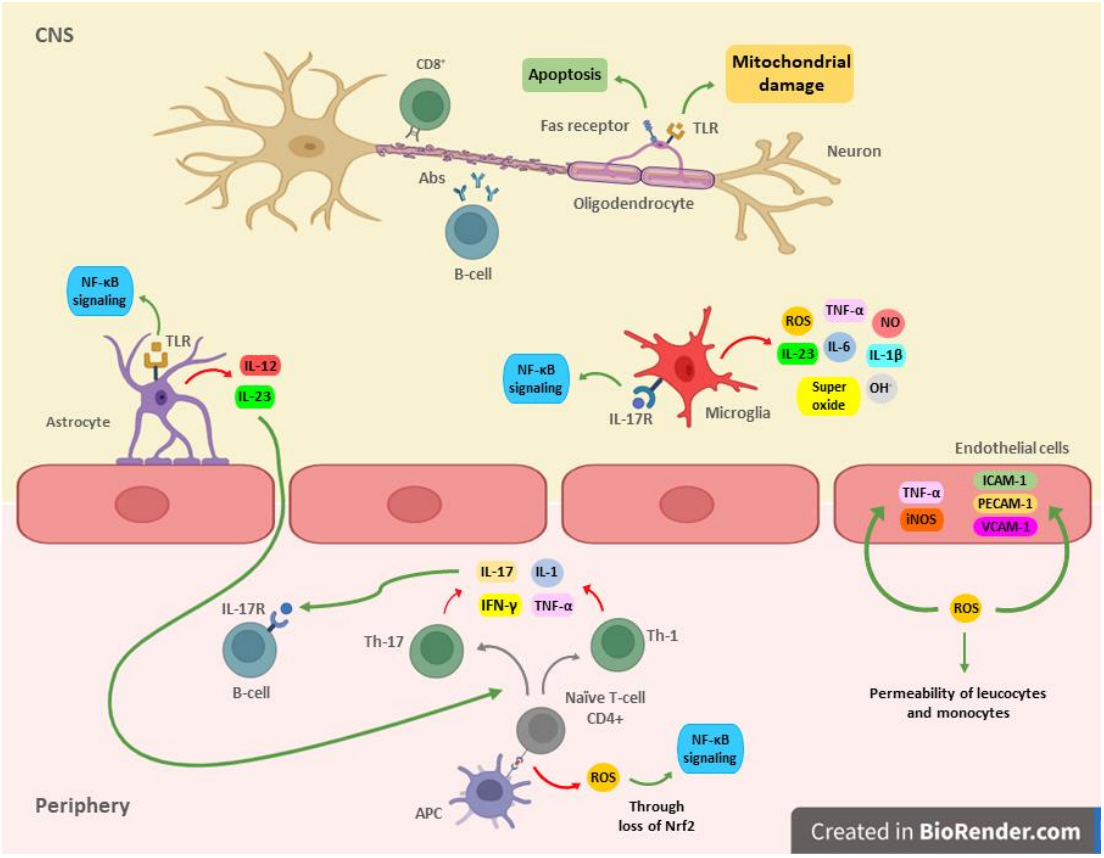

Fig. 1. Pathogenesis of MS

APC interacts with naïve T-cells in peripheral tissues and differentiates them into Th-1 and Th-17 that secrete inflammatory cytokines. ROS are also produced after this interaction and stimulate the secretion of inflammatory cytokines in endothelial cells. In CNS, NF-κB signaling is stimulated in astrocytes and microglia. Inflammatory cytokines are also secreted by neuronal cells. B-cells start to secrete antibodies that attack the myelin sheath, besides the cytotoxic cells. All these events are followed by mitochondrial damage and apoptosis due to the stimulation of TLR and Fas receptors on OLGs. The green arrow represents the stimulation, and the red arrow represents the secretion. Abs, antibodies; APC, Antigen presenting cell; $CD4^+$, T-helper cells; $CD8^+$, cytotoxic cells; CNS, Central nervous system; ICAM-1, Intercellular Adhesion Molecule; IFN-γ, Interferon-gamma; IL, Interleukin; IL-17R, Interleukin 17 receptor; iNOS, Inducible nitric oxide synthase; NF-κB, Nuclear factor kappa B; NO, Nitric oxide; Nrf-2, nuclear factor erythroid 2 - related factor 2; PECAM-1, Platelet endothelial cell adhesion molecule; ROS, Reactive oxygen species; Th-1, T helper lymphocyte-1; Th-17, T helper lymphocyte-17; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor-alpha; T-reg, Regulatory T-cell; VCAM-1, Vascular cell adhesion protein 1.

As shown in **Fig.1**, firstly, activation of T-cells via the antigen-presenting cells (APC) in addition to infiltrating the brain parenchyma, then they are re-activated by the microglial cells [14]. The main autoreactive CD4+ T helper cells involved in MS pathology are Th1 and Th17, which can immigrate to the brain, stimulating the glial cells to start the inflammatory cascade [16]. Th1 cells are responsible for producing multiple pro-inflammatory cytokines, including interleukin-1 (IL-1), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α). Whereas Th17 produces IL-17 and another pro-inflammatory cytokine **[17]**.

Furthermore, Th1 and Th17 promote inflammation in MS, and it was found that IL-17 receptors (IL-17R) are detected and identified in both acute and chronic MS plaques **[18]**. This was shown in previous studies that revealed a decrease in MS clinical symptoms or severity when performed in IL-17R deficient mice **[19]**.

On the contrary, regulatory T-cells (T-regs), possess a functional suppressive action in healthy conditions. Consequently, this action is slightly impaired in MS pathology **[20]**.

After T-cells' immigration by disrupting the BBB, they allow other peripheral immune cells (B-cells and monocytes) to migrate centrally **[21]**. Then, myelin starts to be damaged, causing neuronal degeneration in some lesions as a result of myelin-specific antibodies secreted by B-cells, attacking myelin sheath. Also, chronic inflammation is seen in MS and continues through innate immune responses of microglia and astrocytes **[22]**.

1.2.2. De-myelination

1.2.2.1. Oxidative stress and tissue damage

Many studies have demonstrated that both neuro-inflammation as well as oxidative stress are key contributors to the development of MS. Specially, oxidative stress plays a crucial role in the disease progression. This is because the myelin sheath is composed mainly of lipids (around 70%). This makes it very susceptible to damage caused by free radicals, a process known as lipid peroxidation **[23]**.

In patients with MS, Reactive oxygen species (ROS) regulate the endothelial cells, which serve as the link between the brain and peripheral tissues. ROS alters the structure of these cells, making the BBB more permeable. This heightened permeability enhances the infiltration of immune cells like leukocytes and monocytes to enter the brain. Once inside, these immune cells produce more ROS, which further activate T-lymphocytes and monocytes, and then induce neuro-inflammation, phagocytosis of myelin sheath, loss of oligodendrocyte (OLG), and thereby neuronal and axonal destruction **[24]**. In endothelial cells, ROS stimulates the expression of adhesion molecules—including vascular cell adhesion molecule-1 (VCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), and intercellular adhesion molecule-1 (ICAM-1)—as well as pro-inflammatory mediators such as TNF-α and inducible nitric oxide synthase (iNOS) **[25, 26]**.

The interaction between T-cells and APCs leads to the production of intracellular ROS. ROS can influence the adaptive immune response, where high levels of ROS lead to the production of pro-inflammatory cytokines due to depletion of nuclear factor erythroid 2-related factor 2 (Nrf2). Additionally, ROS can regulate the activity of nuclear factor Kappa-B (NF-κB) proteins, which are important transcription factors involved in inflammation and immunity **[27]**.

Microglia share in the damage of myelin by generating excessive quantities of superoxide, hydroxyl radicals (HO^-), hydrogen peroxide (H_2O_2), and nitric oxide (NO) **[28]**.

1.2.2.2. Glial activation and gliosis

In the CNS, microglia play an important role in innate immune responses as they participate in the removal of apoptotic cells and myelin debris **[29]**.

The activation of microglia, especially the M2 type, occurs after the activation of their receptors by undefined pathogens or antigens or due to the phagocytosis of myelin or myelin debris. Also, microglia are found to express IL-17 receptors (IL-17R), which results in activation and proliferation as a response to IL-17 stimulation. The inflammation of microglia is considered one of the early signs of axonal injury **[30]**.

After microglial activation, they proliferate and start their crucial role in de-myelination through the secretion of NO, ROS, and pro-inflammatory cytokines like TNF-α, IL-1β, IL-6, and IL-23 [31]. NF-κB is one of the significant inflammatory signaling pathways in microglia **[32]** and its activation shares to the release of the aforementioned pro-inflammatory cytokines **[33]**. This inflammatory response doesn't follow the regulatory mechanism, and the neuronal functions are affected, resulting in the progression of neurodegeneration and hence demyelination **[22]**.

Astrocytes also have an important role in MS pathogenesis as they are the most abundant neuronal cells in the CNS. They are responsible for the immunological responses, as well as keeping the BBB structure, integrity, and brain homeostasis. They also have a role in scar formation, which is important to keep boundaries between healthy and inflamed tissues to restrict the spreading of inflammation cells and thereby neuronal loss and demyelination. Astrocytes respond to neurodegeneration and infections by astrogliosis, where they change in morphology and increase in number **[34]**. On the other hand, activated astrocytes secrete pro-inflammatory cytokines, which propagate microglial activation and neurodegeneration. Demyelinating lesions in MS are characterized by noticeable astrocyte proliferation and activation. Like microglia, astrocytes also express toll-like receptor (TLR) molecules **[35]**.

OLGs and astrocytes are connected by gap junction proteins. These connections help maintain the ionic balance between the two cell types. A disruption in this balance can contribute to neurodegeneration due to impaired function of OLGs **[36]**.

1.2.2.3. Oligodendrocyte death

OLGs are the cells that are responsible for the production and maintenance of the myelin sheath. Patients with MS were found to have myelin repair impairment due to OLGs' death, which leads to myelin loss. OlG's death occurs due to necrosis and apoptosis, resulting in demyelination. OLGs express Fas receptors (cell surface receptors) that are responsible for the apoptosis process **[37]**.

Also, it is found that IFN-γ expression in OLGs may lead to demyelination. OLG destruction is developed due to glial activation and gliosis (microglia and or astrocytes) or mitochondrial stress (oxidative stress) **[38]**.

Glial activation leads to the expression of TLR, which in turn activates the NF-κB signaling, which can stimulate the secretion of IL-12 and IL-23 that augment the interaction of undifferentiated T-cells with APCs, causing them to differentiate into helper T cells (e.g., Th1 and Th17) that can migrate into the CNS and secrete their pro-inflammatory cytokines. In addition, astrocytic and microglial NF-κB create a local inflammatory environment by inducing the secretion of cytokines and chemokines, causing OLGs damage **[39]**.

Moreover, mitochondrial damage in OLGs results from the activation of TLR on OLGs themselves, leading to damage to OLG myelin and the underlying axon **[40]**.

1.2.3. Axonal Loss

Axonal degeneration is the main reason for increasing disability in patients with MS. It is associated with the progression of RRMS. Axonal degeneration results from the direct attack of inflammatory and immune factors or as a secondary to the direct attack of myelin **[41]**. Pro-inflammatory cytokines, free radicals, and matrix metalloproteinases (MMPs) can damage axons, as well as the OLGs **[42]**.

2. Animal Models of MS

Owing to the auto-immune nature of MS pathology, the experimental autoimmune encephalomyelitis (EAE) model is the most widely used animal model in MS **[43]**.

EAE is induced in SJL/J and C57BL/9 mice by triggering some proteins that stimulate an autoimmune T-cell response, where it resembles MS pathology **[44]**.

Also, the cuprizone (Cup) intoxication model is another model that is widely used due to the ease of administration and disease development. The Cup model is applied mainly on C57BL/6 mice owing to the reproducible demyelination, re-myelination, accompanied by microgliosis and astrogliosis simulating MS pathology **[45]**.

It can be applied where animals are fed with cup in their standard diet, resulting in OLGs' death and activation of glial cells that occur naturally with de-myelination **[46]**.

The demyelination process occurs in response to OLGs' death in the Cup model and by direct attack on the myelin sheath as in the EAE model **[47]**.

The cup model resembles MS pathology in the innate immune response nature of resident cells (microglia, macrophages, and astrocytes) which are involved as well as the adaptive immune response that occurs in MS. Normally, microglia play a protective role where it helps in debris clearance **[48]**, but throughout the pathogenesis, this mechanism is impaired in the cup model of MS and therefore re-myelination is inhibited **[49]**.

3. Disease-Modifying Therapies

Management of MS is mainly based on the prevention of damage through immunomodulatory agents or the repair of damage that occurred in CNS neurons through remyelinating agents. Currently, treatments for RRMS include interferon-β, glatiramer acetate, mitoxantrone, Cladribine, Fingolimod, and Teriflunomide, along with newer options like monoclonal antibodies like natalizumab, alemtuzumab, and rituximab. These agents primarily target inflammation and exert their neuroprotective effect through this anti-inflammatory effect without targeting healthy nerve cells directly. Therefore, true neuroprotection would involve a therapy that can reach the brain and directly support neurons, axons, and OLGs. Estrogens show promise in MS treatment, as research from basic science to clinical studies suggests that estrogens may offer both anti-inflammatory and direct neuroprotective benefits. They can easily be taken orally, have a good safety profile, and are relatively affordable **[50]**. Therapies for MS also include treatment of relapses and symptomatic treatment. Steroids are the most established treatment option for MS relapses that target reducing inflammation and speeding up recovery. However, the short courses of steroids have been implicated in adverse effects **[51]**. In addition, physical activity and rehabilitation are useful in MS management **[52]**. In the last few decades, it was noted that there has been an increase in the

usage of complementary and alternative medicine for the treatment of multiple disorders, believing that they are safer alternatives with fewer adverse effects **[53]**.

4. Estrogen

Estrogen, existing in the body as one of these three forms: estrone, estradiol, or estriol (E_3), is a steroid hormone secreted by the ovaries and associated with the female reproductive system, which is responsible for developing female sexual characteristics **[54]**. These three types of estrogen work by regulating gene transcription by nuclear Estrogen receptors (ERs), ER-α and ER-β, which show different transcriptional properties. Those types differ in how they interact with ERs. Estradiol is the most biologically active and abundant estrogen in the body. It is present in two forms: α and β, 17β-estradiol being the more active form. Estradiol plays a crucial role in the regulation of the menstrual cycle and pregnancy. It exerts its effects by binding to specific receptors and activating intracellular signaling pathways **[55, 56]**. Estradiol is the most commonly used form of estrogen hormone replacement therapy. Recently, Estradiol showed promising benefits approved by the Food and Drug Administration (FDA) in the treatment of postmenopausal symptoms and in decreasing the risk of complications related to menopause as osteoporosis and cardiovascular diseases. Synthetic estrogens have been recently designed to have better absorption and effects by modifying the chemical structure to be more compatible for oral, intramuscular, and topical use **[57]**.

4.1. Role of Estrogen in MS

MS is more prevalent in females than in males; this prevalence may be 3 times higher in females than in males, and recent evidences suggest that the gender gap is getting bigger **[58]**. The sex hormones' involvement in disease pathophysiology could be supported by the evidence of decreasing disease incidence in the last trimester of pregnancy, where estrogen is at its highest percentage. During the postpartum period, low estrogen levels may predispose to disease progression. Also, administration of 17β-estradiol helped in decreasing disease severity in EAE models in male and female animals **[13, 59]**.

Although only a minority of patients with MS have lower testosterone levels than the normal range **[60, 61]**, these findings suggest that testosterone may be protective in young men who are genetically predisposed to MS **[59]**. The incidence of MS before puberty is very rare, and the significant increase in MS cases among females after puberty strongly suggests that female sex hormones play a crucial role in the development or progression of this disease. Early puberty is associated with a higher risk of developing adult-onset MS and an earlier disease onset. Furthermore, puberty impacts the disease course, with increased relapse rates around menarche in contrast to later menarche, which is characterized by less severe disability in progressive MS **[62, 63]**. It has been observed that patients with MS, as well as other autoimmune diseases such as Rheumatoid Arthritis (RA) and psoriasis, often experience a temporary clinical improvement during pregnancy **[64]**. This is seen in MS by the evidence that the frequency of relapses is significantly reduced due to a shift in the immune system towards a less inflammatory state, likely influenced by hormonal changes, particularly the increase in E3 and progesterone. However, the post-partum period often witnesses a rebound in MS activity as hormone levels drop sharply, leading to an increased risk of relapses, especially in women with a history of frequent relapses before or during pregnancy and those with higher levels of disability **[65]**. These observations are valid as pregnancy is

characterized by biological changes that mediate immunomodulatory and neuroprotective effects. Regarding MS pathology, one of the main effects of estrogen is the systemic shift from Th-1 type cellular immunity towards Th-2 humoral immunity. This may be helpful for pregnancy in addition to general immune suppression to decrease the chance of fetal rejection. Also, this facilitates the passive transport of antibodies, which is supported by a shift towards Th-2 humoral immunity. In addition, pregnancy is characterized by the presence of potentially neuroprotective hormones, including estrogen, progesterone, and prolactin, which have an important role in the development of neurons and OLGs **[66]**. Those well-established clinical observations drew attention to the concept that sex hormones have a significant role in MS pathogenesis **[67]**. Some studies showed that sex hormones prevented the induction of the condition in EAE animal models, while others showed that sex hormones ameliorate the symptoms and progression of the disease, similar to the effect of late pregnancy, in which it is the peak period of female sex hormones **[13]**. A study observed 254 women with MS for a year post-delivery. It was found that relapse rates significantly decreased (by nearly 80%) during the third trimester of pregnancy. This reduction in relapses during late pregnancy is more substantial than that achieved with commonly used MS treatments like Interferon-beta, Glatiramer Acetate (33%), and Natalizumab (66%) **[68]**. Another long-term study found that women with MS who had at least one pregnancy after their diagnosis reached a stage of severe disability (wheelchair dependence) later in their disease course (around 6.1 years later) compared to those who did not have children **[69]**. While estrogens likely play a key role in the improvement of MS during late pregnancy, it's important to remember that other factors associated with pregnancy may also contribute to this positive effect. However, menopause, characterized by declining estrogen levels, is associated with shifts in the immune system. These shifts include an increase in pro-inflammatory cytokines (for example, IL-17) and a decrease in anti-inflammatory cytokines, which are similar to the immunological imbalances observed in MS. Some studies suggest that estrogen deficiency during menopause might contribute to worsened MS symptoms in some women because estrogen deficiency has been linked to increased levels of Th17 cells. This includes reports of increased symptom severity after menopause, although a direct causal link remains unclear. The overlap between the typical age of active MS and menopause raises questions about the potential influence of hormonal changes on disease course **[70]**. It was found that female sex hormones have this suppressive effect on the pathological process of MS, owing to the inhibition of autoimmune inflammation and neuroprotection. Another mechanism by which female sex hormones reduce the activity of the autoimmune process in MS is by suppressing the production of certain pro-inflammatory cytokines like TNF-α and IFN-γ **[13]**. Estrogen receptors ERα and ERβ play crucial roles: ERα in astrocytes reduces inflammation, while ERβ in OLGs and microglia promotes remyelination and neuroprotection. Estrogens may influence microglia, potentially shifting them towards a less inflammatory state, which could involve indirect effects on B cells **[71]**. As shown in **Fig. 2**, in the CNS, 17-β estradiol originates from astrocytes, neurons, and blood circulation. In microglia, estradiol can activate ER-α and ER-β, exerting its anti-inflammatory effects through the inhibition of inducible nitric oxide synthase (iNOS) expression and the suppression of TNF and IL-6. In another way, it can activate the rapid signaling of calcium (Ca^{2+}), cyclic adenosine monophosphate (cAMP), and extracellular signal-regulated kinases (ERK) through the

membrane receptor G protein-coupled receptor 30 (GPR30) in addition to the negative regulation of inflammasome (NLPR3) signaling pathway, resulting in suppression of IL-1β secretion. In astrocytes, estradiol exerts its anti-inflammatory property as it can suppress chemokine expression through the activation of ER-α **[72]**.

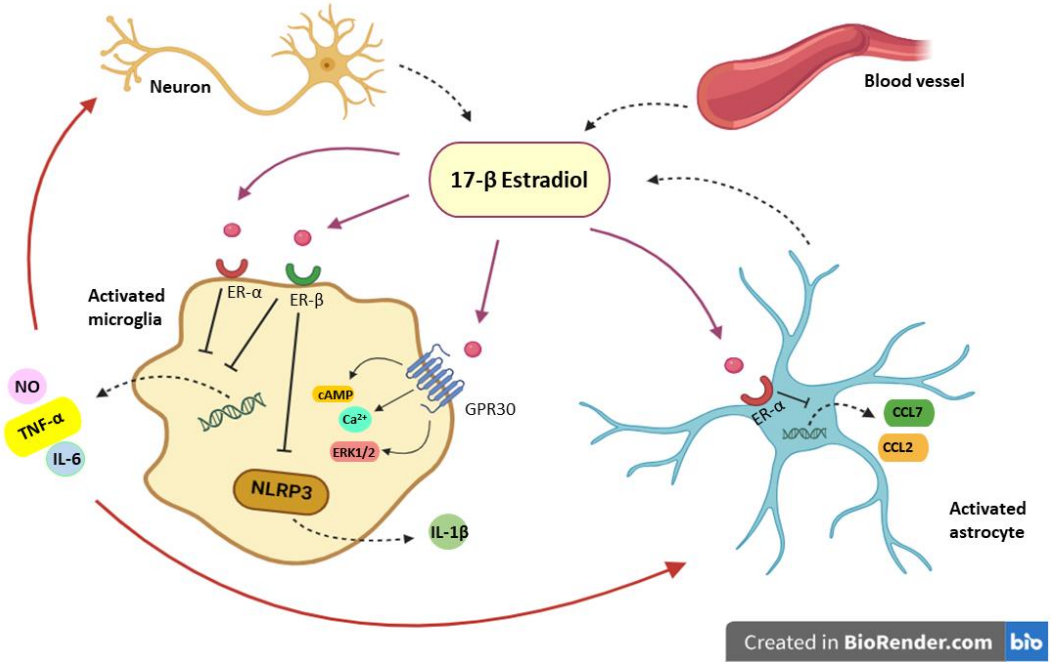

Fig. 2. Functions and signaling of Estradiol in CNS cells

In the CNS, Estrogen derives mainly from neurons, blood circulation and astrocytes. It interacts with its receptors (α, β) on microglia and astrocytes. In astrocytes, estradiol activates ER-α and inhibits CCL secretions, which are involved in MS pathogenesis. While in microglia, it inhibits the transcription of inflammatory cytokines (IL-6 and TNF-α) and NO. In addition to the inhibition of IL-1β through suppression of the NLPR3 pathway. Moreover, estrogen activates the signaling of Ca^{2+}, cAMP, and ERK through the activation of GPR30. The dashed arrow represents the secretion. The purple arrow represents the interaction of 17-β Estradiol with its receptors. The red arrow represents the effect. cAMP, Cyclic adenosine monophosphate; CCL, Chemokine ligand; ER, Estrogen receptor; ERK, Extracellular signal-regulated kinases; GPR30, G-protein coupled receptor 30; IL, Interleukin; NLRP3, inflammasome signaling pathway; NO, Nitric oxide; TNF-α, Tumor necrosis factor-alpha.

While clinical trials investigating androgens in MS are limited, the potential of estrogens to modulate MS has drawn significant attention due to their widespread use in various clinical settings. Recently, E_3, which is produced by the placenta throughout pregnancy, was found to have an obvious effect on EAE. E_3 primarily interacts with ERβ, which can have different effects on gene activity compared to ERα. E_3 treatment was found to be effective also after disease onset in the EAE model **[50, 73]**, encouraging multiple trials using E_3 as a major candidate in the treatment of MS owing to its potent effect on the immune system as well as the CNS **[74]**. Estrogen therapy, including oral contraceptives and E3, has shown promise in reducing relapse rates, MRI activity, and preserving grey matter volume in women with MS. Combined therapy with estriol or oral contraceptives and disease-modifying drugs like Glatiramer or Interferon-β may enhance cognitive performance by preserving gray matter.

Furthermore, co-administration of E3 with disease-modifying treatments may reduce neurofilament light chain levels, suggesting protection against axonal injury **[75]**. A study in female MS patients found that daily E3 treatment for six months significantly reduced the number and size of brain lesions in those with RRMS. However, these improvements were temporary and reversed after treatment ended. The study was continued with the same group of patients with RRMS, this time adding progesterone to the estriol treatment. Over four months, this combined therapy led to a further decrease in the number and size of brain lesions **[76]**. Another study revealed that analysis of blood samples from patients with RRMS treated with estriol showed a shift in immune cell populations, with a decrease in Th1 cells and an increase in Th2 cells. This shift was accompanied by changes in cytokine production, including increased levels of IL-5 and IL-10, and decreased levels of TNF-α. These findings suggest that estriol may have direct anti-inflammatory effects within the brain **[77]**. In a large study, women with RRMS were treated with glatiramer acetate plus either estriol or a placebo. Estriol significantly reduced relapses, improved cognitive function, and reduced fatigue compared to the placebo. Higher estriol levels were associated with fewer relapses and brain lesions. Further analysis showed that areas of the brain preserved from damage during estriol treatment were linked to better cognitive performance **[78]**. Another study found that high doses of estriol decreased the levels of MMP-9 in women with RRMS. MMP-9 is a protein involved in inflammation within the CNS. The reduction in MMP-9 levels was associated with a decrease in the number of active brain lesions detected on MRI scans **[79]**. Also, it was found that adding 20 or 40 µg of ethinyl estradiol to interferon-β treatment in people with RRMS significantly reduced the number of active brain lesions compared to interferon-β alone. The lower dose of ethinyl estradiol (20 µg) showed a smaller, non-significant reduction in lesions. The higher dose of ethinyl estradiol (40 µg) was associated with better cognitive performance compared to placebo when combined with interferon-β **[80]**. Studies in animal models found that estriol doses similar to those seen in late pregnancy offered protection from MS-like disease. However, achieving the same level of protection with estradiol required significantly higher doses, highlighting the importance of finding the right type and dose of estrogen for effective MS treatment **[65, 81]**. In addition, Tibolone, an estrogenic compound, shows promise in reducing inflammation and demyelination in EAE, suggesting potential therapeutic applications for MS **[82]**. These studies highlight the importance of both the type and dosage of estrogen when considering its use as a potential treatment for MS.

4.1.1. Immunomodulatory Properties of Estrogen

In the periphery, estrogens negatively impact the antigen presentation by APCs in addition to shifting the Th-1 response into the Th-2 pattern with the contribution of T-reg cells **[59]**. While in the CNS, estradiol can limit immune-mediated damage by reducing chemokines, cytokines, dendritic cell function, and NO production **[83]**. The peripheral immune cells' migration capacity is also negatively impacted also owing to the role of estrogen in downregulating MMP-9 production as shown in **Fig. 3 [84]**. It was shown that treatment with estradiol activates Th-2 type immune response by the stimulation of IL-4 or IL-10 and suppression of TNF-α from immune cells **[83]**. Estrogen induces CD4+/CD25+ T-reg cells in the EAE model, which also has an anti-inflammatory function **[85]**.

4.1.2. Neuroprotective Impact of Estrogen

The neuroprotective property of estrogen is

suggested to be due to the activation of astrocytic GPR30 in reducing the release of inflammatory cytokines and restoring the autophagy imbalance **[86]**. Estrogen was also found to increase glutamate uptake by astrocytes and which protects neurons from excitotoxicity **[87]**. It was also shown to have a protective role against OLGs from cytotoxicity **[88]**. As shown in **Fig.4**, Estrogen contributes to the formation of dendritic spines and synapses in CA1 pyramidal cells of the hippocampus, resulting in improved cognitive memory function **[89]**. Estrogen has a protective property toward neurons and OLGs by inhibiting their apoptosis. This may be linked to increasing glutamate uptake of astrocytes and thereby decreasing their excitotoxicity. Also, estrogens have a role in synaptogenesis and neurotrophic factor induction **[59]**. Generally, estrogens can modulate microglia activity, reducing inflammation and potentially promoting a more regenerative phenotype. Also, in non-immune demyelination models, estrogens consistently promote remyelination and improve nerve conduction **[90]**. The anti-inflammatory property of estrogen, mediated through estrogen receptor (ER) alpha (α), also has a neuroprotective mechanism. However, this wasn't easy to demonstrate in EAE due to the presence of the anti-inflammatory effect. Recent studies suggest that the neuroprotective effect in EAE may be mediated by ER-beta (ER-β), without an anti-inflammatory effect **[91] (Fig. 4.)**.

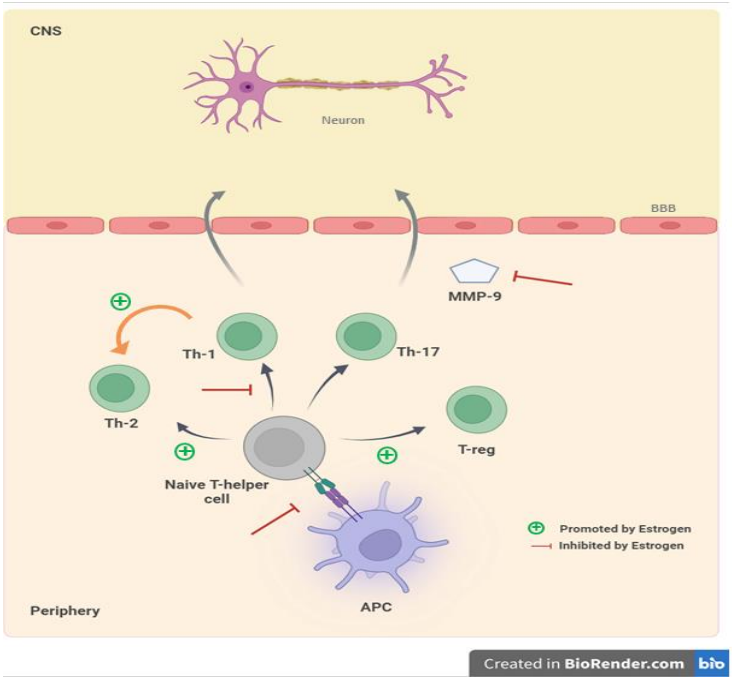

Fig. 3. Immunomodulatory role of Estrogen in peripheral tissues

Estrogen possesses an immunomodulatory function in peripheral tissues as it decreases the interaction of APC with naïve T-helper cells. Estrogen also stimulates the differentiation of T-cells into Th-2, which secretes anti-inflammatory cytokines, and T-reg cells, which have functional anti-inflammatory cells in healthy conditions. In addition to shifting the Th-1 response into the Th-2 pattern response. MMP-9 helps in immune cell infiltration into the BBB, so when inhibited by estrogen, this indirectly exerts an anti-inflammatory response. APC, Antigen-presenting cell; BBB, blood-brain barrier**;** CNS, Central nervous system**;** MMP-9, Matrix metalloproteinase**;** Th-1, T helper lymphocyte 1; Th-2, T helper lymphocyte 2; Th-17, T helper lymphocyte 17**;** T-reg, Regulatory T-cells.

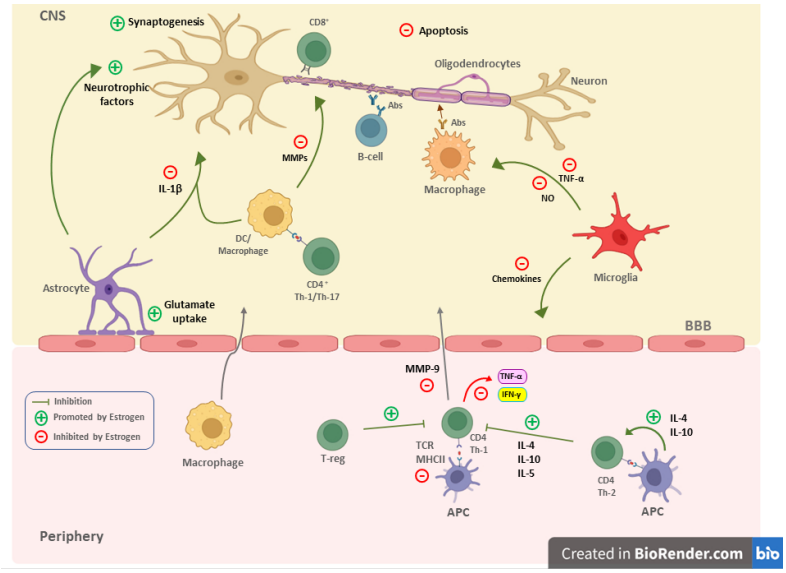

Fig. 4. Mechanism of Estradiol treatment in MS and EAE

In the peripheral immune system, estrogens exert immunomodulatory effects by inhibiting antigen presentation by APCs and shifting immune responses from a Th1 toward a Th2 pattern with the help of T-regulatory cells. The migratory capacity of peripheral immune cells is inhibited by downregulating MMP production, in particular MMP-9, and reducing chemokines. In the CNS, estrogen limits immune-mediated damage by reducing inflammatory cytokines, chemokines, and NO production. The neuroprotective effects of estrogens also include the protection of neurons and OLGs from apoptosis, which may be linked to the ability of estrogens to decrease excitotoxicity by increasing glutamate uptake of astrocytes. Estrogens also induce neurotrophic factors and promote synaptogenesis. The green arrow represents the effect, and the red arrow represents the secretion. Abs, antibodies; APC, antigen-presenting cell; BBB, blood-brain barrier; $CD4^+$, T-helper cells; $CD8^+$, cytotoxic cells; CNS, central nervous system; DC, dendritic cell; IL, interleukin; IFN-γ, Interferon-gamma; MHC, major histocompatibility complex; MMP: matrix metalloproteinase; NO: nitric oxide; TCR, T cell receptor; Th, T-helper lymphocyte; TNF-α, tumor necrosis factor-alpha; T-reg, regulatory T-cells.

4.2. Potential Adverse Events and Limitations of Estrogen Use

Although estrogen may offer some potential benefits for individuals with MS, it's crucial to acknowledge the possible adverse events and limitations associated with the use of natural and synthetic estrogen. Before starting estrogen treatment, doctors need to check your health carefully. This includes looking for any increased risk of serious conditions like breast cancer, uterine cancer, heart problems, stroke, and blood clots (deep vein thrombosis and pulmonary embolism). It's also very essential to perform regular checkups while you are on estrogen therapy **[92]**. The hormonal changes that occur during estrogen therapy may result in irregular menstrual bleeding, breast tenderness, mood swings, headache, nausea, and bloating. Other Potential Risks also include gallbladder disease and liver problems **[62, 63, 65, 75]**. Research from the 1970s to today has consistently shown estriol to be the safest estrogen. It's widely used in Europe and Asia for menopausal symptoms. Unlike estradiol, it was found that it is mainly responsible for possible uterine endometrial proliferation **[50]**.

Conclusion

Estrogen and its receptors have been

implicated in various hypotheses concerning the pathogenesis of MS, making them a central focus of clinical treatment. Estrogen receptors play a significant role in MS treatment by inhibiting neuroinflammation through various pathways. Estradiol prevents inflammation by blocking interactions between antigen-presenting cells and T-cells, reducing pro-inflammatory cytokines, and decreasing immune cell infiltration into the blood-brain barrier. It also enhances anti-inflammatory effects via T-reg and their cytokines. Additionally, estrogen improves glutamate uptake in astrocytes, reduces excitotoxicity, promotes synaptogenesis, and stimulates neurotrophic factors. In microglia, estrogen binding decreases inflammatory cytokine secretion. Despite these benefits, further research is needed to fully understand the mechanisms and potential risks. A comprehensive approach coordinating multiple mechanisms and stable estrogen regulation may offer a more effective treatment for MS.

Abbreviations

APC, Antigen presenting cell; BBB, blood-brain barrier; cAMP, Cyclic adenosine monophosphate; CCL, Chemokine ligand; CD, Cluster of differentiation; CNS, Central nervous system; CSF, Cerebrospinal fluid; Cup, Cuprizone; E_3, Estriol; EAE, Experimental autoimmune encephalomyelitis; ER, Estrogen receptor; ERK, Extracellular signal-regulated kinases; FDA, Food and Drug Administration; GPR30, G-protein coupled receptor 30; ICAM-1, Intercellular Adhesion Molecule 1; IFN-γ, Interferon-gamma; IL, Interleukin; IL-17R, Interleukin 17 receptor; iNOS, Inducible nitric oxide synthase; MHC, Major histocompatibility complex; MMP, Matrix metalloproteinase; MRI, Magnetic resonance imaging; MS, Multiple sclerosis; NF-κB, Nuclear factor kappa B; HO_-, hydroxyl ion; H_2O_2, hydrogen peroxide; NO, Nitric oxide; Nrf-2, nuclear factor erythroid 2 - related factor 2; OLGs, Oligodendrocytes; PECAM-1, Platelet endothelial cell adhesion molecule; PPMS, Primary progressive multiple sclerosis; RA, Rheumatoid arthritis; NLRP3, NOD-like receptor family, pyrin domain containing 3; ROS, Reactive oxygen species; RRMS, Relapsing-remitting multiple sclerosis; SPMS, Secondary progressive multiple sclerosis; TCR, T-cell receptor; Th, T helper lymphocyte; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor-alpha; T-reg, Regulatory T-cells; VCAM-1, Vascular cell adhesion protein 1.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed are included in the main manuscript. All figures included in this manuscript were designed by the authors. These figures are uploaded again separately for high resolution.

Competing interests

The authors declare that no competing interests exist.

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Author's Contribution

M.I.: Conceptualization, Validation, Writing original draft. A.G.: Conceptualization, Validation, Writing - review & editing, Visualization, Supervision. E.M.: Conceptualization, Validation, Writing - review & editing, Visualization, Supervision. M.T.: Conceptualization, Validation, Writing - review & editing, Visualization, Supervision.

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