Archives of Autoimmune Diseases

Nair et al., Arch Autoimmune Dis 2020;
1(2):37-43.

Review Article

Role of estrogen in neuroimmunomodulation in the periphery and onset of autoimmune dysfunction

Rahul S. Nair, Hannah P. Priyanka*

Inspire Lab, Institute of Advanced Research in Health Sciences Tamil Nadu Government Multi Super Speciality Hospital, Omandurar Government Estate, Chennai-600002, India

*Author for correspondence:
Email: hannahpriyanka@gmail.com

Received date: September 07, 2020
Accepted date: November 20, 2020

Citation: Nair RS, Priyanka HP. Role of estrogen in neuroimmunomodulation in the periphery and onset of autoimmune dysfunction. Arch Autoimmune Dis 2020; 1(2):37-43.

Abstract

Maintenance of neuroendocrine-immune homeostasis is tightly regulated by the active involvement of neural, endocrine and immune mediators. As age progresses, this bidirectional regulation losses its robustness due to the influence of several factors especially, gonadal hormones. Among the hormones, estrogen has gained significant attention due to its diverse effects as a potent modulator on the cellular and systemic level which influences both physiological and psychological functions. The mechanistic implications through which estrogenic hormones promote the onset of age-associated diseases are complex especially, its effects on the immune system which is vital for the maintenance of health. This review aims to understand the mechanisms of estrogen-mediated neuroendocrine-immune interactions to facilitate healthy aging, and extent of its effects on the onset of autoimmune dysfunction that may aid the development of better treatment strategies for auto-immune disorders.

Keywords: Estrogen; Neural-immune; Lymphoid organs; Autoimmune disorder

Introduction

Neuroendocrine-immune communication is an intricate interdependent network between the three super systems: the nervous system, the endocrine system, and the immune system. This bidirectional communication is necessary for the maintenance of homeostasis [1,2]. The trophic hormones from the pituitary are under tight regulation by the hypothalamic neuroendocrine system through the release of neurotransmitters, neuropeptides, and hormones; and modulate endocrine functions to maintain physiological homeostasis [1,3]. In turn, the immune system tightly regulates systemic functions, through release of cytokines and chemokines that can cross the blood-brain barrier [4].

Hormones play a diverse role, through its modulatory effects on the neural-immune network throughout reproductive life. Among the several hormones, estrogen has drawn a significant attention because of its pleiotropic effects on neural-immune communication. The effects of estrogen on cellular functions are influenced by receptor subtype status, dose, and duration of exposure [5,6]. Various studies have demonstrated that estrogen treatment confers neuroprotection, but in some cases, it has shown contradictory results [7]. Estrogen has shown modulatory effects on the immune system through a number of pathways and understanding the interdependence of these pathways is a necessity to appreciate the onset of the diseases. Estrogen is recognized as an important immunomodulator, showing significant effects on cellular proliferation, hematopoietic cell differentiation, and cytokine production [8-11].

In this review, we discuss the role of estrogen in influencing neural-immune interactions in the peripheral lymphoid organs (thymus, spleen and lymph nodes), and its involvement in the onset of auto-immune dysfunction contributing to the development and progression of auto-immune disorder with focus on the rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS).

Estrogen-induced Neuroimmunomodulation in the Lymphoid Organs

Several studies have established that nerve fibers such as noradrenergic (NA) and peptidergic fibers, extensively innervate the primary (bone marrow and thymus) and secondary lymphoid organs (spleen, lymph nodes, and lymphoid tissues). These nerve fibers release neural mediators to modulate immune functions. The presence of α- and β-adrenergic receptors (ARs) on T and B lymphocytes, macrophages, and other immune cells have been demonstrated and their role in immunomodulation has been studied by our lab and others [12-15]. Estrogen also modulates the immune system via its receptors, through modulation of the intracellular signalling cascade [6]. The estrogen receptors (ERs) are expressed differentially on lymphocytes with ERβ expressed predominantly on CD4+ T cells while ERβ on B cells that may be involved in maturation and differentiation of T cells in thymus and alteration of B lymphopoiesis in bone marrow [16]. Also, the presence of ERs on the natural killer (NK) and dendritic cells implicates that innate immunity is also regulated in addition to acquired immunity via NF-κB signaling mechanism [17]. Studies also showed a modulatory effect of estrogen on the activation and proliferation of lymphoid cells, thus determining the capability for cytokine and antibody production [18]. Estrogen augments the shift towards Th2 cytokine profile, thereby enhancing humoral immunity and decreasing cell-mediated immunity by suppressing the proliferation of CD4+ T cells involving ERβ-associated mechanism [19].

Estrogen attenuates neuro-immune communication in thymus

Studies have established that estrogen reduces the early thymic progenitor cell population and facilitates the arrest of T cell maturation mediated by ER-β and Fas/FasL pathway that may suppress the neural-immune network [20,21]. Treatment of early middle-aged rats for 30 days with estrogen prevented age- and ovariectomy (OVX)-related increase in the tyrosine hydroxylase (TH) expression in the thymus depending on the dose of estrogen, further accompanied by differential expression of nerve growth factor (NGF) and enhanced expression of intracellular signaling molecules such as p-ERK, p-CREB, and p-Akt that may alter sympathetic NA modulation of thymopoiesis [22]. Estrogen also significantly enhanced lipid peroxidation and production of free radicals, despite an increase in the activities of anti-oxidant enzymes, providing inference that the reactive species may promote sympathetic NA degeneration in thymus and facilitating inflammatory environment [23].

Estrogen-induced neural-immune communication in spleen

Estrogen has shown diverse effects on sympathetic NA neuronal expression and immune responses in the spleen of early middle-aged rats implanted with 17β-estradiol slow release pellets (0.6 μg or 300 μg) for 30 days [24]. Of particular interest is the dose-dependent role of estrogen in modulating neural-immune interactions in the immune effector cells from the spleen. Treatment with lose dose of estrogen (0.6 μg) reduced interferon-gamma (IFN-γ) production and enhanced concanavalin A (Con A)-induced lymphoproliferation, interleukin-2 (IL-2) production, expression of intracellular signaling markers (p-ERK, p-CREB, and p-Akt), activities of anti-oxidant enzymes and nitric oxide (NO) production in the spleen [24]. Treatment with high dose of estrogen (300 μg) however, suppressed Con A- induced lymphoproliferation but increased the expression of p-TH, NGF, intracellular signaling molecules (p-ERK and p-CREB), and activities of anti-oxidant enzymes [24]. Despite these protective effects of estrogen, the production of lipid peroxidation and protein carbonyl formation were enhanced, implicating that the estrogen-mediated oxidative stress may compromise immune functions with advancing age [25]. Studies, both *in vitro* and *in vivo*, have shown similar dose-dependent effects of estrogen on splenocyte proliferation and cytokine production [6,12,13]. *In vitro*, treatment of splenocytes with lower dose of estrogen (10^{-10} M-10^{-12} M) enhanced the lymphoproliferation and production of IFN-γ, whereas treatment with higher doses (10^{-6} M-10^{-8} M) had no effect indicating dose-dependency [6]. Also, co-treatment of 17β-estradiol (E_2) with α1- or α2-adrenergic receptor (AR) agonists and antagonists had differential effects on immune cell proliferation and intracellular signalling molecules suggesting that E_2 can modulate αAR-mediated immune responses [12]. Treatment of splenocytes with estrogen and β2-AR agonist modulated β2-AR-induced immunosuppression through ERα involving cell survival (ERK, PKA and PKC) and inflammation-inducing (NF-κB and NO) pathways that may promote regulation of Th1/Th2 immunity and inflammation during aging [13].

Estrogen-induced neural-immune interactions in lymph nodes

In middle-aged OVX female rats, estrogen treatment (300 μg slow release pellets/30 days) induced significant modulation in the neural-immune network in the skin-draining (inguinal and axillary) and gut-draining (mesenteric) lymph nodes. There was a decline in the lymphocyte proliferation, IFN-γ, tumor necrosis factor-alpha (TNF-α) production, and p-Akt/Total Akt expression in the inguinal and axillary lymph node of early middle-aged female rats [26]. Once again, the dose dependency of the immunomodulatory effects of estrogen on the immune effector cells from the lymph nodes is observed in this study. Contrary to the high dose estrogen treatment, low dose estrogen [0.6 μg slow release pellets/30 days] in OVX rats shown a significant increase in lymphoproliferation, IFN-γ and TNF-α, ROS production, expression of p-NF-κB (p50 and p65), p-mTOR, and p-Akt/Total Akt and decreased cytochrome C oxidase activity concomitant with *in vitro* findings [26]. These studies reiterate the findings that estrogen can act in a dual manner on immune effector cells and influence neural mediators based on the concentration, type of activation and duration of exposure. Another significant finding of this study was that estrogen treatment enhanced malondialdehyde (MDA) formation and NO production. Estrogen may play a role in promoting the development of inflammatory milieu in the inguinal and axillary lymph nodes through enhanced IFN-γ and TNF-α production, intracellular signaling markers such p-Akt, p-mTOR, cytochrome-c oxidase activity, and NO production aided by an increase in lipid peroxidation in the lymphocytes isolated from skin-draining lymph nodes of early middle-aged female rats [26]. In the gut-draining lymph nodes however, estrogen augmented expression of target-derived growth factor expression such as p-TH and NGF in early middle-aged female rats thereby restoring age-associated decline in compensatory mechanisms. Activity of antioxidant enzymes were also enhanced in a dose-dependent manner suggesting neuroprotective effects of estrogen in the mesenteric lymph nodes (MLN) [22]. The expression of the intracellular signaling molecules (p-ERK and p-CREB) was enhanced by treatment with estrogen that may aid in truncating

bacterial translocation and inflammation [27,28]. Differences in the estrogen-induced Neuroimmunomodulation in these lymph nodes may be attributed due to the differences in the functionality of the stromal cells that determines the trafficking of cells of the immune system [29].

Estrogen and Auto-immune Disorders

The differential effects of estrogen on the immune system implicate it's involvement in the onset of autoimmune-disorders. The role of estrogen is complicated and in some disorders, estrogen has shown to be immunostimulatory while in others as inhibitory [8,9]. Estrogen-mediated effects in disease like SLE worsen during pregnancy; others including MS, RA, uveitis and thyroiditis improve, likely due to the maternal shift from a Th1 to Th2 immune response. The diseases that are critically dependent on the T cell-dependent Th1 response; benefit from this diversion, while in SLE a shift to the Th2 propagates the autoantibody response leading to worsening of the disease state [8,9,16]. Studies have shown that estrogen activates B cell function and influences T and B cell lymphopoiesis [16,21]. Reduction in thymic cellularity causing thymic atrophy is observed in treatment with high dose of estrogen [21]. The effect of estrogen on the homeostasis of the immune system is complex, and depends on the cell/tissue type, dose and duration of exposure, expression of receptor subtypes and physiological or pathological contexts [6,30].

RA

RA is the common rheumatic autoimmune disorder with a high female to male incidence both in middle and old age, thereby implicating the role of estrogen in the onset of systemic dysfunction [31-33]. The role of estrogen and its metabolites in the onset of RA is complex, as it is well-established that estrogenic hormones can both stimulate and suppress chronic inflammatory arthritis. The development of collagen-induced arthritis has been prevented with estrogen treatment in mouse models of arthritis using DBA/1LAcJ mice and Lewis rats [34]. Similarly, 2-methoxyestradiol, a naturally occurring estrogen metabolite, conferred a protective effect in murine models for collagen-induced arthritis [35].

In clinical studies, serum 17β-estradiol concentrations were significantly higher in post-menopausal women (n=13) with long-standing RA, compared to controls [36]. Also, inflammatory arthritis is severe in women compared to men (incidence and severity), as evidenced by a multinational cohort study of 6,004 patients with RA [37]. In RA patients, the estrogen concentration in the synovial fluid from knee joints was observed to be 2-2.5 times higher as compared to osteoarthritis patients [38]. *In vitro* studies have shown that estrogen metabolites such as 16-hydroxyestrone and 2-hydroxyestradiol stimulated the proliferation of cultured human monocytes at low and high concentrations [39]. Estrogen stimulates the intra-articular monocytes, that express both ER-α and ER-β in the knee joints of RA patients, thereby contributing to hyperplasia and synovial inflammation [39,40].

Different factors are associated with the protective vs. detrimental effects of estrogen in RA. In RA women, it has been reported that pregnancy is protective for development and progression of RA. It may be due to the presence of pregnancy steroid, estriol or regulated by excessive estrogen [41]. The expression of the ERα is found to be higher than ERβ in the synovial tissues from RA patients, and inflammation enhances receptor expression to further induce proinflammatory cytokines TNFα, IL-1β, and IL-6 by PBMCs [42]. Certain case-controlled studies have shown that oral-contraceptives prevent mild-RA disease from progressing to severe condition in women [43].

Hormone replacement has beneficial effects on postmenopausal osteoporosis in RA, without activating the underlying rheumatic disease and treatment with estradiol twice a week with norethisterone enhanced lumbar spine bone mineral density in postmenopausal RA patients [44]. Patients with high serum estradiol concentrations have improved articular index and pain index compared to controls [45]. A longer period of treatment in RA women (n=88) who undertook randomized hormone replacement plus vitamin D3 with supplemental calcium/vitamin D3 and calcium alone for 2 years; resulted in reduced erythrocyte sedimentation rate, lower disease activity score and delayed the progression of bone destruction [46]. Hormone replacement was associated with significantly higher insulin-like growth factor I and decreased soluble IL-6 receptor, but standard measures of humoral and cell-mediated immune responses were not affected [47,48]. In RA patients, the hormone replacement for a 2-year period was found to be beneficial, however patient predisposed to coronary heart disease has risk of the hormone treatment, thereby preclude long-term replacement with estrogen for the majority of postmenopausal RA patients [49-51].

Estrogen receptor modifiers and estrogen receptor ligands have in-turn drawn attention in treatment of osteoporosis and modulate RA. Treatment with raloxifene in ovariectomised mice with collagen-induced arthritis has shown sustained suppression of the progression of arthritis and failure to develop new joint involvement. Despite of inflammatory condition, depriving estrogen in the treated mice has sustained bone mineral density [52]. Sex-based differences have been observed in the male and female RA patients indicating that gonadal hormones affect the disease pathogenesis differentially. Con A-induced interleukin (IL)-2 and IL-17 and p-CREB expression were elevated in peripheral blood mononuclear cells from both men and women with RA. However, IFN-γ expression was suppressed in women and p-STAT-3 expression was enhanced in men [53].

SLE

SLE is another chronic systemic autoimmune disease that affects women in childbearing condition, and it can affect any organ in the body [54,55]. Elevated serum estrogen concentration exacerbates the disease state [56]. It has been reported that estrogen accelerates the progression of disease while estrogen removal ameliorates disease in female lupus-prone mice, treated with hormone replacement and hormone deprivation regimens [57].

In animal models of SLE, treatment of ovariectomised NZB/W mice with ERβ agonist 4,4',4"-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT), enhanced the levels of autoantibodies and proteinuria, and the animals succumbed to the disease sooner compared to controls. ERβ agonist 2,3-bis(p-hydroxyphenyl) propionitrile (DPN) treated mice however, showed reduced anti-dsDNA autoantibodies. These studies show the proinflammatory role of ERα and the anti-inflammatory role of ERβ in SLE models [58]. Concomitantly studies have shown that in male and female SLE animals, the deficiency of ER-α was associated with decreased autoantibody expression, glomerulonephritis, and improved the survival rate [59]. Interestingly, improvement in ERα-deficient lupus-prone female mice were significantly high compared to male mice [60]. Treatment of ERα-deficient mice with estradiol led to

increased serum Th2 cytokine profile, causing increased kidney damage and death [61].

Estrogen signaling contributes to the activation or repression of a number of immunomodulatory cytokines. In SLE, various defects were observed in the phenotype signaling, homeostasis, metabolism and function of T-cells; and estrogen was found to modulate T-cell signaling and activation [62-64]. Increased levels of potent estrone metabolites were observed in SLE patients, implying epigenetic changes via the estrogen receptors [65,66]. Peripheral blood mononuclear cells (PBMCs) express ERα and ERα transcripts and T-cells from SLE patients exhibit binding of ER proteins to the ERE sites [67]. ER expression by PBMCs from patients with SLE is altered showing increased ERα mRNA levels but reduced ERαβ transcripts [68]. In lupus, intracellular ERα and ERβ showed an enhanced variability of ER expression in T-cells compared to healthy controls, indicating a pro-inflammatory role of ERα and anti-inflammatory role of ERβ in SLE patients.

Another determining factor in SLE disease pathogenesis is the imbalance between Th17 and regulatory-T cells (Tregs) [69,70]. Studies have shown that interleukin-6 (IL-6) with low dose TGF-β, influences naïve CD4+ differentiation to Th17 cells rather than Tregs, thereby inhibiting TGF-β induced Treg differentiation [71]. Combination of IL-6 with IL-1β initiates FoxP3 degradation, thus implicating Il-6 as a crucial cytokine in cellular differentiation [72]. In SLE patients, increased levels of IL-6 found in the serum and urine correlates with disease progression [73-76]. Estrogen (E2) stimulates IL-6 expression by biliary epithelial cells in mice and humans [77]. IL-6 production is controlled genetically in an age and gender dependent manner. Clinical studies have reported that IL-6 production increases with age and is a dominant cytokine in women [78]. Thus, IL-6 is a critical inflammatory cytokine, which shifts the balance from Tregs to Th17. These studies indicate that estrogen plays a critical role in immune cell differentiation and function and impacts the resultant immune responses.

MS

MS is an autoimmune disease where the myelin tissue of the CNS is under the attack of autoreactive T-cells that leads to axonal demyelination and dysfunction. The neuroprotective effects of estrogen in both human and EAE mouse model of MS, are mediated through transition of the immune response from Th1 to Th2 type and suppressing immune activation [79-82]. Significant correlation in the progesterone/17β-estradiol ratios were observed during follicular and luteal phases of the menstrual cycle in women with relapsing-remitting MS using brain MRI studies [83]. The relapse rate of MS is reduced in the third trimester of pregnancy, but enhanced 3-months post-partum [79]. Treatment with estriol in non-pregnant women has showed improvement in the disease lesions, presumably due to the shift from Th1 to Th2 immune response [80]. Estrogen treatment ameliorates experimental autoimmune encephalitis, and has been shown to reduce pro-inflammatory Th1, Th17 cells, IFN-γ, IL-17, TNF, and other molecules including iNOS and MCP-1 through ER-α. Estrogen also induces anti-inflammatory cytokines IL-10 and TGF-β and promotes expansion of Tregs possibly through ER-β. Estrogen suppresses CD4 T cell expansion, increases T cell apoptosis by promoting Th1 to Th2 shift and protects grey matter from atrophy. Estrogen can exert a dual role in MS pathogenesis similar to SLE by exerting pathogenic effects through ERα and neuroprotective effects through ERβ [84]. Thus, the role of estrogen in autoimmune diseases have shown to be modulated by differential expression of estrogen receptor subtypes, levels of expression of estrogen and its metabolites and down-stream signals that may contribute to inflammatory or anti-inflammatory signals.

Conclusion

With advancing age, the estrogen plays crucial role involving diverse effects on the neural-immune network that may promote development of autoimmune diseases such as RA, SLE, MS, etc. The alterations in estrogen levels may induce modulatory effects on the neural-immune network in the peripheral lymphoid organs that leads to the development of autoimmune diseases. Through various findings, it has been observed that the estrogen behaves in a dual manner with differential effects mediating dysregulation in the neural-immune communication in peripheral lymphoid organs, in contrast to its neuroprotective property in the central nervous system. Understanding the extent of estrogenic alterations and its influence on systemic functions might provide a better insight on the role of estrogen in neuroimmunomodulation and pave the way for development of better therapeutic targets to prevent the onset of autoimmune dysfunction.

Acknowledgement

Supported by the Department of Science and Technology, Government of India, New Delhi under the Inspire Faculty Award Scheme of AORC (IFA15/LSBM-154).

References

1. Meites J. Neuroendocrine biomarkers of aging in the rat. Experimental Gerontology. 1988 Jan 1;23(4-5):349-58.
2. Ader R, Felten DL, Cohen N (Eds.) Psychoneuroimmunology. (3rd edition) Academic Press, New York. 2001.
3. Downs JL, Wise PM. The role of the brain in female reproductive aging. Molecular and Cellular Endocrinology. 2009 Feb 5;299(1):32-8.
4. Banks WA. The blood-brain barrier in neuroimmunology: tales of separation and assimilation. Brain, Behaviour, and Immunity. 2015 Feb 1;44:1-8.
5. Burns KA, Korach KS. Estrogen receptors and human disease: an update. Archives of Toxicology. 2012 Oct 1;86(10):1491-504.
6. Priyanka HP, Krishnan HC, Singh RV, Hima L, ThyagaRajan S. Estrogen modulates in vitro T cell responses in a concentration- and receptor-dependent manner: effects on intracellular molecular targets and antioxidant enzymes. Molecular Immunology. 2013 Dec 31;56(4):328-39.
7. Wise PM, Dubal DB, Rau SW, Brown CM, Suzuki S. Are estrogens protective or risk factors in brain injury and neurodegeneration? Reevaluation after the Women's health initiative. Endocrine Reviews. 2005 May 1;26(3):308-12.
8. Nalbandian G, Kovats S. Estrogen, immunity & autoimmune disease. Current Medicinal Chemistry-Immunology, Endocrine & Metabolic Agents. 2005 Feb 1;5(1):85-91.
9. Cutolo M, Capellino S, Sulli A, Serioli B, Secchi ME, Villaggio B, et al. Estrogens and autoimmune diseases. Annals of the New York Academy of Sciences. 2006 Nov;1089(1):538-47.
10. Lang TJ. Estrogen as an immunomodulator. Clinical Immunology. 2004 Dec 1;113(3):224-30.

11. Cohen-Solal JF, Jeganathan V, Hill L, Kawabata D, Pinto-Rodriguez D, Grimaldi C, Diamond B. Hormonal regulation of B-cell function and systemic lupus erythematosus. Lupus. 2008 Jun;17(6):528-32.

12. Priyanka HP, ThyagaRajan S. Selective modulation of lymphoproliferation and cytokine production via intracellular signaling targets by α1-and α2-adrenoceptors and estrogen in splenocytes. International Immunopharmacology. 2013 Nov 1;17(3):774-84.

13. Priyanka HP, Singh RV, Pratap UP, ThyagaRajan S. Estrogen modulates β2-adrenoceptor-induced cell-mediated and inflammatory immune responses through ER-α involving distinct intracellular signaling pathways, antioxidant enzymes, and nitric oxide. Cellular Immunology. 2014 Nov 1;292(1-2):1-8.

14. Nance DM, Sanders VM. Autonomic innervation and regulation of the immune system (1987–2007). Brain, Behavior, and Immunity. 2007 Aug 1;21(6):736-45.

15. Madden KS. Catecholamines, sympathetic innervation, and immunity. Brain, Behavior, and Immunity. 2003 Feb;17:S5.

16. Cunningham M, Gilkeson G. Estrogen receptors in immunity and autoimmunity. Clinical Reviews in Allergy & Immunology. 2011 Feb 1;40(1):66-73.

17. Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. Cellular Immunology. 2015 Apr 1;294(2):63-9.

18. McMurray RW. Estrogen, prolactin, and autoimmunity: actions and interactions. International Immunopharmacology. 2001 Jun 1;1(6):995-1008.

19. Maret A, Coudert JD, Garidou L, Foucras G, Gourdy P, Krust A, et al. Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor α expression in hematopoietic cells. European Journal of Immunology. 2003 Feb;33(2):512-21.

20. Yao G, Hou Y. Thymic atrophy via estrogen-induced apoptosis is related to Fas/FasL pathway. International Immunopharmacology. 2004 Feb 1;4(2):213-21.

21. Zoller AL, Kersh GJ. Estrogen induces thymic atrophy by eliminating early thymic progenitors and inhibiting proliferation of β-selected thymocytes. The Journal of Immunology. 2006 Jun 15;176(12):7371-8.

22. Ravichandran KA, Karrunanithi S, Hima L, Pratap UP, Priyanka HP, ThyagaRajan S. Estrogen differentially regulates the expression of tyrosine hydroxylase and nerve growth factor through free radical generation in the thymus and mesenteric lymph nodes of middle -aged ovariectomized female Sprague–Dawley rats. Clinical and Experimental Neuroimmunology. 2017.

23. Roy D, Cai Q, Felty Q, Narayan S. Estrogen-induced generation of reactive oxygen and nitrogen species, gene damage, and estrogen-dependent cancers. Journal of Toxicology and Environmental Health, Part B. 2007 May 30;10(4):235-57.

24. Kale P, Mohanty A, Patil A, Mishra M, Pratap UP, Priyanka HP, et al. Estrogen modulates neural–immune interactions through intracellular signaling pathways and antioxidant enzyme activity in the spleen of middle-aged ovariectomized female rats. Journal of Neuroimmunology. 2014 Feb 15;267(1-2):7-15.

25. Kasturi BS, MohanKumar SM, Sirivelu MP, Shin AC, Mohankumar PS. Chronic estradiol-17β exposure suppresses hypothalamic norepinephrine release and the steroid-induced luteinizing hormone surge: Role of nitration of tyrosine hydroxylase. Brain Research. 2013 Feb 1;1493:90-8.

26. Pratap UP, Sharma HR, Mohanty A, Kale P, Gopinath S, Hima L, et al. Estrogen upregulates inflammatory signals through NF-κB, IFN-γ, and nitric oxide via Akt/mTOR pathway in the lymph node lymphocytes of middle-aged female rats. International Immunopharmacology. 2015 Dec 1;29(2):591-8.

27. Li X, Rana SN, Kovacs EJ, Gamelli RL, Chaudry IH, Choudhry MA. Corticosterone suppresses mesenteric lymph node T cells by inhibiting p38/ERK pathway and promotes bacterial translocation after alcohol and burn injury. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2005 Jul;289(1):R37-44.

28. Hofmann C, Dunger N, Schölmerich J, Falk W, Obermeier F. Glycogen synthase kinase 3-β: a master regulator of toll-like receptor-mediated chronic intestinal inflammation. Inflammatory Bowel Diseases. 2010 Nov 1;16(11):1850-8.

29. Pabst O, Wahl B, Bernhardt G, Hammerschmidt SI. Mesenteric lymph node stroma cells in the generation of intestinal immune responses. Journal of Molecular Medicine. 2009 Oct 1;87(10):945.

30. Lewis-Wambi JS, Jordan VC. Estrogen regulation of apoptosis: how can one hormone stimulate and inhibit?. Breast Cancer Research. 2009 Jun;11(3):1-2.

31. Alpízar-Rodríguez D, Pluchino N, Canny G, Gabay C, Finckh A. The role of female hormonal factors in the development of rheumatoid arthritis. Rheumatology. 2017 Aug 1;56(8):1254-63.

32. Islander U, Jochems C, Lagerquist MK, Forsblad-d'Elia H, Carlsten H. Estrogens in rheumatoid arthritis; the immune system and bone. Molecular and Cellular Endocrinology. 2011 Mar 15;335(1):14-29.

33. Alpízar-Rodríguez D, Finckh A. Environmental factors and hormones in the development of rheumatoid arthritis. InSeminars in immunopathology 2017 Jun 1 (Vol. 39, No. 4, pp. 461-468). Springer, Berlin Heidelberg.

34. Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. The American Journal of Pathology. 2008 Sep 1;173(3):600-9.

35. Brahn E, Banquerigo ML, Lee JK, Park EJ, Fogler WE, Plum SM. An angiogenesis inhibitor, 2-methoxyestradiol, involutes rat collagen-induced arthritis and suppresses gene expression of synovial vascular endothelial growth factor and basic fibroblast growth factor. The Journal of Rheumatology. 2008 Nov 1;35(11):2119-28.

36. Straub RH, Härle P, Atzeni F, Weidler C, Cutolo M, Sarzi-Puttini P. Sex hormone concentrations in patients with rheumatoid arthritis are not normalized during 12 weeks of anti-tumor necrosis factor therapy. The Journal of Rheumatology. 2005 Jul 1;32(7):1253-8.

37. Sokka T, Toloza S, Cutolo M, Kautiainen H, Makinen H, Gogus F, et al. Women, men, and rheumatoid arthritis: analyses of disease activity, disease characteristics, and treatments in the QUEST-RA study. Arthritis Research & Therapy. 2009 Feb 1;11(1):R7.

38. Rovensky J, Kvetnansky R, Radikova Z, Imrich R, Greguska O, Vigas M, et al. Hormone concentrations in synovial fluid of patients with rheumatoid arthritis. Clinical and Experimental Rheumatology. 2005 May 1;23(3):292-6.

39. Capellino S, Montagna P, Villaggio B, Soldano S, Straub RH, Cutolo M. Hydroxylated estrogen metabolites influence the proliferation of cultured human monocytes: possible role in synovial tissue hyperplasia. Clinical & Experimental Rheumatology. 2008 Sep 1;26(5):903.

40. Capellino S, Riepl B, Rauch L, Angele P, Cutolo M, Straub RH. Quantitative determination of steroid hormone receptor positive cells in the synovium of patients with rheumatoid arthritis and

osteoarthritis: is there a link to inflammation?. Annals of the Rheumatic Diseases. 2007 Jan 1;66(1):53-8.

41. Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. The American Journal of Pathology. 2008 Sep 1;173(3):600-9.

42. Straub RH. The complex role of estrogens in inflammation. Endocrine Reviews. 2007 Aug 1;28(5):521-74.

43. Spector TD, Hochberg MC. The protective effect of the oral contraceptive pill on rheumatoid arthritis: an overview of the analytical epidemiological studies using meta-analysis. British Journal of Rheumatology. 1989;28:11.

44. Hall GM, Daniels M, Huskisson EC, Spector TD. A randomised controlled trial of the effect of hormone replacement therapy on disease activity in postmenopausal rheumatoid arthritis. Annals of the Rheumatic Diseases. 1994 Feb 1;53(2):112-6.

45. MacDonald AG, Murphy EA, Capell HA, Bankowska UZ, Ralston SH. Effects of hormone replacement therapy in rheumatoid arthritis: a double blind placebo-controlled study. Annals of the Rheumatic Diseases. 1994 Jan 1;53(1):54-7.

46. D'Elia HF, Larsen A, Mattsson LA, Waltbrand E, Kvist G, Mellström D, et al. Influence of hormone replacement therapy on disease progression and bone mineral density in rheumatoid arthritis. The Journal of Rheumatology. 2003 Jul 1;30(7):1456-63.

47. d'Elia HF, Mattsson LÅ, Ohlsson C, Nordborg E, Carlsten H. Hormone replacement therapy in rheumatoid arthritis is associated with lower serum levels of soluble IL-6 receptor and higher insulin-like growth factor 1. Arthritis Research & Therapy. 2003 Sep 1;5(4):R202.

48. Forsblad d' Elia H, Carlsten H. The Impact of Hormone Replacement Therapy on Humoral and Cell-mediated Immune Responses In Vivo in Post-menopausal Women with Rheumatoid Arthritis. Scandinavian Journal of Immunology. 2008 Dec;68(6):661-7.

49. US Preventive Services Task Force. Hormone therapy for the prevention of chronic conditions in postmenopausal women: recommendations from the U.S. Preventive Services Task Force. Annals of Internal Medicine. 2005;142:885-60.

50. Buyon JP, Petri MA, Kim MY, Kalunian KC, Grossman J, Hahn BH, et al. The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. Annals of Internal Medicine. 2005 Jun 21;142(12_Part_1):953-62.

51. Grady D. HERS Research Group. Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). JAMA. 2002;288(1):49-57.

52. Jochems C, Lagerquist M, Hakansson C, Ohlsson C, Carlsten H. Long-term anti-arthritis and anti-osteopenic effects of raloxifene in established experimental postmenopausal polyarthritis. Clinical and Experimental Immunology 2008;152:593-7.

53. Pratap U, Hima L, Kannan T, Thyagarajan C, Priyanka HP, Vasantharekha R, et al. Sex-Based Differences in the Cytokine Production and Intracellular Signaling Pathways in Patients With Rheumatoid Arthritis. Archives of Rheumatology. 2020;35.

54. Tsokos GC. Systemic lupus erythematosus. The New England Journal of Medicine. 2011;365:2110-21.

55. Moulton VR, Suarez-Fueyo A, Meidan E, Li H, Mizui M, Tsokos GC. Pathogenesis of human systemic lupus erythematosus: a cellular perspective. Trends in Molecular Medicine. 2017 Jul 1;23(7):615-35.

56. Mccarty DJ, Manzi S, Medsger Jr TA, Ramsey-Goldman R, Laporte RE, Kwoh CK. Incidence of systemic lupus erythematosus race and gender differences. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 1995 Sep;38(9):1260-70.

57. Lahita RG. The immunoendocrinology of systemic lupus erythematosus. Clinical Immunology. 2016 Nov 1;172:98-100.

58. Li J, McMurray RW. Effects of estrogen receptor subtype-selective agonists on autoimmune disease in lupus-prone NZB/NZW F1 mouse model. Clinical Immunology. 2007 May 1;123(2):219-26.

59. Bynote KK, Hackenberg JM, Korach KS, Lubahn DB, Lane PH, Gould KA. Estrogen receptor-α deficiency attenuates autoimmune disease in (NZB× NZW) F 1 mice. Genes & Immunity. 2008 Mar;9(2):137-52.

60. Svenson JL, EuDaly J, Ruiz P, Korach KS, Gilkeson GS. Impact of estrogen receptor deficiency on disease expression in the NZM2410 lupus prone mouse. Clinical Immunology. 2008 Aug 1;128(2):259-68.

61. Feng F, Nyland J, Banyai M, Tatum A, Silverstone AE, Gavalchin J. The induction of the lupus phenotype by estrogen is via an estrogen receptor-α-dependent pathway. Clinical Immunology. 2010 Feb 1;134(2):226-36.

62. Katsuyama T, Tsokos GC, Moulton VR. Aberrant T cell signaling and subsets in systemic lupus erythematosus. Frontiers in Immunology. 2018 May 17;9:1088.

63. Perl A. Metabolic control of immune system activation in rheumatic diseases. Arthritis & Rheumatology. 2017 Dec;69(12):2259-70.

64. Moulton VR, Tsokos GC. T cell signaling abnormalities contribute to aberrant immune cell function and autoimmunity. The Journal of Clinical Investigation. 2015 Jun 1;125(6):2220-7.

65. Lahita RG. The immunoendocrinology of systemic lupus erythematosus. Clinical Immunology. 2016 Nov 1;172:98-100.

66. Cutolo M, Sulli A, Straub RH. Estrogen metabolism and autoimmunity. Autoimmunity Reviews. 2012 May 1;11(6-7):A460-4.

67. Kassi EN, Vlachoyiannopoulos PG, Moutsopoulos HM, Sekeris CE, Moutsatsou P. Molecular analysis of estrogen receptor alpha and beta in lupus patients. European Journal of Clinical Investigation. 2001 Jan;31(1):86-93.

68. Inui A, Ogasawara H, Naito T, Sekigawa I, Takasaki Y, Hayashida Y, et al. Estrogen receptor expression by peripheral blood mononuclear cells of patients with systemic lupus erythematosus. Clinical Rheumatology. 2007 Oct 1;26(10):1675-8.

69. Giang S, La Cava A. Regulatory T cells in SLE: biology and use in treatment. Current Rheumatology Reports. 2016 Nov 1;18(11):67.

70. Mizui M, Tsokos GC. Targeting regulatory T cells to treat patients with systemic lupus erythematosus. Frontiers in Immunology. 2018 Apr 17;9:786.

71. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. European Journal of Immunology. 2010 Jul;40(7):1830-5.

72. Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, et al. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity. 2008 Jul 18;29(1):44-56.

73. Linker-Israeli M, Deans RJ, Wallace DJ, Prehn J, Ozeri-Chen T, Klinenberg JR. Elevated levels of endogenous IL-6 in systemic lupus erythematosus. A putative role in pathogenesis. The Journal of Immunology. 1991 Jul 1;147(1):117-23.

74. Ripley BJ, Goncalves B, Isenberg DA, Latchman DS, Rahman A. Raised levels of interleukin 6 in systemic lupus erythematosus correlate with anaemia. Annals of the Rheumatic Diseases. 2005 Jun 1;64(6):849-53.

75. Sabry A, El-Agroudy A, Sheashaa H, Hawas S, El-Shahat FB, Barakat N. Co-administration of ketoconazole and tacrolimus therapy: a transplanted rat model. International Urology and Nephrology. 2006 Dec 1;38(3-4):713-8.

76. Li Y, Tucci M, Narain S, Barnes EV, Sobel ES, Segal MS, et al. Urinary biomarkers in lupus nephritis. Autoimmunity Reviews. 2006 Jul 1;5(6):383-8.

77. Isse K, Specht SM, Lunz III JG, Kang LI, Mizuguchi Y, Demetris AJ. Estrogen stimulates female biliary epithelial cell interleukin-6 expression in mice and humans. Hepatology. 2010 Mar;51(3):869-80.

78. Isse K, Specht SM, Lunz JG, Kang L-I, Mizuguchi Y, Demetris AJ. Estrogen stimulates female biliary epithelial cell interleukin-6 expression in mice and humans. Hepatology. 2010;51:869-80.

79. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T, Pregnancy in Multiple Sclerosis Group. Rate of pregnancy-related relapse in multiple sclerosis. New England Journal of Medicine. 1998 Jul 30;339(5):285-91.

80. Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, et al. Treatment of multiple sclerosis with the pregnancy hormone estriol. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 2002 Oct;52(4):421-8.

81. Bebo BF, Fyfe-Johnson A, Adlard K, Beam AG, Vandenbark AA, Offner H. Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. The Journal of Immunology. 2001 Feb 1;166(3):2080-9.

82. Duncan GS, Brenner D, Tusche MW, Brüstle A, Knobbe CB, Elia AJ, et al. 2-Methoxyestradiol inhibits experimental autoimmune encephalomyelitis through suppression of immune cell activation. Proceedings of the National Academy of Sciences. 2012 Dec 18;109(51):21034-9.

83. Pozzilli C, Falaschi P, Mainero C, Martocchia A, D'Urso R, Proietti A, et al. MRI in multiple sclerosis during the menstrual cycle: relationship with sex hormone patterns. Neurology. 1999 Aug 1;53(3):622-.

84. Pozzilli C, Falaschi P, Mainero C, Martocchia A, D'Urso R, Proietti A, et al. MRI in multiple sclerosis during the menstrual cycle: relationship with sex hormone patterns. Neurology. 1999 Aug 1;53(3):622.