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Sex Differences in Stroke across the Lifespan: The Role of T Lymphocytes

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Abstract

Stroke is a sexually dimorphic disease. Ischemic sensitivity changes throughout the lifespan and outcomes depend largely on variables like age, sex, hormonal status, inflammation, and other existing risk factors. Immune responses after stroke play a central role in how these factors interact. Although the post-stroke immune response has been extensively studied, the contribution of lymphocytes to stroke is still not well understood. T cells participate in both innate and adaptive immune responses at both acute and chronic stages of stroke. T cell responses also change at different ages and are modulated by hormones and sex chromosome complement. T cells have also been implicated in the development of hypertension, one of the most important risk factors for vascular disease. In this review, we highlight recent literature on the lymphocytic responses to stroke in the context of age and sex, with a focus on T cell response and the interaction with important stroke risk factors.

Keywords

Immune Response; Lymphocytes; Stroke; Sex Differences; T cells

Introduction

Stroke is the fifth leading cause of death (Murphy et al. 2015) and the most common cause of disability (Roger et al. 2011) in the United States. The economic burden of stroke was \$33.0 billion in 2013 (American Heart Association Statistics Committee and Stroke Statistics Subcommittee 2015), making the prevention and treatment of stroke a critical public health issue.

Stroke is a disease that exhibits sexual dimorphism throughout the lifespan (Mallick & O'Callaghan 2010) (Niewada et al. 2005; Appelros et al. 2009). Accumulating data from both the laboratory and clinical studies have shown that sex differences in stroke are highly

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dependent on age and hormonal status (Liu & McCullough 2012). It is widely accepted that estrogen, one of the most important female gonadal hormones, is neuroprotective and contributes to the “female resistant” stroke phenotype seen in women throughout most of the lifespan. Intriguingly, neonatal boys have a higher stroke incidence and poorer long-term outcomes than girls suffering from stroke, conversely, elderly women have both a higher incidence of stroke and more detrimental sequela than age-matched men (Hurn et al. 2005; American Heart Association Statistics Committee and Stroke Statistics Subcommittee 2015). Little is known as to why neonatal and geriatric stroke show such striking sexual differences, as circulating hormone levels are equivalently low at both ends of the age spectrum.

The immune system plays a critical role in stroke outcomes, during both the acute stroke event and long-term post-stroke recovery. Emerging data has shown that post-stroke inflammation is shaped by both sex chromosomes and gonadal hormones (Stamova et al. 2012; Liu & McCullough 2012; Manwani et al. 2014; McCullough et al. 2016). The contribution of these factors appears to differ based on age, and a complex interaction between hormones and genetic factors has been recently recognized (McCullough et al. 2016; Manwani et al. 2013). T lymphocytes have been shown to play a significant role in the immune response to stroke. The involvement of estrogen in sex differences in stroke has been extensively reviewed (Ritzel et al. 2013; Pernis 2007; Koellhoffer & McCullough 2013). In this review, we will focus on the T-lymphocyte response to stroke as a possible contributing factor to the sexual dimorphism seen in stroke outcomes across the lifespan.

The Importance of T cells in the Immune Response to Stroke

Inflammatory responses to stroke are initiated by multiple mediators both inside the affected vessel and within the ischemic parenchyma (Kim et al. 2016), a process involving microglial activation and leukocyte aggregation, adhesion and migration into the injured brain. Monocytes, neutrophils and lymphocytes that are recruited into the brain collaborate with microglia to exacerbate the inflammatory responses. The principal inflammatory mechanisms of stroke are summarized in Figure 1 (for a comprehensive review see (Benakis et al. 2016)).

One of the important components of the immune response to stroke is the activation of T lymphocytes that participate in both the innate and adaptive immunity. T lymphocytes have a crucial role in the immunopathology of ischemic stroke and have been shown to have both pro- and anti-inflammatory functions (Gagliani & Huber 2017), contributing to brain damage as well as functional recovery after stroke (S. Wang et al. 2016). In stroke, T cells are classically activated by specific pathogens or antigens presented on antigen presenting cells (APCs), acting as a major driver of the adaptive immune response. However, evidence also shows that certain classes of naïve T cells can also respond to the inflammatory milieu by secreting reactive oxygen species (ROS) and inflammatory cytokines in an antigen-independent manner, contributing to the early, innate immune response (Unutmaz et al. 1994). Traditionally, two main types of T cells have been recognized, divided by their different effector actions. Cytotoxic T cells (CD8⁺ T Cells) can destroy infected cells by cytotoxic mechanisms, and Helper T cells (CD4⁺ T Cells) can release cytokines to

coordinate and modulate the adaptive and innate immune response. T cells of different subtypes have differential effects in inflammation and are summarized in Table 1 and the mechanisms by which T cells affect stroke are clarified in Figure 2.

CD8⁺ T cells not only exert a traditional cytotoxic effect in immunity but also have recently been shown to play a regulatory role via CD8 T-regulatory cells (CD8 Tregs; CD8⁺ CD122⁺). These cells secrete the anti-inflammatory cytokine IL-10 and have an important role in stroke immune tolerance and stroke outcome (Bodhankar et al. 2015; Bodhankar et al. 2015(1)). However, the vast majority of experimental studies that have examined Tregs after stroke have only examined their function in young, male animals. B cells, which are the lymphocytes that secrete antibodies, also have important immunomodulatory effects. Specifically, B regulatory cells (B regs) participate in the suppression of immune responses by the secretion of IL-10, TGF- β and the expression of pro-apoptotic surface proteins (Berthelot et al. 2013). These cells have been shown to participate earlier than Tregs in inflammatory modulation. In a female mouse model of multiple sclerosis, estrogen treatment induced the differentiation of Bregs, promoting an anti-inflammatory response in microglia (Benedek et al. 2016). In the absence of Tregs, Bregs coordinated the immune responses in experimental autoimmune encephalomyelitis (EAE) replacing the Treg role (Subramanian et al. 2011).

Another type of lymphocyte implicated in the response to stroke is $\gamma\delta$ T cell (Shichita et al. 2009; He et al. 2014). The $\gamma\delta$ T cell subtype is characterized by the presence of different T cell receptor (TCR) chains than those found on classical CD4⁺ and CD8⁺ T lymphocytes ($\alpha\beta$ TCR). $\gamma\delta$ T cells can act as helper lymphocytes, and also express specific immune receptors, including Toll-like receptors (TLRs) and an invariant TCR that allows them to respond immediately after injury to pathogen-associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs), giving $\gamma\delta$ T cells innate immune-like features (Prinz et al. 2013). $\gamma\delta$ T cells can also secrete IL-17, a pro-inflammatory cytokine that acts in concert with IL-23 to recruit monocytes and neutrophils to sites of inflammation. Neutralization of IL-17 reduced neutrophil infiltration and protected against ischemic stroke in murine models, but the sex of the animals was not specified, and all mice were young adults (Gelderblom et al. 2012). How the IL-17 signaling axis changes with age has not been well studied in experimental models, but recent evidence has shown that the IL-17 axis changes with aging in humans (Rawji et al. 2016). As the treatment with estrogen can upregulate IL-17 levels in activated splenocytes, future studies need to control for age and hormone status, especially in a disease like stroke, which primarily affects the elderly (Schmitt et al. 2013). $\gamma\delta$ T cells can be activated by microglia through TLR-2, 4 and 9 *in vitro* (Derkow et al. 2015), and can be detected as early as 16 hours in meninges after stroke (Benakis et al. 2016), where they may possibly coordinate the innate and adaptive immune responses. In humans, an imbalance of Th17 and $\gamma\delta$ T cells, as well as an increase in their principal product IL-17, have been reported after stroke. This increase was due to a decrease in immunosuppressive Treg cells and their products, including IL-10 and TGF- β (Hu et al. 2014; Ruhnau et al. 2016).

Sex differences in the adaptive immune system have been recognized for many years. Estrogen has a significant impact on the function of immune cells, leading to dramatic

immunological changes after menopause, and contributes to the predominance of autoimmune diseases in females (Selmi et al. 2012). Importantly, several immune genes are located on the X chromosome, including FoxP3, a master regulator of Tregs, and CD40L, a molecule that is critical for T cell activation (Spolarics 2007; Bianchi et al. 2012). Some of the major discoveries in sex differences in lymphocytes and the most important findings so far in stroke have been summarized in Table 2.

Sex Differences in Ischemic Injury before Puberty

Various injuries to the fetus and neonate can occur in peripartum period, including infection, trauma, and anoxia. Hypoxic ischemic encephalopathy (HIE), also known as intrapartum asphyxia, is a brain injury caused by oxygen deprivation to the fetus, often the result of cord compromise and a global hypoxic event (i.e. umbilical cord strangulation). HIE is the primary cause of preterm birth (Lauterbach et al. 2001), neonatal death, and long-term disability, and is one of the most important causes of cerebral palsy and seizures (Caffarelli et al. 2016). Neonates with HIE present with abnormalities shortly after delivery, principally in muscle tone, posturing, and the development of seizures (Kharoshankaya et al. 2016). Clinically, HIE is a sexually dimorphic disease as boys have been shown to have a higher incidence and poorer post-HIE outcome than girls (Mayoral et al. 2009). Sex differences in the immune response to HIE (Hill & Fitch 2012), including the lymphocytic response, have been documented. A recent study from our laboratory found that neonatal male mice have higher levels of circulating cytokines (TNF- α , IL-1 β), increased microglial activation, and more brain infiltration of lymphocytes and myeloid cells, compared to females 3 days after HIE, indicating an enhanced inflammatory response in HIE males (Mirza et al. 2015).

Together with pH values, increased lymphocyte counts in neonates is a reliable marker for HIE detection (Bracci et al. 2006). A small cohort study reported that human neonates who have suffered from asphyxia show an increase in T cells in the circulation up to day 3 after birth, with counts normalizing after 7 days (Wang & Lu 2008). This finding was supported by another study in which an increased $\alpha\beta$ and $\gamma\delta$ T cells was detected in neonates suffering from pre-term asphyxia (Krolak-Olejniak & Mazur 2004). Sex differences in the immune responses to HIE, especially the T lymphocytic response, warrants further investigation. Modifying T lymphocytes after HIE could reduce long-term disability in children of both sexes.

The vast majority of childhood strokes (defined as strokes occurring in children older than 1 month of age) are acute ischemic strokes (AIS). AIS is one of the main causes of cerebral palsy and other cognitive impairments in children. More than 90% of clinical studies on stroke in children older than 1 year have shown that males have a higher stroke incidence (Mallick & O'Callaghan 2010). Boys also have poorer outcomes than girls after the injury occurs, with higher mortality and more severe long-term cognitive deficits, an effect also observed in HIE (Lauterbach et al. 2001; Mayoral et al. 2009). Interestingly, the protective ischemic phenotype seen in female newborns and children is unlikely due to gonadal hormones (Golomb et al. 2009). In fact, girls and boys have similar levels of estrogen until around 5 years old, when estrogen levels begin to climb in girls, becoming significantly higher during pre-puberty and adolescence (Germany 1973). The mechanisms underlying

this female protective phenotype are still unknown. Several cellular mechanisms have been proposed for this intrinsic female protection, including differences in immune regulation of microglia in the CNS (Pimentel-coelho et al. 2013). These mechanisms are summarized in Table 3.

Stroke in Young Adult Females and Risk Factors

Young adult females have been reported to have a protective stroke phenotype when challenged with ischemic injury, and this has been largely attributed to the effects of estrogen. It is well known that estrogen, a major gonadal hormone with high blood concentration in young women, has neuroprotective and anti-inflammatory effects (for review see (Ritzel et al. 2013; Koellhoffer & McCullough 2013; Villa et al. 2016; Pernis 2007; Gyenes et al. 2010)). In addition, males have higher stroke incidence compared to women during reproductive ages (Liu et al. 2009; Mozaffarian et al. 2015). However, estrogen is not the sole factor that shapes the sex difference in young adults; recent evidence suggests that other mechanisms, including sex-specific T cell function (Table 2), may contribute to the sexual dimorphism seen in ischemic stroke. Several important risk factors for stroke are considered “sex-specific risks”, as they occur only in females (e.g. pregnancy). Other risk factors are considered stronger and more prevalent in women (e.g. hypertension) because they show differential effects in men and women as regards their contribution to stroke risk, while others are considered similar in both sexes (Bushnell, L. D. McCullough, et al. 2014; Bushnell, L. McCullough, et al. 2014). We will specifically discuss potential contributions of T cells to several of these risk factors and direct readers to the published American Heart Association guidelines for further details.

Female-Specific Stroke Risk Factors

Risk factors that are only present in women include Poly Cystic Ovary Syndrome (PCOS), pregnancy and pregnancy complications such as preeclampsia, gestational diabetes, and gestational hypertension. PCOS is a female-specific disease characterized by abnormal sex hormone levels, ovarian cyst formation and an increased risk of infertility. PCOS is a risk factor for the development of cardiovascular disease (de Groot et al. 2011; Gill 2015), is highly related to metabolic syndrome, and has been shown to promote inflammation (Zierau et al. 2016; Z. Wang et al. 2016). Studies on the risk of stroke and cardiovascular disease in PCOS patients have led to contradictory results, as several studies have shown no significant increase in the risk for stroke while others reported PCOS as a risk factor in these patients (Meyer et al. 2005; Carmina et al. 2005; Solomon et al. 2002; Shroff et al. 2007; Wild et al. 2010; Okoroh et al. 2015). The blood pressure, body mass index, testosterone levels and atherogenic lipid profile in the women with PCOS are usually abnormal, which increases the risk for stroke (de Groot et al. 2011). Moreover, treatments (oral contraceptives, metformin, etc.) that PCOS patients receive are also likely to influence the risk of stroke (Anderson et al. 2014; Okoroh et al. 2015).

Although stroke during pregnancy is uncommon, pregnancy increases the risk of stroke and stroke contributes to about 12% of maternal deaths (Bushnell, L. D. McCullough, et al. 2014), with the highest risk seen in the third trimester and the 12 weeks following delivery.

Conditions that increase stroke risk include gestational hypertension, diabetes and, especially, pre-eclampsia. Pre-eclampsia (PE) is a pregnancy complication characterized by new onset hypertension and signs of end-organ dysfunction. In a normotensive woman, PE usually begins after 20 weeks of gestation. PE can lead to placental ischemia and intra-uterine growth retardation and is one of the leading causes of pre-term birth and neonatal and maternal morbidity/mortality. The etiology of PE is unclear but likely involves a loss of immune tolerance between the mother and the fetus (Amaral et al. 2015), generating a massive immune response against fetal antigens characterized by an upregulation of Th1 cells. CD4⁺ T cells play a role in the long-term effects of PE on maternal health. Placental ischemia generates an imbalance in the Treg/Th17 ratio and leads to endothelial dysfunction. This chronic inflammation and endothelial modification increase the risk of later-life hypertension, increasing the risk of cerebrovascular disease and stroke (LaMarca et al. 2013; Amaral et al. 2015). Adoptive transfer of pre-eclamptic CD4⁺ cells into normal pregnant rats has shown to have detrimental effects and leads to kidney damage and high blood pressure in the mother (Novotny et al. 2012).

Female-Prevalent Stroke Risk Factors

Women have a higher incidence of migraine, atrial fibrillation (due to their prolonged lifespan), diabetes mellitus, depression, and hypertension, which are all factors that are known to increase the risk of stroke to varying degrees (Bushnell, L. D. McCullough, et al. 2014). Hormonal status plays a major role in the development of these risk factors, and diabetes mellitus and obesity are related to metabolic syndrome, which in women is closely related to PCOS and the use of oral contraceptives (Mani et al. 2013). Hypertension (HTN) is one of the most important risk factors for stroke. The incidence of HTN increases with age and is higher in males before the age of 55 years; however, this epidemiology changes after this age and HTN becomes more prevalent in elderly females vs. males (Tipton & Sullivan 2014).

Hypertension may have an immunological origin (Sandberg et al. 2015), as T cell infiltration into the kidney and vasculature was seen concomitant with the development of elevated blood pressure (Harrison et al. 2011; Ma et al. 2014). In male Rag1^{-/-} transgenic mice that lack B and T lymphocytes, hypertension did not develop after Angiotensin-II (Ang-II) stimulation (Guzik et al. 2007). The hypertensive response was restored after the adoptive transfer of male T cells but not of male B cells, implicating T cells in the development of HTN. Sex differences were also observed in this animal model of hypertension. While the transfer of adult male T cells into male Rag1^{-/-} generated hypertension and an increased Th17 profile, the transfer of female T cells had the opposite effect due to a higher secretion of IL-10 that suppressed the development of hypertension (Ji et al. 2014). Interestingly, female Rag1^{-/-} hosts were resistant to Ang-II-mediated hypertension induction after adoptive transfer of male T cells (Pollow et al. 2014). To define the chromosomal contribution of sex differences in the generation of hypertension, the four-core genotype (FCG) mice were used. The FCG mice are a transgenic mouse model that does not depend on the Y chromosome to generate a male phenotype, as the Sry gene, which encodes for the testis determinant factor, is translocated to chromosome 3 (Itoh et al. 2015) and is no longer on the Y chromosome. As a result, gonadal females (XX or XY-sry without Sry gene) or

gonadal males (XX+sry or XY+sry, with Sry gene on chromosome 3) can be generated, which allows for the differentiation of hormonal and chromosomal effects (Arnold & Chen 2009; Corre et al. 2014). It was shown that gonadectomy of XX-males and XX-females resulted in increased blood pressure after Ang-II treatment, indicating a significant chromosomal influence that was masked by either female or male gonadal hormones (Ji et al. 2010).

In humans, the incidence of hypertension increases after menopause, which may be related to elevated basal inflammation, as levels of proinflammatory cytokines increase at a much higher rate in post-menopausal women compared to age-matched men (Deswal et al. 2001), resulting in an elevated basal inflammation (Abu-Taha et al. 2009) that can be reversed with hormone therapy (Miller et al. 2003). Interestingly, genes closely related to blood pressure control, such as Angiotensin 2 receptor (AT2R) and the angiotensin converting enzyme 2 (ACE2), are located on the X-chromosome, implying a sex chromosome contribution in the etiology of hypertension (Koike et al. 1994; Tipnis et al. 2000). In summary, T cells are involved in the development of hypertension potential after infiltration to the kidneys, a process influenced by both sex chromosomes and sex hormones. Much more work needs to be done in this area to optimize drug development for the treatment of hypertension (the most common modifiable risk factor for stroke), as effective treatments may differ by sex.

Patients with depression have a higher risk of stroke (Pan et al. 2011), but the mechanism mediating this interaction and the influence of sex are not known. After puberty, depression occurs more frequently in women than men, and T cells may play a role in this sexual dimorphism. For example, women exposed to domestic violence had a stress-related increase in CD4+ T cells, a decrease in Tregs and enhanced transformation of naïve CD4+T cells to effector memory cells (Kalokhe et al. 2016). T lymphocytes have known effects on brain function and behavior (Toben & Baune 2015), as Treg depletion increased anxiety-like behavior and CD4 T cell depletion worsened performance in cognitive tests such as Morris water maze (Herkenham & Kigar 2016). How T cells can modify mood states is unclear, but may be an important factor in stroke recovery.

Stroke in the Elderly

In contrast to young women, older women (over 65 years old) have an increased incidence of stroke and higher morbidity and mortality after stroke compared to age-matched men (Appelros et al. 2009). It has been proposed that the higher rates of stroke, as well as the poorer recovery from stroke in senior women, is due to a decrease in circulating gonadal hormones (Engler-Chiurazzi et al. 2016). However, the risk of stroke does not increase in women until several decades past menopause. Aging increases basal levels of chronic inflammation and levels of circulating pro-inflammatory cytokines, a phenomenon termed “inflammaging”. Inflammaging is a risk factor for morbidity and mortality in the elderly population, independent of disease-specific pathology (Franceschi & Campisi 2014). Under these circumstances, the system becomes pro-inflammatory, increasing the risk of developing neurological diseases (Cribbs et al. 2012). Importantly, chromosomal effects, risk factors developed in adulthood, and the organizational effects of hormones may contribute to the pro-inflammatory changes after menopause.

Permanent Effects of Sex: Hormones vs. Chromosomes

Females are largely “ischemia resistant” during most of their lifespan, perhaps due to the neuroprotective and anti-inflammatory effects of estrogen. In contrast, males are ischemic sensitive during most of the lifespan, with aging being a notable exception. Males also undergo gonadal senescence, and some studies suggest that the waning levels of testosterone contribute to a reduction in stroke risk in men during aging. Gonadectomy in male rodents is protective against ischemic injury, implying a sensitizing role of testosterone in males; in contrast, studies have shown that middle-aged rodents treated with testosterone have a better outcome than untreated animals after stroke, demonstrating an age-dependent response to stroke (Cheng et al. 2009) and rendering the role of testosterone in ischemic stroke outcome unclear. The activational effects of hormones in ischemic stroke are well known (for review (Koellhoffer & McCullough 2013)), yet hormones can also have organizational effects, which are generated by exposure to the hormone early in development, causing tissue patterning and programming that are independent of continued hormone levels, such as sex-specific areas of brain development (Arnold & Breedlove 1985).

Previous studies from our group show that the major differences in ischemic sensitivity in young animals were due to hormones (particularly estrogen) rather than the sex chromosome complement. In these experiments, adult FCG animals were subjected to MCAO, showing that the presence of female gonads was the key factor in sexual dimorphism after stroke as animals that had female gonads (ovaries) and high serum estrogen levels had better stroke outcome than males with testes. Gonadectomy leads to equivalent ischemic damage in all animals regardless of chromosome complement, suggesting that at least in young animals, ischemic sensitivity is driven primarily by gonadal hormones (Manwani et al. 2014). Another study compared (XX) and transgenic XO mice (with either maternal or paternal loss of the second X-chromosome) and found no differences in stroke outcomes regardless of the number of X chromosomes (Turtzo et al. 2011). However, chromosomal contributions to stroke could not be ruled out entirely, as only young mice were examined, and the epigenetic milieu is known to change with aging. Women with Turner’s syndrome, which is characterized by the lack of one of the X chromosomes in females (XO), are three times more likely to develop cerebrovascular disorders than healthy women but these women also suffer from premature ovarian failure and the associated early loss of estrogen, which could independently increase vascular risk (Gravholt et al. 1998; Gravholt 2005). Very new and intriguing data from our laboratory has shown that unlike the findings seen in young FCG mice (where hormones drive ischemic sensitivity), the outcome of stroke seems to be completely dependent on the sex chromosome complement in aged FCG animals. Aged acyclic females and aged males of both genotypes (XX and XY) were compared, and mice with an XX genotype (regardless of gonadal sex; ovaries or testes) had larger infarcts and poorer outcomes compared to XY animals, despite equivalent hormone levels (estrogen or testosterone) (McCullough et al. 2016). These results strongly suggest that the chromosomal complement (XX vs. XY) contributes to ischemic stroke sensitivity in aged animals. As stroke is a disease of the aged, sex chromosomes could play an important role in ischemic sensitivity in the population at risk for stroke, demonstrating the importance of using appropriate animal models that recapitulate the human disease.

Studies on human blood suggest that there is epigenetic regulation and coordination of T and B cell pathways in response to ischemic stroke or atherosclerotic diseases (Barr et al. 2015). It is well known that estrogen can alter epigenetic profiles and that altered gene expression may generate differential responses to a broad spectrum of diseases. Epigenetic regulation of a variety of genes (including those involved in the immune response) may play a role in ischemic sexual dimorphism. Recent data show H3K4 methylation of over 200 genes in T cells has sexually dimorphic changes after an ischemic insult in the mouse brain (Shen et al. 2015) (He et al. 2013). Analysis of human female transcriptome and methylome in CD14, CD19, CD4, and CD8 cells revealed large differences in methylated regions when their genetic signature was compared with a male database of immune cells, specifically in genes related to cell signaling, molecular transport, cell-mediated immune response, organism injury and cardiovascular diseases. (Mamrut et al. 2015).

Lack of estrogen has been proposed as the mechanism underlying the immune changes in females after menopause, which has prompted examination of Hormonal Therapy (HT) as a treatment for stroke in post-menopausal patients. However, clinical trials of estrogen treatment have failed, likely due to the fact that the HT was started many years after menopause. Subsequent experimental studies have shown that estrogen treatment is protective in aged or ovariectomized female animals only when started immediately after the endogenous hormone levels decreased (Liu et al. 2009; Suzuki et al. 2007), suggesting the beneficial effects of exogenous estrogen depends on the timing of the initiation of HT. After menopause, women tend to manifest impaired immune responses as hormone levels diminish; however, HT during menopause may correct this immunological imbalance (Abdi et al. 2016). One study showed that after vaccination against influenza virus, young females generated a more robust B and T cell response than the post-menopausal women; however, when estrogen treatment was administered to the elderly females, a significant increase of IgG titers was induced, suggesting estrogen up-regulated the humoral immune response (Engelmann et al. 2016).

Adaptive Immune Response in Females

Sex hormones shape the development and function of the immune system. Estrogen not only affects the development of T and B cells (Pennell et al. 2012; Hill et al. 2011), but also enhances their function after vaccination (Fairweather et al. 2008). However, estrogen is also associated with an increased risk of autoimmune diseases (Selmi et al. 2012). It was reported that estrogen's effect varies at different concentrations, as estrogen has immunomodulatory effects at high doses by increasing the secretion of IL-4 from Th2 cells, while at lower doses it promotes the differentiation of naïve T cells to Th1 and the subsequent secretion of the proinflammatory cytokine interferon γ (IFN γ) (Pennell et al. 2012).

The X-chromosome harbors several immune-related genes. Although the expression of genes on the second X chromosome is silenced (Chow et al. 2005), some of these genes can escape from X-chromosome inactivation (XCI) with aging. It has been reported that 15% of X-linked genes can escape XCI, resulting in a higher expression of these genes in females (Gubbels Bupp 2015; J. Wang et al. 2016). Females have a higher expression of several immune receptors related to the innate response against virus infection, like TLR-7 and

TLR-8 (Krieg 2007; Cervantes et al. 2012) as well as FoxP3, a transcription factor in regulatory T helper cells (Zheng & Rudensky 2007). The X-chromosome is also rich in microRNA that could significantly affect the immune system after menopause (Zhao et al. 2016). The X-chromosome has a different pattern of gene expression after ischemic stroke in women vs. men, including genes involved in the regulation of proliferation, differentiation, motility and apoptosis, and also genes needed for the differentiation of endothelial cells and the formation of BBB (Stamova et al. 2012). The second X chromosome may be an extra tool that females have to handle ischemic insults as it may provide females an immunological advantage over men. For example, the mRNA expression of genes from the X chromosome in female lymphocytes is different than that in males (by RNA patterns). Also, female patients with Systemic Lupus Erythematosus, an autoimmune disease with predominance in women, have a bi-allelic expression of certain immune genes (J. Wang et al. 2016). These studies suggest a better and faster response of female lymphocytes to injury and immune regulation.

Immunosenescence in Females

Sex differences exist in immune-senescence, an age-associated decline of the immune system and host defense mechanisms (Farage et al. 2012). One mRNA transcriptome study found that females had more altered pathways after menopause compared with elderly men, and importantly some of these changes led to a decrease in T cell response but an increase in pro-inflammatory responses. Specifically, the authors found increased activity in pathways important for T cell regulation, like CTLA-4 and iCOS-iCOS ligand pathways, and an increase in their cytotoxicity, implying the development of a more pro-inflammatory milieu in older women (Marttila et al. 2013).

Aged female mice have a higher basal level of inflammation, which directly translates into larger infarcts and more robust inflammatory responses to ischemic injury (Liu et al. 2009). Females respond differently to stroke at different ages. Compared with males, female mice have less peripheral immune cell infiltration into the brain after stroke at young and middle age, but more with aging (Manwani et al. 2013). Further studies are warranted to clarify the T cell phenotypes in stroke and aging. Some of the most important effects of menopause on human lymphocytes are summarized in Table 4. There is an increase in pro-inflammatory markers in the blood of postmenopausal women, including IL-1 β , IL-6, and TNF α , with a decrease in CD4 T cells and B lymphocytes. This implies an age-dependent imbalance in immune homeostasis. This immunosenescence is reflected in cellular and humoral immune responses and has been implicated in chronic inflammatory diseases such as hypertension, diabetes mellitus, autoimmune diseases, heart disease and atherosclerosis (Gameiro et al. 2010). Although the increase of autoimmunity in post-menopausal women may suggest that sex steroids have an important immunomodulatory role, immunological changes occur with or without hormonal therapy in comparison with young females (Gameiro & Romao 2010). Findings from these studies indicate that estrogen deprivation is only one of the factors that contribute to immunosenescence and there may be other mechanisms to be explored.

Conclusions and Future Perspectives

The immune system is an important contributor to ischemic outcome and is likely the “bridge” linking the CNS and periphery. We focused on sex differences in T cells in this review and discussed how T cells differ in males and females at baseline, how T cells are altered in stroke from work done predominately in male-specific studies, how sex differences in T cells contribute to stroke sensitivity, and finally, how T cells can contribute to common stroke risk factors like hypertension.

Sex differences exist in ischemic stroke sensitivity throughout the lifespan and are due to complex interactions with the immune system that we are only beginning to understand. Age has a major effect on stroke outcome, and how sex influences outcome after stroke remains to be studied. The effects of the hormonal milieu, and changes in hormone levels throughout the lifespan, influence the activity of T cells in both males and females. Identifying these changes will be integral to the development of successful therapeutic agents for both sexes.

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Abbreviations

ACE2	Angiotensin convert enzyme 2
AIS	arterial ischemic stroke
Ang-II	Angiotensin II
APCs	antigen presenting cells
AT2R	Angiotensin II receptor
BBB	blood brain barrier
BDNF	brain-derived neurotrophic factor
Breg	B regulatory cells
CNS	Central nervous system
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
DAMPS	Damage associated molecular patterns
EAE	Experimental Autoimmune Encephalomyelitis
ER	Estrogen Receptor
FCG	Four core genotype
HIE	hypoxic–ischemic encephalopathy

HT	hormone therapy
HTN	Hypertension
IFNγ	Interferon γ
iCOS	Inducible T-cell co-stimulator
IL	interleukin
LPS	lipopolysaccharide
MCAO	middle cerebral artery occlusion
MHC-II	major histocompatibility complex II
OC	oral contraception
PAMPs	Pathogen associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PCOS	poly-cystic ovary syndrome
PE	pre-eclampsia
PMA/Io	phorbol 12-myristate 13-acetate/Ionomycin stimulation
PPAR	peroxisome proliferator-activated receptors
ROS	reactive oxygen species
TCR	T cell receptor
TGF-β	transforming growth factor β
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	T-regulatory cells
vWF	von Willebrand factor
XCI	X-chromosome inactivation

References

- Abdi F, et al. Effects of hormone replacement therapy on immunological factors in the postmenopausal period. *Climacteric : the journal of the International Menopause Society*. 2016; 19(3):234–9. [Accessed October 7, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27086591>. [PubMed: 27086591]
- Abu-Taha M, et al. Menopause and ovariectomy cause a low grade of systemic inflammation that may be prevented by chronic treatment with low doses of estrogen or losartan. *Journal of immunology* (Baltimore, Md : 1950). 2009; 183(2):1393–1402.

- Amaral LM, et al. Preeclampsia: Long-term consequences for vascular health. *Vascular Health and Risk Management*. 2015; 11:403–415. [PubMed: 26203257]
- American Heart Association Statistics Committee, Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation*. 2015
- Anderson SA, Barry JA, Hardiman PJ. Risk of coronary heart disease and risk of stroke in women with polycystic ovary syndrome: A systematic review and meta-analysis. *International Journal of Cardiology*. 2014; 176(2):486. Available at: <http://dx.doi.org/10.1016/j.ijcard.2014.06.079>. [PubMed: 25096551]
- Appelros P, Stegmayr B, Terént A. Sex differences in stroke epidemiology: a systematic review. *Stroke; a journal of cerebral circulation*. 2009; 40(4):1082–90. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19211488>.
- Arnold AP, Breedlove SM. Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. *Hormones and behavior*. 1985; 19(4):469–98. [Accessed October 9, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/3910535>. [PubMed: 3910535]
- Arnold AP, Chen X. What does the “four core genotypes” mouse model tell us about sex differences in the brain and other tissues? *Frontiers in Neuroendocrinology*. 2009; 30(1):1–9. Available at: <http://dx.doi.org/10.1016/j.yfrne.2008.11.001>. [PubMed: 19028515]
- Barr TL, et al. Systemic Transcriptional Alterations of Innate and Adaptive Immune Signaling Pathways in Atherosclerosis, Ischemia Stroke, and Myocardial Infarction. *Journal of bioanalysis & biomedicine*. 2015; 7(2):029–034. [Accessed October 9, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27213032>. [PubMed: 27213032]
- Benakis C, et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal $\gamma\delta$ T cells. *Nature Medicine*. 2016; 13(10):720–726. Available at: <http://www.nature.com/doifinder/10.1038/nm.4068> <http://www.ncbi.nlm.nih.gov/pubmed/22968153>.
- Benedek G, et al. Estrogen induces multiple regulatory B cell subtypes and promotes M2 microglia and neuroprotection during experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology*. 2016; 293:45–53. Available at: <http://dx.doi.org/10.1016/j.jneuroim.2016.02.009>. [PubMed: 27049561]
- Berthelot J-M, et al. Regulatory B cells play a key role in immune system balance. *Joint Bone Spine*. 2013; 80(1):18–22. [PubMed: 22858147]
- Bianchi I, et al. The X chromosome and immune associated genes. *Journal of Autoimmunity*. 2012; 38(2–3):J187–J192. [Accessed November 2, 2016] Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0896841111001259>. [PubMed: 22178198]
- Bodhankar S, Chen Y, Lapato A, Dotson AL, et al. PD-L1 Monoclonal Antibody Treats Ischemic Stroke by Controlling Central Nervous System Inflammation. *Stroke*. 2015; 46(10):2926–2934. [PubMed: 26306753]
- Bodhankar S, Chen Y, Lapato A, Vandenbark AA, et al. Regulatory CD8+CD122+ T-cells predominate in CNS after treatment of experimental stroke in male mice with IL-10-secreting B-cells. *Metabolic Brain Disease*. 2015; 30(4):911–924. [Accessed October 9, 2016] Available at: <http://link.springer.com/10.1007/s11011-014-9639-8>. [PubMed: 25537181]
- Bracci R, Perrone S, Buonocore G. The Timing of Neonatal Brain Damage. *Neonatology*. 2006; 90(3):145–155. [Accessed September 15, 2016] Available at: <http://www.karger.com/?doi=10.1159/000092517>.
- Bushnell C, McCullough LD, et al. Guidelines for the Prevention of Stroke in Women: A Statement for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke*. 2014; 45(5):1545–1588. [Accessed October 9, 2016] Available at: <http://stroke.ahajournals.org/content/early/2014/02/06/01.str.0000442009.06663.48>. [PubMed: 24503673]
- Bushnell C, McCullough L, et al. Stroke Prevention in Women: Synopsis of the 2014 American Heart Association/American Stroke Association Guideline. *Annals of Internal Medicine*. 2014; 160(12):853. [Accessed October 31, 2016] Available at: <http://annals.org/article.aspx?doi=10.7326/M14-0762>. [PubMed: 24935489]
- Caffarelli C, et al. Progress in pediatrics in 2015: choices in allergy, endocrinology, gastroenterology, genetics, haematology, infectious diseases, neonatology, nephrology, neurology, nutrition,

- oncology and pulmonology. Italian journal of pediatrics. 2016; 42(1):75. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27566421>. [PubMed: 27566421]
- Carmina E, et al. Phenotypic Variation in Hyperandrogenic Women Influences the Findings of Abnormal Metabolic and Cardiovascular Risk Parameters. The Journal of Clinical Endocrinology & Metabolism. 2005; 90(5):2545–2549. [Accessed October 31, 2016] Available at: <http://press.endocrine.org/doi/10.1210/jc.2004-2279>. [PubMed: 15728203]
- Cervantes JL, et al. TLR8: the forgotten relative revindicated. Cell Mol Immunol. 2012; 9(6):434–438. Available at: <http://dx.doi.org/10.1038/cmi.2012.38>. [PubMed: 23085951]
- Cheng J, et al. Age-dependent effects of testosterone in experimental stroke. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2009; 29(3):486–94. [Accessed October 31, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19002196>.
- Chow JC, et al. Silencing of the mammalian X chromosome. Annual review of genomics and human genetics. 2005; 6:69–92.
- Corre, C., et al. Separate effects of sex hormones and sex chromosomes on brain structure and function revealed by high-resolution magnetic resonance imaging and spatial navigation assessment of the Four Core Genotype mouse model; Brain Struct Funct. 2014. p. 1-20. Available at: <http://dx.doi.org/10.1007/s00429-014-0952-0>
- Cribbs DH, et al. Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study. Journal of neuroinflammation. 2012; 9:179. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3419089&tool=pmcentrez&rendertype=abstract>. [PubMed: 22824372]
- Derkow K, et al. Microglia Induce Neurotoxic IL-17+ $\gamma\delta$ T Cells Dependent on TLR2, TLR4, and TLR9 Activation. PloS one. 2015; 10(8):e0135898. [Accessed October 7, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26288016>. [PubMed: 26288016]
- Deswal, a, et al. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). Circulation. 2001; 103(16):2055–2059. [PubMed: 11319194]
- Engelhardt B, Sorokin L. The blood-brain and the blood-cerebrospinal fluid barriers: Function and dysfunction. Seminars in Immunopathology. 2009; 31(4):497–511. [PubMed: 19779720]
- Engelmann F, et al. Impact of Estrogen Therapy on Lymphocyte Homeostasis and the Response to Seasonal Influenza Vaccine in Post-Menopausal Women. PloS one. 2016; 11(2):e0149045. [Accessed October 7, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26859566>. [PubMed: 26859566]
- Engler-Chiurazzi EB, Singh M, Simpkins JW. From the 90's to now: A brief historical perspective on more than two decades of estrogen neuroprotection. Brain Research. 2016; 1633:96–100. Available at: <http://dx.doi.org/10.1016/j.brainres.2016.06.016>. [PubMed: 26740397]
- Fairweather D, Frisncho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. The American journal of pathology. 2008; 173(3):600–9. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2527069&tool=pmcentrez&rendertype=abstract>. [PubMed: 18688037]
- Farage MA, Miller KW, Maibach HI. Effects of menopause on autoimmune diseases. Expert Review of Obstetrics & Gynecology. 2012; 7(6):557–571. [Accessed October 22, 2016] Available at: <http://www.tandfonline.com/doi/full/10.1586/eog.12.63>.
- Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. The journals of gerontology. Series A, Biological sciences and medical sciences. 2014; 69(Suppl 1):S4–9. [Accessed October 15, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24833586>.
- Gagliani N, Huber S. Basic Aspects of T Helper Cell Differentiation. Methods in molecular biology (Clifton, NJ). 2017; 1514:19–30. [Accessed October 30, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27787789>.
- Gameiro C, Romao F. Changes in the immune system during menopause and aging. Frontiers in bioscience (Elite edition). 2010; 2:1299–303. [Accessed October 7, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20515802>. [PubMed: 20515802]

- Gameiro CM, Romão F, Castelo-Branco C. Menopause and aging: changes in the immune system--a review. *Maturitas*. 2010; 67(4):316–20. [Accessed October 7, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20813470>. [PubMed: 20813470]
- Gelderblom M, et al. Neutralization of the IL-17 axis diminishes neutrophil invasion and protects from ischemic stroke. *Blood*. 2012; 120(18):3793–3802. [PubMed: 22976954]
- Germany W. Plasma Estrogens in Childhood and Puberty under. *Physiologic and Pathologic Conditions*. 1973; 907:901–907.
- Gill D, Veltkamp R. Dynamics of T cell responses after stroke. *Current Opinion in Pharmacology*. 2016; 26:26–32. [PubMed: 26452204]
- Gill SK. Cardiovascular risk factors and disease in women. *Medical Clinics of North America*. 2015; 99(3):535–552. Available at: <http://dx.doi.org/10.1016/j.mcna.2015.01.007>. [PubMed: 25841599]
- Golomb MR, et al. Male predominance in childhood ischemic stroke: Findings from the international pediatric stroke study. *Stroke*. 2009; 40(1):52–57. [PubMed: 18787197]
- Gravholt CH, et al. Morbidity in Turner syndrome. *Journal of Clinical Epidemiology*. 1998; 51(2): 147–158. [PubMed: 9474075]
- Gravholt CH. Turner syndrome in adulthood. *Hormone Research*. 2005:86–93.
- de Groot PCM, et al. PCOS, coronary heart disease, stroke and the influence of obesity: A systematic review and meta-analysis. *Human Reproduction Update*. 2011; 17(4):495–500. [PubMed: 21335359]
- Gubbels Bupp MR. Sex, the aging immune system, and chronic disease. *Cellular Immunology*. 2015; 294(2):102–110. Available at: <http://dx.doi.org/10.1016/j.cellimm.2015.02.002>. [PubMed: 25700766]
- Guzik TJ, et al. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp Med*. 2007; 204(10):2449–2460. Available at: <http://jem.rupress.org/content/204/10/2449.full.pdf>. [PubMed: 17875676]
- Gyenes A, et al. 17 β -estradiol attenuates injury-induced microglia activation in the oculomotor nucleus. *Neuroscience*. 2010; 171(3):677–682. [PubMed: 20870014]
- Harrison DG, et al. Inflammation, immunity, and hypertension. *Hypertension*. 2011; 57(2):132–140. [PubMed: 21149826]
- He S, et al. Histone methyltransferase and histone methylation in inflammatory T-cell responses. *Immunotherapy*. 2013; 5(9):989–1004. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3879725&tool=pmcentrez&rendertype=abstract>. [PubMed: 23998733]
- He, Y., et al. [Accessed November 5, 2016] Novel IL-6-secreting $\gamma\delta$ T cells increased in patients with atherosclerotic cerebral infarction. *Molecular Medicine Reports*. 2014. Available at: <http://www.spandidos-publications.com/10.3892/mmr.2014.2796>
- Herkenham, M., Kigar, SL. [Accessed October 10, 2016] Contributions of the adaptive immune system to mood regulation: Mechanisms and pathways of neuroimmune interactions. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2016. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27613155>
- Hill, CA., Fitch, RH. [Accessed November 5, 2016] Sex differences in mechanisms and outcome of neonatal hypoxia-ischemia in rodent models: implications for sex-specific neuroprotection in clinical neonatal practice; *Neurology research international*. 2012. p. 867531 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22474588>
- Hill L, et al. Differential roles of estrogen receptors α and β in control of B-cell maturation and selection. *Molecular medicine (Cambridge, Mass)*. 2011; 17(3–4):211–20.
- Hu, Y., et al. [Accessed October 9, 2016] Imbalance between IL-17A-producing cells and regulatory T cells during ischemic stroke; *Mediators of inflammation*. 2014. p. 813045 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24991091>
- Hurn PD, Vannucci SJ, Hagberg H. Adult or Perinatal Brain Injury: Does Sex Matter? *Stroke*. 2005; 36(2):193–195. [Accessed November 5, 2016] Available at: <http://stroke.ahajournals.org/cgi/doi/10.1161/01.STR.0000153064.41332.f6>. [PubMed: 15625289]
- Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med*. 2011; 17(7):796–808. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21738161>. [PubMed: 21738161]

- Itoh Y, et al. Four Core Genotypes mouse model: localization of the Sry transgene and bioassay for testicular hormone levels. *BMC Research Notes*. 2015; 8(1):69. [Accessed November 5, 2016] Available at: <http://www.biomedcentral.com/1756-0500/8/69>. [PubMed: 25870930]
- Ji H, et al. Sex chromosome effects unmasked in angiotensin II-induced hypertension. *Hypertension (Dallas, Tex : 1979)*. 2010; 55(5):1275–82. [Accessed October 4, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20231528>.
- Ji H, et al. Sex-specific T-cell regulation of angiotensin II-dependent hypertension. *Hypertension*. 2014; 64(3):573–582. [PubMed: 24935938]
- Kalokhe AS, et al. Intimate Partner Violence is Associated with Increased CD4(+) T-cell activation among HIV-Negative high risk women. *Pathogens & immunity*. 2016; 1(1):193–213. [Accessed October 10, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27668294>. [PubMed: 27668294]
- Kharoshankaya, L., et al. [Accessed October 31, 2016] Seizure burden and neurodevelopmental outcome in neonates with hypoxic-ischemic encephalopathy. *Developmental medicine and child neurology*. 2016. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27595841>
- Kim JY, et al. Inflammation after Ischemic Stroke: The Role of Leukocytes and Glial Cells. *Experimental Neurobiology*. 2016; 25(5):241. [Accessed November 5, 2016] Available at: <http://synapse.koreamed.org/DOIx.php?id=10.5607/en.2016.25.5.241>. [PubMed: 27790058]
- Koellhoffer EC, McCullough LD. The Effects of Estrogen in Ischemic Stroke. *Translational Stroke Research*. 2013; 4(4):390–401. [PubMed: 24323337]
- Koike G, et al. Human type 2 angiotensin II receptor gene: cloned, mapped to the X chromosome, and its mRNA is expressed in the human lung. *Biochemical and biophysical research communications*. 1994; 203(3):1842–50. [Accessed October 10, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7945336>. [PubMed: 7945336]
- Konsman JP, Drukarch B, Van Dam A-M. (Peri)vascular production and action of pro-inflammatory cytokines in brain pathology. *Clinical science*. 2007; 112(1):1–25. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17132137>. [PubMed: 17132137]
- Krieg AM. The Toll of Too Much TLR7. *Immunity*. 2007; 27(5):695–697. [PubMed: 18031693]
- Krolak-Olejnik B, Mazur B. Influence of perinatal risk factors on CD3+/TCR alphabeta and CD3+/TCR gammadelta lymphocytes in cord blood of preterm neonates. *Pediatr Int*. 2004; 46(4):403–408. [Accessed September 15, 2016] Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15310303. [PubMed: 15310303]
- LaMarca B, Cornelius D, Wallace K. Elucidating immune mechanisms causing hypertension during pregnancy. *Physiology (Bethesda, Md)*. 2013; 28(4):225–33. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3742131&tool=pmcentrez&rendertype=abstract>.
- Lauterbach MD, Raz S, Sander CJ. Neonatal hypoxic risk in preterm birth infants: the influence of sex and severity of respiratory distress on cognitive recovery. *Neuropsychology*. 2001; 15(3):411–20. [PubMed: 11499996]
- Lindsberg PJ, Strbian D, Karjalainen-Lindsberg M-L. Mast cells as early responders in the regulation of acute blood–brain barrier changes after cerebral ischemia and hemorrhage. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2010; 30(4):689–702. Available at: <http://dx.doi.org/10.1038/jcbfm.2009.282>. [PubMed: 2009.282]
- Liu F, et al. Changes in experimental stroke outcome across the life span. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2009; 29(4):792–802. [Accessed October 6, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19223913>.
- Liu F, McCullough LD. Interactions between age, sex, and hormones in experimental ischemic stroke. *Neurochemistry International*. 2012; 61(8):1255–1265. Available at: <http://dx.doi.org/10.1016/j.neuint.2012.10.003>. [PubMed: 23068990]

- Ma F, et al. The requirement of CD8+ T cells to initiate and augment acute cardiac inflammatory response to high blood pressure. *Journal of immunology* (Baltimore, Md : 1950). 2014; 192(7): 3365–73. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24600037>.
- Mallick AA, O'Callaghan FJK. The epidemiology of childhood stroke. *European Journal of Paediatric Neurology*. 2010; 14(3):197–205. Available at: <http://dx.doi.org/10.1016/j.ejpn.2009.09.006>. [PubMed: 19879783]
- Mamrut S, et al. Integrative analysis of methylome and transcriptome in human blood identifies extensive sex- and immune cell-specific differentially methylated regions. *Epigenetics*. 2015; 10(10):943–957. [PubMed: 26291385]
- Mani H, et al. Diabetes and cardiovascular events in women with polycystic ovary syndrome: A 20-year retrospective cohort study. *Clinical Endocrinology*. 2013; 78(6):926–934. [PubMed: 23046078]
- Manwani B, et al. Differential effects of aging and sex on stroke induced inflammation across the lifespan. *Experimental Neurology*. 2013; 249:120–131. [Accessed September 8, 2016] Available at: <http://dx.doi.org/10.1016/j.expneurol.2013.08.011>. [PubMed: 23994069]
- Manwani B, et al. Sex differences in ischemic stroke sensitivity are influenced by gonadal hormones, not by sex chromosome complement. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2014; 7440068(October):1–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25388681>.
- Marsh BJ, Williams-Karnesky RL, Stenzel-Poore MP. Toll-like receptor signaling in endogenous neuroprotection and stroke. *Neuroscience*. 2009; 158(3):1007–1020. [PubMed: 18809468]
- Marttila S, et al. Transcriptional Analysis Reveals Gender-Specific Changes in the Aging of the Human Immune System. *PLOS ONE*. 2013; 8(6):e66229. Available at: <http://www.scopus.com/inward/record.url?eid=2-s2.0-84878862434&partnerID=tZOTx3y1>. [PubMed: 23776639]
- Mayoral SR, Omar G, Penn AA. Sex differences in a hypoxia model of preterm brain damage. *Pediatric Research*. 2009; 66(3):248–253. [PubMed: 19542909]
- McCullough LD, et al. Stroke sensitivity in the aged: sex chromosome complement vs. gonadal hormones. *Aging*. 2016; 8(7):32–41. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27405096>.
- Meyer C, McGrath BP, Teede HJ. Overweight Women with Polycystic Ovary Syndrome Have Evidence of Subclinical Cardiovascular Disease. *The Journal of Clinical Endocrinology & Metabolism*. 2005; 90(10):5711–5716. [Accessed October 31, 2016] Available at: <http://press.endocrine.org/doi/10.1210/jc.2005-0011>. [PubMed: 16046590]
- Miller AP, et al. Hormone replacement therapy and inflammation: Interactions in cardiovascular disease. *Hypertension*. 2003:657–663.
- Mirza, Ma, et al. Sexually dimorphic outcomes and inflammatory responses in hypoxic-ischemic encephalopathy. *Journal of Neuroinflammation*. 2015; 12(1):32. Available at: <http://www.jneuroinflammation.com/content/12/1/32>. [PubMed: 25889641]
- Mozaffarian D, et al. Heart Disease and Stroke Statistics???2016 Update: A Report From the American Heart Association. *Circulation*. 2015; 40(3):1–20. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4376565&tool=pmcentrez&rendertype=abstract>.
- Murphy SL, et al. Mortality in the United States, 2014. *NCHS Data Brief*. 2015; (229):1–8.
- Niewada M, et al. Influence of gender on baseline features and clinical outcomes among 17, 370 patients with confirmed ischaemic stroke in the international stroke trial. *Neuroepidemiology*. 2005; 24(3):123–128. [PubMed: 15637449]
- Novotny SR, et al. Activating autoantibodies to the angiotensin II type I receptor play an important role in mediating hypertension in response to adoptive transfer of CD4+ T lymphocytes from placental ischemic rats. *American journal of physiology. Regulatory, integrative and comparative physiology*. 2012; 302(10):R1197–201. [Accessed October 21, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22461177>.
- Okoroh EM, et al. Assessing the intersection of cardiovascular disease, venous thromboembolism, and polycystic ovary syndrome. *Thrombosis Research*. 2015; 136(6):1165–1168. Available at: <http://dx.doi.org/10.1016/j.thromres.2015.10.022>. [PubMed: 26489726]

- Pan A, et al. Depression and risk of stroke morbidity and mortality: a meta-analysis and systematic review. *JAMA*. 2011; 306(11):1241–9. Available at: <http://jama.jamanetwork.com/article.aspx?articleid=1104406>. [PubMed: 21934057]
- Peerschke EI, Yin W, Ghebrehiwet B. Complement activation on platelets: Implications for vascular inflammation and thrombosis. *Molecular Immunology*. 2010; 47(13):2170–2175. [PubMed: 20621693]
- Pennell LM, Galligan CL, Fish EN. Sex affects immunity. *Journal of Autoimmunity*. 2012; 38(2–3)
- Pernis AB. Estrogen and CD4+ T cells. *Current opinion in rheumatology*. 2007; 19(5):414–420. [Accessed October 15, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17762604>. [PubMed: 17762604]
- Perry VH, Holmes C. Microglial priming in neurodegenerative disease. *Nature reviews. Neurology*. 2014; 10(4):217–24. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24638131>. [PubMed: 24638131]
- Pimentel-coelho PM, Michaud J, Rivest S. Evidence for a Gender-Specific Protective Role of Innate Immune Receptors in a Model of Perinatal Brain Injury. *Journal of Neuroscience*. 2013; 33(28): 11556–11572. [PubMed: 23843525]
- Pinsky DJ, et al. Hypoxia-induced exocytosis of endothelial cell weibel-palade bodies: A mechanism for rapid neutrophil recruitment after cardiac preservation. *Journal of Clinical Investigation*. 1996; 97(2):493–500. [PubMed: 8567972]
- Pollow DP, et al. Sex differences in T-lymphocyte tissue infiltration and development of Angiotensin II Hypertension. *Hypertension*. 2014; 64(2):384–390. [PubMed: 24890822]
- Prinz I, Silva-Santos B, Pennington DJ. Functional development of $\gamma\delta$ T cells. *European Journal of Immunology*. 2013; 43(8):1988–1994. [PubMed: 23928962]
- Rawji KS, et al. Immunosenescence of microglia and macrophages: Impact on the ageing central nervous system. *Brain*. 2016; 139(3):653–661. [PubMed: 26912633]
- Ritzel RM, Capozzi LA, McCullough LD. Sex, stroke, and inflammation: The potential for estrogen-mediated immunoprotection in stroke. *Hormones and Behavior*. 2013; 63(2):238–253. [Accessed September 8, 2016] Available at: <http://dx.doi.org/10.1016/j.yhbeh.2012.04.007>. [PubMed: 22561337]
- Roger VL, et al. Heart disease and stroke statistics-2011 update: A report from the American Heart Association. *Circulation*. 2011; 123(4)
- Ruhnau, J., et al. [Accessed October 9, 2016] Reduced Numbers and Impaired Function of Regulatory T Cells in Peripheral Blood of Ischemic Stroke Patients; Mediators of inflammation. 2016. p. 2974605 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27073295>
- Sandberg K, Ji H, Hay M. Sex-specific immune modulation of primary hypertension. *Cellular Immunology*. 2015; 294(2):95–101. [PubMed: 25498375]
- Schmitt V, Rink L, Uciechowski P. The Th17/Treg balance is disturbed during aging. *Experimental gerontology*. 2013; 48(12):1379–86. [Accessed October 30, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24055797>. [PubMed: 24055797]
- Selmi C, et al. The X chromosome and the sex ratio of autoimmunity. *Autoimmunity Reviews*. 2012; 11(6–7):A531–A537. Available at: <http://dx.doi.org/10.1016/j.autrev.2011.11.024>. [PubMed: 22155196]
- Shen EY, et al. Epigenetics and sex differences in the brain: A genome-wide comparison of histone-3 lysine-4 trimethylation (H3K4me3) in male and female mice. *Experimental Neurology*. 2015; 268:21–29. [PubMed: 25131640]
- Shichita T, et al. Pivotal role of cerebral interleukin-17-producing gammadeltaT cells in the delayed phase of ischemic brain injury. *Nature medicine*. 2009; 15(8):946–950. [Accessed October 7, 2016] Available at: <http://dx.doi.org/10.1038/nm.1999>.
- Shroff R, et al. Young Obese Women with Polycystic Ovary Syndrome Have Evidence of Early Coronary Atherosclerosis. *The Journal of Clinical Endocrinology & Metabolism*. 2007; 92(12): 4609–4614. [Accessed October 31, 2016] Available at: <http://press.endocrine.org/doi/10.1210/jc.2007-1343>. [PubMed: 17848406]

- Solomon CG, et al. Menstrual cycle irregularity and risk for future cardiovascular disease. *The Journal of clinical endocrinology and metabolism*. 2002; 87(5):2013–7. [Accessed October 31, 2016] Available at: <http://press.endocrine.org/doi/10.1210/jcem.87.5.8471>. [PubMed: 11994334]
- Spolarics Z. the X-Files of Inflammation. *Shock*. 2007; 27(6):597–604. Available at: <http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00024382-200706000-00002>. [PubMed: 17505297]
- Stamova B, et al. The X-chromosome has a different pattern of gene expression in women compared with men with ischemic stroke. *Stroke*. 2012; 43(2):326–334. [PubMed: 22052522]
- Strbian D, et al. Cerebral mast cells regulate early ischemic brain swelling and neutrophil accumulation. *Journal of Cerebral Blood Flow & Metabolism*. 2006; 26:605–612. [PubMed: 16163296]
- Subramanian S, et al. Oestrogen-mediated protection of experimental autoimmune encephalomyelitis in the absence of Foxp3+ regulatory T cells implicates compensatory pathways including regulatory B cells. *Immunology*. 2011; 132(3):340–347. [PubMed: 21091909]
- Suzuki S, et al. Timing of estrogen therapy after ovariectomy dictates the efficacy of its neuroprotective and antiinflammatory actions. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(14):6013–8. Available at: <http://www.pnas.org/content/104/14/6013>. [PubMed: 17389368]
- Tipnis SR, et al. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *The Journal of biological chemistry*. 2000; 275(43):33238–43. [Accessed October 10, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10924499>. [PubMed: 10924499]
- Tipton AJ, Sullivan JC. Sex differences in T Cells in hypertension. *Clinical Therapeutics*. 2014; 36(12):1822–1900. Available at: <http://dx.doi.org/10.1016/j.clinthera.2014.07.011>.
- Toben C, Baune BT. An Act of Balance Between Adaptive and Maladaptive Immunity in Depression: a Role for T Lymphocytes. *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology*. 2015; 10(4):595–609. [Accessed October 31, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26133121>. [PubMed: 26133121]
- Turtzo LC, Siegel C, McCullough LD. X chromosome dosage and the response to cerebral ischemia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2011; 31(37):13255–9. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3179179&tool=pmcentrez&rendertype=abstract>. [PubMed: 21917808]
- Unutmaz D, Pileri P, Abrignani S. Antigen-independent activation of naive and memory resting T cells by a cytokine combination. *The Journal of experimental medicine*. 1994; 180(3):1159–64. [Accessed November 4, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8064232>. [PubMed: 8064232]
- Villa, A., et al. Estrogens, Neuroinflammation and Neurodegeneration; *Endocrine reviews*. 2016. p. er20161007 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27196727>
- Wang J, et al. Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X. *Proceedings of the National Academy of Sciences of the United States of America*. 2016; 113(14):E2029–38. [Accessed October 9, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27001848>. [PubMed: 27001848]
- Wang J, Lu Q. Expression of T subsets and mIL-2R in peripheral blood of newborns with hypoxic ischemic encephalopathy. *World journal of pediatrics : WJP*. 2008; 4(2):140–4. [Accessed September 12, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18661772>. [PubMed: 18661772]
- Wang S, Zhang H, Xu Y. Crosstalk between microglia and T cells contributes to brain damage and recovery after ischemic stroke. *Neurological research*. 2016; 38(6):495–503. [Accessed October 9, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27244271>. [PubMed: 27244271]
- Wang, Z., et al. [Accessed October 9, 2016] Quercetin Decreases Insulin Resistance in a Polycystic Ovary Syndrome Rat Model by Improving Inflammatory Microenvironment. *Reproductive sciences (Thousand Oaks, Calif)*. 2016. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27634381>

- Wild RA, et al. Assessment of Cardiovascular Risk and Prevention of Cardiovascular Disease in Women with the Polycystic Ovary Syndrome: A Consensus Statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *The Journal of Clinical Endocrinology & Metabolism*. 2010; 95(5):2038–2049. [Accessed October 31, 2016] Available at: <http://press.endocrine.org/doi/10.1210/jc.2009-2724>. [PubMed: 20375205]
- Zhao L, et al. Sex differences in metabolic aging of the brain: Insights into female susceptibility to Alzheimer's disease. *Neurobiology of Aging*. 2016; 42:69–79. Available at: <http://dx.doi.org/10.1016/j.neurobiolaging.2016.02.011>. [PubMed: 27143423]
- Zheng Y, Rudensky AY. Foxp3 in control of the regulatory T cell lineage. *Nature Immunology*. 2007; 8(5):457–462. Available at: <http://www.nature.com/ni/journal/v8/n5/full/ni1455.html><http://www.nature.com/ni/journal/v8/n5/pdf/ni1455.pdf>. [PubMed: 17440451]
- Zierau L, et al. Coexistence of asthma and polycystic ovary syndrome: A concise review. *Respiratory medicine*. 2016; 119:155–159. [Accessed October 9, 2016] Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27692138>. [PubMed: 27692138]

Research Highlights

- Sex differences in stroke exist throughout lifespan.
- Immune responses to stroke are shaped by age, sex, hormones, and chromosomes.
- Activation of T cells contributes to stroke sensitivity and outcomes.

Figure 1. Inflammatory mechanisms involved in stroke

Ischemic stroke occurs when a blood vessel that supplies one area of the brain is occluded. The obstruction of the vessel generates hypoxia/ischemia, activates the complement system and platelets, and stimulates endothelial cells leading to the exocytosis of Weibel-Palade bodies, the secretion of Von Willebrand Factor (vWF) and P/E-Selectin, and the subsequent release of leukocytes. In the perivascular space, mast cells are also activated to secrete histamine, $\text{TNF}\alpha$, and proteases. As a result, the blood-brain barrier (BBB) integrity is compromised, facilitating the infiltration of leukocytes into the brain parenchyma. Concomitantly, blood vessel obstruction deprives brain tissue of oxygen and glucose, resulting in brain damage and neuronal death. The damaged neurons release danger signals that bind to TLRs and scavenger receptors on microglia, leading to their activation. Ongoing inflammation results in the infiltration of more leukocytes into the brain tissue and exacerbates the innate immune response. The activated antigen presenting cells carry CNS danger molecules to lymphocytes and consequently the adaptive immune response is also activated (Peerschke et al. 2010; Pinsky et al. 1996; Strbian et al. 2006; Lindsberg et al. 2010; Engelhardt & Sorokin 2009; Iadecola & Anrather 2011; Marsh et al. 2009; Perry & Holmes 2014; Konsman et al. 2007).

Figure 2. T cell responses after stroke

In the early phase of stroke, T cell mediated inflammation occurs through an antigen independent mechanism (Brown) by which $\gamma\delta$ T cells are activated due to inflammatory factors released from myeloid cells and microglia (IL-23, IL-6, IL-12). In the delayed phase of stroke, antigen dependent responses occur, highlighted by Th1 (IFN γ) and Th17 (Th17) responses (Pink), which enhance the inflammatory responses already occurring in the brain. The activated Treg cells (blue) secrete IL-10 and TGF β to regulate the overall inflammation by suppressing pro-inflammatory T helper cell activation and the subsequent inflammatory cascade (Gill & Veltkamp 2016).

Table 1

Cell subtypes

	Secreted Cytokines/specific markers	Normal function	Exacerbated response
<i>CD4⁺ Subset</i>			
Th1	IFN γ IL-2 TNF α	Cell-mediated immunity and Inflammation. Intracellular pathogens	Autoimmunity. Inflammation
Th2	IL-4, IL-5, IL-6, IL-10, IL-13	Antibody-mediated immunity. B cell coordination. Response against parasites	Asthma, Allergy
Th17	IL-17 (A/F), IL-21, IL-22	Extracellular bacteria. Fungi	Autoimmunity. Inflammation
Treg	TGF- β , IL-10, FoxP3	Immune tolerance. Lymphocyte homeostasis. Regulation of immune response	Autoimmunity. Inflammation. Cancer
<i>CD8 Subset</i>			
Cytotoxic T Lymphocytes	Granzymes, Perforins	Cytotoxic activity. Elimination of infected cells.	Cancer. Infections
CD8 Treg	Perforins. CD122, ICOSL	Self-tolerance. Limit functions of Follicular T cells.	Autoimmunity. Inflammation
<i>$\gamma\delta$ T cells</i>			
$\gamma\delta$ T cells in spleen	IL-4, IL-13, IFN γ	Spleen and liver surveillance	Autoimmunity. Inflammation
$\gamma\delta$ T cells in epitheliums	IL-17, IL-22, IFN γ , TGF β	Epithelial surveillance (Uterus, vagina, tongue, lungs, gut)	Autoimmunity. Inflammation

Table 2

Sex differences in the lymphocyte response to different stimuli

Age	Species	Insult	Sex differences	Reference
Neonate	Wistar Rat	LPS	Increase of Th1 profile in male splenocytes after activation.	(Kosyrevva 2014)
Adult	Mouse	PPAR γ AGONIST	PPAR γ inhibits Th17 differentiation only in Male. Modulates Th1, Th2, and Th17 differentiation in female T cells based on different level of estrogen exposure <i>in vitro</i> .	(Park et al. 2016)
Adult	Mouse	Ang-II derived HTN	Males have increased blood pressure due to an increase of IL-17 after induced hypertension.	(Tipton & Sullivan 2014)
Adult	Mouse	Ang-II derived HTN	Females have higher Treg response after Angiotensin II-induced hypertension.	(Tipton & Sullivan 2014)
Adult	Human	SMOKING	Elevated Th1/Th2 immune function among female smokers compared to male smokers.	(Guaderrama et al. 2011)
Adult	Human	STROKE	Lower Treg efficacy to modulate immune response after stroke in females	(Yan et al. 2012)
Adult	Mouse	MCAO	IL-4 is required for female neuroprotection during the estrus phase of the cycle.	(Xiong et al. 2015)
Adult	Mouse	MCAO	CD4+ cells are higher in males. CD8+ cells are higher in females after stroke.	(Banerjee et al. 2013)
Adult	Mouse	MCAO	Sham males have more CD4 T cells in spleen than females.	(Banerjee et al. 2013)
Adult	Mouse	EAE/ESTROGEN	Estrogen increases the differentiation to B reg cells, also increasing M2 differentiation in microglia and neuroprotection.	(Benedek et al. 2016)
Adult	Mouse	MCAO	Splenectomy reduces infarct volume and neuroinflammation in male but not female mice in experimental stroke.	(Dotson et al. 2015)

Table 3

Mechanisms potentially underlying the sexual dimorphism in HIE

Mechanism	Finding	Importance	Reference
Microglia			
Microglia cell number	Microglia are necessary for brain masculinization	Males have more microglia than females at early stages of the brain development	(Lenz et al. 2013)
TLRs pathways with TRIF signaling	mRNAs of TLR receptors are increased after HIE and TRIF-deficient male neonatal mice have a larger atrophy when compare with females and wild-type mice	TLR-3/TLR-4-TRIF pathways have been involved in mediating ischemic protection	(Pimentel-Coelho et al. 2013) (Pan et al. 2014) (Halder et al. 2015)
Fractalkine/CX3CR1 pathway	Neonatal female CX3CR1 KO mice were more affected by an ischemic stroke than wild-type mice. The effect is not observed in males	Fractalkine (CX3CL1/CX3CR1) pathway is one of the endogenous inhibitory signaling of microglia in the healthy brain	(Pimentel-Coelho et al. 2013)
NFκB	Males express higher basal levels of NFκB, and hypoxia induces a NFκB increase in male cells but a decrease in females	Important transcription factor in immune cells like microglia and macrophages	(Zhao & Eghbali-Webb 2002)
MHC-II expression	Females have a lower expression of MHC-II in microglia 3 days after HIE	MHC-II is a classic activation marker	(Mirza et al. 2015)
Neurons			
ERα and BDNF/Trk-B protection pathway in hippocampus	ERα knockout (KO) mice were not sensitive to Trk-B agonists, and present similar infarct sizes when to compare to males	ERα can activate the BDNF protection pathway after ischemia in females, but not males	(Cikla et al. 2016)
Peripheral infiltration			
Leukocytes	Females have lower infiltration of peripheral cells after HIE	Peripheral infiltration is highly related to the overall stroke injury	(Mirza et al. 2015)

Table 4

Changes in lymphocytes profiles after menopause

Postmenopausal effect in women Lymphocytes	Reference
Postmenopausal women exhibit a reduced number of total lymphocytes, mainly B and CD4+ T lymphocytes	(Giglio et al. 1994)
CD4+/CD8+ ratio and the numbers of circulating B cells are decreased, while Natural Killer cells increase.	(Kumru et al. 2004)
Increase IL-1 β , IL-6, IL-10, and TNF- α .	(Deguchi et al. 2001) (Kamada et al. 2001) (Vural et al. 2006) (Yasui et al. 2007)
IFN- γ levels gradually decrease with age. The production of IL-10 increases during the postmenopausal period.	(Deguchi et al. 2001)
Altered pathways in women highlighting lower T-cell defense and more inflammation in elderly females	(Marttila et al. 2013)
Women of reproductive age were shown to have significantly higher CD4+ T-cell and lower Natural killer cell counts, and at older ages, post menopause reference ranges were comparable for both sexes	(Jentsch-Ullrich et al. 2005)
Faster progression of immunosenescence in men with a more pronounced decline in T cells including naive CD4+ and CD8+CD28+, B cells, T-cell proliferative capacity, and IL-6 secretion, and a weaker increase in CD4+ memory T cells and Natural killer cells	(Hirokawa et al. 2013)
Age-related alterations in T-cell cytokine secretion are sex-specific, as IFN γ and IL-17 secretion were decreased in elderly men after <i>in vitro</i> stimulation but not in women	(Goetzl et al. 2010)
Elder female T cells have increased secretion of IL-10, IL-2, IL-13, IL-4 and IFN γ , compared with young after PMA/Io stimulus <i>in vitro</i>	(Pietschmann et al. 2003)