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Sex-specific Immune Responses in Stroke

Anik Banerjee, M.S.[1,2], **Louise D. McCullough, M.D./ Ph.D.**[1,*]

[1]Department of Neurology, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX, 77030, USA

[2]UTHealth Graduate School of Biomedical Sciences, University of Texas MD Anderson Cancer Center, Houston, TX,77030, USA

Abstract

In both acute and chronic disease, functional differences in host immune responses arise from a multitude of intrinsic and extrinsic factors. Two of the most important factors affecting the immune response are biological sex and aging. Ischemic stroke is a debilitating disease that predominately affects older individuals. Epidemiological studies have shown that older women have poorer functional outcomes compared to men, in part due to the older age at which they experience their first stroke and the increased co-morbidities seen with aging. The immune response also differs in men and women, which could lead to altered inflammatory events that contribute to sex differences in post-stroke recovery. Intrinsic factors including host genetics and chromosomal sex play a crucial role both in shaping the host immune system and in the neuroimmune response to brain injury. Ischemic stroke leads to altered intracellular communication between astrocytes, neurons, and resident immune cells in the central nervous system (CNS). Increased production of cytokines and chemokines orchestrate the infiltration of peripheral immune cells and promote neuroinflammation. To maintain immunosurveillance, the host immune and CNS are highly regulated by a diverse population of immune cells which are strategically distributed within the neuro-vascular unit and become activated with injury. In this review, we provide a comprehensive overview of sex-specific host immune responses in ischemic stroke.

Introduction:

Over the past decade, many studies have highlighted the importance of clinical and public health initiatives directed at addressing stroke disparities in women[1,2]. Stroke incidence is higher in men as compared to women throughout most of the lifespan, however, the prevalence of stroke in women is significantly higher due to their increased longevity[3]. A multitude of pre-clinical and clinical studies have shown that post-stroke outcomes (i.e., functional recovery, post-stroke inflammatory conditions, quality of life, and depression) are worse in women compared to men[4]. Interestingly, recent retrospective studies using

*Correspondence to Dr. Louise D. McCullough, Department of Neurology, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX, 77030, USA. Louise.D.McCullough@uth.tmc.edu.

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a claimed database of insured Americans found that in the 25 to 34 and 35 to 44-year age groups, more women had strokes than men (incidence rate ratio: men: women, 0.70 [95% confidence interval (CI), 0.57-0.86]; and 0.87 [95% CI, 0.78-0.98], respectively). However, younger women had better outcomes compared with age-matched men[5]. In contrast, in the 45 to-74-year-old age group, more men had strokes. A recent systematic review confirmed these findings, but noted the risk was highest in women ≤35 years (44% more women with ischemic strokes[6]). This suggests that factors such as pregnancy (a time of high risk for women) or other non-atherosclerotic risk factors may be contributing to the increase in incidence in younger women. Sex differences in both risk factors and in post-stroke outcomes may be secondary to chronic hormonal effects from life exposure to sex hormones, or from acute effects of circulatory hormones primarily during the reproductive years. Most studies suggest that older post-menopausal women have poorer outcomes after stroke compared to older men, suggesting potential contributions from factors beyond gonadal hormone exposure[7]. More recently, sex chromosomal effects (XX vs. XY) have been recognized in pre-clinical models, and X-linked genes can regulate the neuroinflammatory response to stroke[8]. As the presence of sex differences are apparent in the context of ischemic stroke, recent studies have focused on the mechanistic pathways that may play a contributory role[9].

Aging contributes to an enhanced basal state of inflammation in the host, often referred to as “inflammaging”. This also contributes to a functional decline in the immune system or immunosenescence[10]. Many independent reports have shown that the host immune response towards foreign or self-antigens are distinct among males and females[11,12]. Following ischemic stroke, the brain is exposed to a myriad of inflammatory signals leading to neuroinflammation. The protective effects of the blood brain barrier (BBB) in the naïve non-diseased brain actively protects the host from pathological immune cell infiltration into the brain[13]. Impairment in the BBB enhances ischemic injury, allowing for the infiltration of immune cells and serum factors into the brain, contributing to secondary ischemic damage[14], edema, hemorrhagic transformation, and disruption of the neurovascular unit[15]. Mechanisms through which sex and sex hormones influence the integrity of the BBB may potentially play a contributory role in the sex-specific differences observed in peripheral infiltration of immune cells post-stroke[16] and in the extent of ischemic damage. Sex differences in the activation states of both the innate and adaptive immune compartments, regulated by many physiological factors, may lead to differences in post-stroke outcomes (Figure 1). Over the past decade, studies have shed light on how the host immune system responds in a sex-specific manner following acute ischemic injury[11] which will be the focus of this review.

Innate and Adaptive Immunity in Stroke

Mechanisms of Innate Immunity and Stroke

Innate immunity is the host’s first line of defense to prevent invading pathogens at physical (i.e., primarily tight junctions and secretive mucin located on the skin or mucosal surfaces) and anatomical barriers (i.e., skin, epithelial barriers, etc.). Phagocytes (i.e., monocytes, neutrophils, etc.), and a wide array of transient receptor-mediated immune activation

pathways (i.e., Toll-like-receptors (TLRs), pattern recognition receptors (PRRs), Purinergic receptors (PRs), etc.)[17] are components of innate immunity. In contrast to adaptive immunity, innate immunity relies on rapid activation through transient and ubiquitous receptors on innate immune cells that sense the host environment for foreign- and self-antigens referred to as damage-associated molecular patterns (DAMPs)[18]. Innate immune cells include dendritic cells, macrophages, and monocytes along with unconventional subsets of lymphocytes such as gamma-delta (γδ) T cells which can be activated independently of primary innate immune activation. These cells are found in higher quantities in the blood of women and decline with age in both sexes[19]. Upon antigenic challenge, females respond more efficiently for the clearance of pathogens compared to males, however, females have significantly higher incidence of activation-induced immunopathology and autoimmune diseases[20]. Activation of specific genes associated with TLR pathways and antiviral type I interferon (IFN) responses differ between males and females. Females express significantly higher levels of TLRs contributing to increased activation of pathogen-mediated immunity and pathogen clearance[21]. Gonadal steroids can cause direct effects on immune cell function and development, however, sex differences may arise from inherit imbalance in the expression of genes encoded on the X and Y chromosomes. Multiple genes related to the immune activation and TLR-mediated signaling pathways are encoded on the X chromosome leading to higher expression levels in females[22].

Microglia

Microglia drive the infiltration of systemic immune cells towards the site of brain injury through cytokine-mediated recruitment, initiating neuroinflammation. There are confirmed sex differences in microglia numbers and transcriptomic signatures early in development and in neuroinflammatory environments[23]. For example, endogenous processing of antigen presentation and higher expression of major histocompatibility complex (MHC I) and MHCII are more potently activated in males[24]. There are sex specific effects on microglial development that begin in the neonatal period suggesting that early life development of innate immunity potentially dictates immune-associated disease outcomes later in life[25]. There are large number of studies showing the influence of sex-specific microglial responses following experimental stroke[26]. Young male rodents have larger infarcts compared to young females, an effect primarily mediated by estrogen[27] as it is lost with ovariectomy and with aging (reproductive senescence)[28]. Interestingly, this reversal in outcomes with aging may be mediated in part by microglia as basal immune cell activation and inflammation are significantly higher in aged females[29,30].

Sex hormones contribute to both sex-specific proliferation of microglia and the sex-specific chemotactic signals produced from microglia, resulting in differential immune cell infiltration into the brain in males and females[31]. Specific chemotactic surface ligands such as chemokine ligand (CCL4), (CCL20), and CD206 differ in males and females within the hippocampus, amygdala, and the cortex after stroke, suggesting differences in systemic immune cell recruitment[32]. In addition, levels of specific cytokines including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and C-X-C motif (CXC)L10 differ in males and females further supporting the presence of sex differences in microglial-specific inflammation[33]. Interestingly, one study found that ovariectomy (with the subsequent loss

of circulating estrogen) did not significantly alter microglial gene expression patterns in the brains of adult female mice. Further studies investigating how the sex-specific landscape of the brain shapes basal and injury-induced activation states of microglia are needed.

Neutrophils

Neutrophils increase in the brain following ischemic stroke, and positively correlate with stroke severity, infarction volume, and post-stroke outcomes[34,35]. Most studies have highlighted the detrimental role of neutrophils in ischemic stroke including disruption of the BBB, enhanced cerebral edema, and increased brain injury[36]. However, potential beneficial effects of neutrophil infiltration, such as enhanced clearance of necrotic cells have also been reported[37]. One hallmark feature of neutrophils is their ability to produce neutrophil extracellular traps (NETs), implicated in clot formation, thrombosis[38] and reduced effectiveness of tissue-type plasminogen (tPA)-induced thrombolysis. Microbial cues and endogenous danger signals can also potentiate the highly regulated process of NETosis[39]. Neutrophils isolated from females undergo greater NETosis after calcium induced *ex vivo* stimulation compared to males[40].

Recent advancements in single-cell technology have highlighted sex-specific transcriptional profiles of circulating neutrophils in young adult men and women. Circulating neutrophils in males were more developmentally immature and had a higher threshold for activation compared to those from age-matched females, leading to a distinct neutrophil-specific immune-metabolic signatures[41]. Genes specific to neutrophil activation and migration were both time and sex-specific; females showed differentially expressed genes acutely following cardioembolic stroke, however, this was not seen in males[42]. In contrast, in experimental stroke models, aged males had greater brain infiltration of neutrophils compared to age-matched females. This was further corroborated by the significantly higher levels of neutrophil specific cytokines, monocyte chemoattractant protein-1 (MCP-1) and granulocyte colony stimulating factor (G-CSF) in the circulation of aged males, potentially contributing to an increased incidence of hemorrhagic transformation[43]. The discrepancies among these studies may be due to the progression of aging and its effects on neutrophil biology, or in the clinical vs. pre-clinical models examined (as humans have significantly more neutrophils than rodents). Although multiple studies have highlighted the presence of sex differences in the neutrophilic response to stroke, mechanistic approaches incorporating both sexes, and deeper consideration of biological variables such as hormonal or chromosomal influences on sex-specific neutrophilic signatures are needed.

Mechanisms of Adaptive Immunity and Stroke

The components of the adaptive immune system that have been the most well studied in stroke include T and B lymphocytes[44]. Cell-mediated responses are primarily comprised of the activation of T cells toward a cytotoxic phenotype, or differentiation into specific T cell subtypes (i.e., T-helper (T_H)) to modulate the immune response through the production of subset specific cytokines[45]. In contrast, “unconventional” T cell subsets, including T_H17 and γδ T cells are activated and differentiated independently of antigen presentation from peripheral tissues[46]. Once activated, B cells differentiate into antibody producers to promote complement activation, antigen neutralization, antigen opsonization, and the apoptosis of

other phagocytic immune cells for DAMP or PAMP clearance[47]. T and B cell deficient mice have less stroke induced inflammation and better outcomes[48]. Sex differences in lymphocyte subset diversity have been extensively documented in adult humans. In non-diseased states, males show significantly lower numbers of T_H cells in the circulation compared to age-matched females, however, males have higher numbers of cytotoxic T cells[49]. In contrast to confirmed sex differences in T cell biology, sex-specific B cell immunity has been less studied. A few studies have reported that B cells numbers and immunoglobulin levels are higher in females[50].

Dendritic Cells

Dendritic Cells (DCs) act as sentinels of the host innate immune system[51], they are professional antigen presenting cells (APCs) expressing MHCII and are considered to bridge the gap among innate and adaptive immune systems. During homeostatic conditions DCs are positioned strategically near the cerebral spinal fluid (CSF)-blood barrier. They can migrate towards draining cervical lymph nodes and activate immunogenic T cell responses or promote host tolerance by exhibiting tolerogenic characteristics[52]. With stroke there is a temporally regulated migration and maturation of peripheral DCs, primarily from the bone marrow, into the ischemic area[53]. DC activation state, efficacy of antigen presentation, and surface marker phenotypes are distinct between the sexes. Plasmacytoid DCs (pDCs) from females produce significantly higher levels of IFN-α after TLR activation compared to males[54]. In multiple pre-clinical studies females exhibit greater expression of TLRs on DCs compared to age-matched males, leading to more effective antigen sensing, processing, and presentation[55,56]. The differences in TLR expression seen between males and females may be X chromosome mediated as many classes of TLRs, including TLR2, TLR3, and TLR7, are on the X chromosome[21].

The specific lineage of DCs (i.e., CD11b and CD103) is highly sexually dimorphic in peripheral tissues (i.e., spleen) with advancing age in mice[57]. A hallmark feature of DCs is their ability to perform antigen presentation to initiate T-cell mediated immune activation in the host[58]. However, downstream sex-specific DC-mediated T cell activation may differ between the sexes. For example, Cytomegalovirus (CMV) infection in females elicited a profound TLR specific-DC response resulting in an IFN-γ mediated T_H1 response compared to males, which resulted in faster and more effective viral clearance[59]. T_H1 responses in the host are protective in the acute phases of infection and enhance effective viral removal, however, these may be detrimental chronically, as reflected by the higher incidence of auto-immune diseases in women[60]. Studies investigating the specific role of DCs in ischemic stroke are needed.

T cells

The infiltration of lymphocytes, specifically T cells, in the brain after stroke has been extensively documented. During acute stroke, infiltrating T cells orchestrate the adhesion of platelets to the cerebral endothelium resulting in thrombo-inflammation and larger infarcts[61]. Sex differences in leukocyte-platelet interactions in patients with atherosclerosis, a major risk factor for stroke, have been documented. Females had significantly more leukocyte-platelet aggregates which were detrimental to recovery[62].

Conway et al. demonstrated IL-10, an anti-inflammatory cytokine, secreted by regulatory T cells (T_{reg}) and T_H2 cells differs by sex. The exacerbated IL-10 production seen in females was associated with poorer recovery and immunosuppression which was not evident in males[63]. The effects of excessive IL-10 production have been associated with post-stroke immunosuppression, potentially increasing the incidence of post-stroke infections[64]. IL-10 production from cytotoxic T cells was significantly elevated after stroke in female compared male mice[65]. Separately, Ahnstedt et al. demonstrated that although there was temporal elevation of T_H and T-cytotoxic cells in the brain across both sexes at post-stroke day 15, the relative frequencies of T-cytotoxic cells and T_{reg} were significantly higher in males[4], as was hemorrhagic transformation. These sex-specific differences in adaptive immunity after stroke were correlated with greater stroke induced cognitive deficits. Future studies investigating how chromosomal and hormonal effects influence T cell transcriptomics, the TCR repertoire, and T cell signaling in the context of stroke in both sexes are warranted.

Unconventional T Cells: γδ T Cells

Unconventional innate T cells characterized by an invariant T cell receptor in γδ T cells have become increasingly recognized as a contributor to ischemic damage[66,67]. These cells produce IL-17 which exacerbates neuroinflammation through induction of G-CSF and other chemokines which foster the recruitment of pro-inflammatory immune cells such as neutrophils into the brain. γδ T cells are a relatively minor subset of T lymphocytes in the peripheral blood (PB), comprising only 1–5% of the circulating lymphocytes[68]. However, γδ T cells are abundant at barrier sites such as the skin, gut, lung, and reproductive tract and up to 20% of intraepithelial lymphocytes in the human colon express the γδ TCRs[69]. IL-17 plays a key role in infection and autoimmunity[70] and meningeal IL-17 producing γδ T cells induce anxiety-like behavior in mice[71]. In clinical studies, post-mortem brains from stroke patients had higher IL-17 positive lymphocytes[72]. Zhang et al. found that γδ T cells and a specific subset of IL-17 producing T_H cells (T_H17) were significantly increased in the circulation of stroke patients suggesting that these cells traffic from peripheral tissues[73]. Notably, γδ T cells are highly abundant in the lamina propria of the gut and migrate toward the leptomeninges following stroke[74]. Several studies have highlighted the importance of commensal-derived signals from the gut microbiome that regulate the migration of IL-17 producing γδ T cells from the gut to the brain that increase post stroke inflammation[67,75]. Various extrinsic factors including host genetics, environmental conditions, and sex have the potential to shape the composition of the gut microbiome. Future studies are warranted to investigate if sex-specific differences in the host microbiome can influence the migration of IL-17 producing γδ T cells into the brain after stroke.

B cells

B cells are classified as effector cells and are involved in antigen presentation and antibody production. Early studies documented the presence of immunoglobulins within the CSF of human stroke survivors at chronic timepoints after stroke[76,77]. Selective depletion of B cells led to larger infarct volumes, poorer recovery, and higher overall mortality[48]. The early infiltration of B cells may be beneficial acutely after stroke by promoting immunosuppression and production of neurotrophins, but detrimental chronically, due to enhanced autoantibody production. However, future studies are essential to investigate the

diminishing effect of these protective neurotrophic B cells in the chronic phases of stroke[78]. Aging and sex can both cause a decline of B cell lymphopoiesis resulting in a decrease in systemic B cells, leading to a reduction in B cell-specific neurotrophic signaling and an augmentation of the detrimental effects of B cell antibody production and cognitive decline[79]. B Cell Maturation Antigen Protein (BCMA) regulates B cell proliferation, survival, and plasma cell formation[80]. As sex differences have been documented in antibody production, recent studies have manipulated BCMA to investigate sex specific B cell activation and antibody production in experimental autoimmune encephalomyelitis (EAE) models. Male mice lacking BCMA had significantly worse disease with increased demyelination, higher infiltration of inflammatory T cells and macrophages, and enhanced detrimental neuroinflammation compared to females[81]. These findings suggest that mechanisms that regulate B cell proliferation, survival, and differentiation are highly sex-specific in their immunogenic potential in the context of neuroinflammation. It has been well documented that IL-10 producing regulatory B cells (B_{reg}) cells play a protective role following ischemic stroke, although sex-specific effects were not investigated[82]. Females had a significant increase in overall B cell numbers after stroke, but a specific decrease in B_{reg} numbers in the spleen compared to males, possibly due to differential migration into the brain[83]. Similarly, Benedek et al. showed an estrogen-mediated increase in B_{reg} numbers in the female brain after EAE[84]. As aged females are disproportionately affected by stroke, future studies investigating how sex and aging alter B cells are needed.

Conclusions

The neuroimmune landscape encompasses tissue-resident microglia which play a role in immune surveillance and in maintenance of the neurovascular unit. In addition, a wide array of immune cell types resides within the leptomeninges, the choroid plexus, and the cerebral endothelia. With ischemia, a culmination of pro-inflammatory events results in the activation of innate and adaptive immunity. Accordingly, mounting evidence in pre-clinical (Table 1) and clinical studies (Table 2) demonstrate that sex differences in immune responses following stroke are evident and contribute to post-stroke outcomes.

Advancements in single-cell sequencing technologies will clarify hormonal and chromosomal effects in the regulation of immune sex-specific transcriptomics and intra-immune cell communication within the CNS. Despite the growing recognition of the importance of sex and gender in diseases such as stroke[113], women remain underrepresented in clinical trials[114], and females remain underutilized in experimental studies. Pre-clinical studies should utilize both sexes to investigate mechanisms of immune activation and immune cell transcriptomic signatures in the context of ischemic stroke. Unfortunately, despite repeated recommendations, the sex and age of animals used in preclinical research remains underreported[115], and little progress has been made[116]. Clinical studies should include comprehensive documentation of sex-specific co-morbidities (e.g., obesity, cardiovascular disease, diabetes mellitus, etc.) and social factors (isolation and pre-stroke functional status) that may contribute to stroke incidence and outcomes.

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Non-standard Abbreviations and Acronyms

CI	Confidence Interval
BBB	Blood Brain Barrier
TLRs	Toll-Like Receptors
PRRs	Pattern Recognition Receptors
PRs	Purinergic Receptors
DAMPs	Damage-associated Molecular Patterns
(γδ) T Cells	Gamma-delta T Cells
TCR	T cells receptors
CD	Cluster of Differentiation
IFN	Type I Interferon
MHC	Major Histocompatibility Complex
CCL	Chemokine Ligand
IL	Interleukin
TNF	Tumor Necrosis Factor
CXCL	(C-X-C) Motif Ligand
NETs	Neutrophil Extracellular Traps
tPA	Tissue-type Plasminogen
MCP-1	Monocyte Chemoattactant-1
G-CSF	Granulocyte Colony Stimulating Factor
T_H	T-helper
DCs	Dendritic Cells
CNS	Central Nervous System
CSF	Cerebral Spinal Fluid
pDCs	Plasmacytoid DCs

CMV	Cytomegalovirus
T_{reg}	Regulatory T Cells
PB	Peripheral Blood
BCMA	B Cell Maturation Antigen Protein
EAE	Experimental Autoimmune Encephalomyelitis
B_{reg}	Regulatory B Cells
PPAR	Peroxisome proliferator-activated receptor
CaMMK	Ca^{2+}/calmodulin-dependent protein kinase
ICAM	Intracellular Adhesion Molecules
PTGS2	Prostaglandin-endoperoxide synthase 2
WBC	White Blood Cells
CRP	C-reactive Protein
S100B	S100 Calcium Binding Protein B
NIHSS	NIH Stroke Scale/Score
FABP4	Fatty Acid Binding Protein 4
T2D	Type 2 Diabetes
ACS	Acute Coronary Syndrome

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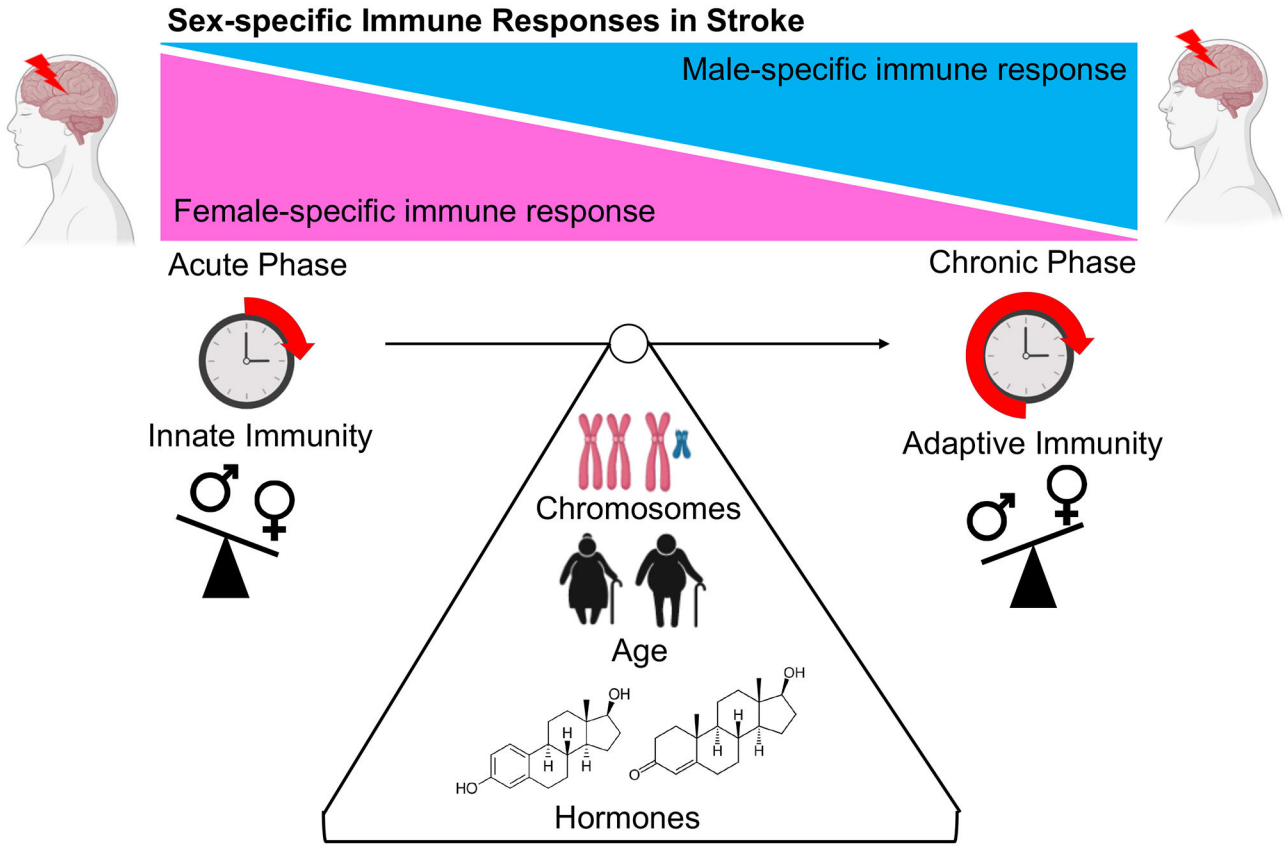

Figure 1.
Physiological factors that influence sex-specific immune responses after ischemic stroke. Figure made in ©BioRender - biorender.com.

Table 1.

Pre-clinical studies examining sex-specific responses in ischemic stroke.

Study	Species and Strain	Age	Key Findings
Villapol et al.[85]	C57BL6/J Mice	Post-natal day 9 (P9)	Neonatal ischemia models showed that female mice had significantly smaller infarcts compared to males as far as 3 months post-ischemia. Increased activation of microglia/macrophages and higher myeloid-specific brain infiltrates were observed in males.
Borgus et al.[86]	Sprague-Dawley Rats	Not reported	Sex and region-specific differences in adenosine, a ubiquitous neuromodulator, were seen.
Ahnstedt et al.[87]	C57BL/6N Mice	20-22 months	Independent of ischemic damage, aged males had significantly increased peripheral immune responses in the gut and exacerbated neuroinflammation in the sub-acute phase of stroke.
Dotson et al.[88]	C57BL/6J or PPARα knockout (KO) Mice (C57BL/6J background)	2-4 months	PPARα, a nuclear receptor transcription factor, plays a key role in perpetuating sex-specific peripheral immune responses following ischemic stroke.
Ahnstedt et al.[89]	C57BL/6J Mice	Young (3-4 months) and middle-aged (15 - 16 months)	Female mice had elevated levels of pro-inflammatory T cells and suppressed levels of anti-inflammatory T_{regs} in adipose tissue. The degree of adiposity, a major risk for ischemic stroke, may be phenotypically distinct across sexes.
Manwani et al.[90]	C57BL/6 Mice	Young (5-6 months), middle aged (14-15 months), and aged (20-22 months)	Heterogenic age- and sex-specific changes were evident in the post-stroke inflammatory milieu. Higher inflammatory macrophages and less lymphocytic infiltration in cycling females. Exacerbated splenic contraction in aged females. Age-specific increases in pro-inflammatory T cells, DCs, and microglia in the brain.
Davis et al.[91]	Sprague-Dawley Rats	18 months	Sex-specific effects of leukemia inhibitory factor treatment in mice seen following ischemic stroke. Sex differences in peripheral splenic responses and production of pro-inflammatory cytokine including IL-3 in females.
Jackson et al.[92]	Wister Rats	2-3 months	Sex differences in diabetes, a major risk factor for ischemic stroke. Higher pro-inflammatory T_H17 in female brains after stroke. Expansion of T cell subsets was sex-dependent and regulated by the sexual dimorphic effects of diabetes.
McCullough et al.[93]	C57BL/6J XYM and wild-type C57BL/6J Mice	18-20 months	X chromosome compliment (XX) regulates ischemic damage in aged animals (worse in females). Sex-specific effects of innate immune responses following ischemic stroke.
Mirza et al.[94]	C57BL/6 Mice	Post-natal day 10 (P10)	Sex-based innate immune response differed with increase microglial activation and infiltration of peripheral leukocytes following HIE in males.
Xiong et al.[95]	BALB/cJ and IL-4 KO (BALB/c-IL-4^{tm2Nnt}/J) Mice	2-3 months	Neuroprotective mechanisms after stroke in female mice are mediated by IL-4. Females exhibit activation of anti-inflammatory M2 microglia as well as reduced in infiltration of peripheral leukocytes.
Nguyen et al.[96]	C57BL/6 and BALB/c Mice	Young (3-4 months) and aged (18 months)	Sex-based differences in cytokines and chemokines within the infarct area at the liquefactive stage of necrosis after stroke.
Dotson et al.[97]	C57BL/6J Mice	Not reported	Splenectomy prior to experimental stroke abolished sex differences in infarct volume and stroke-induced monocyte and microglia activation.
Wang et al.[98]	C57BL/6J Mice	2-3 months	Adoptive transfer of peripheral immune cells (CD4/ CD8/ CD11b), prior to stroke had no effects on post-stroke inflammation. Suggested that other immunoregulatory factors exert sex-based effects of the spleen on post-stroke outcomes.
Raval et al.[99]	Sprague-Dawley Rats	Young (6-7 months) and retried breeder (9-13 months)	Sex differences in the inflammasome related to the reproductive hormones may contribute to sex-based differences in regulation of inflammatory states, seen in microglia activation and immune cell infiltration in the brain post-stroke.

Study	Species and Strain	Age	Key Findings
Liu et al.[100]	CaMKK β knockout (KO) and CaMK IV KO Mice (C57BL/6J background)	3-4 months	Pharmacological inhibition of CaMMK downstream signaling exacerbated stroke outcomes as characterized by BBB impairment and activation of pro-inflammatory responses seen only in females following stroke.
Li et al.[101]	Fischer Rats	2 months	Female rats exhibited a significantly higher inflammatory response following stroke in infarcts across all severities. However, severe infarcts within the cerebral regions in males were significantly larger compared to female counterparts.

Table 2.

Clinical studies examining sex-specific responses in ischemic stroke.

Study	Male/Female Ratio	Mean Age	Comorbidities	Key Findings
Ross et al.[102]	Males: 96 Females: 96	Males: 73.2 Females: 77.3	**Males:** Hypertension: 78 Diabetes: 39 Hyperlipidemia: 57 Smoking: 44 TIA/stroke: 38 **Females:** Hypertension: 83 Diabetes: 27 Hyperlipidemia: 58 Smoking: 28 TIA/stroke: 44	Significant age- and sex-based changes were seen in circulating lymphocytes and myeloid cells after stroke.
Xu et al.[103]	Males: 10 Females: 10	Males: 60 Females: 60	Not reported	CCL20, ICAM1, and PTGS2 identified as sex specific targets in ischemic stroke patients. Males showed a CD8+ T cell response; females had a more monocytic response to stroke.
Zhu et al.[104]	Males: 10 Females: 10	Males: 60.2 Females: 60.2	Not reported	IL-1α, IL-1β, IL-6, IL-8, CXCL1, CXCL2, CXCL20, CCL4, ICAM1, and PTGS2 were linked to protective effects in female stroke patients and contributed to post-stroke immune and apoptotic sex differences.
Lasek-Bal et al.[105]	Males: 63 Females: 75	Males: 70.4 Females: 75.4	**Males:** Hypertension: 59 Atrial Fibrillation: 21 Carotid Artery Stenosis: 7 Coronary Disease: 33 Diabetes: 23 Hemorrhagic Transformation: 2 Lipid Disorders: 21 **Females:** Hypertension: 68 Atrial Fibrillation: 23 Carotid Artery Stenosis: 7 Coronary Disease: 38 Diabetes: 26 Hemorrhagic Transformation: 3 Lipid Disorders: 23	Sex differences in WBC, platelet counts, CRP, S100B and IL-6 levels seen 24 hours after stroke.
Trott et al.[106]	Males: 70 Females: 59	Males: 62 Females: 62	Not reported	A positive correlation was observed in WBC change and absolute NIHSS was seen in female ischemic stroke patients.
Aleksandrova et al.[107]	Quartiles of FABP4 in plasma **Q1** (7.8-10.2 ng/mL): Males: 37.1% Females: 62.9% **Q2** (12.0-14.5 ng/mL):	Quartiles (Q) of FABP4 in plasma **Q1** Males and Females: 560 **Q2**	Quartiles (Q) of FABP4 in plasma **Q1** Males and Females Physically active: 21.6% Self-reported Hypertension: 29.6% Current smoker: 25.9%	FABP4 and T2D were significantly associated with higher risk of stroke in males vs. females.

Study	Male/Female Ratio	Mean Age	Comorbidities	Key Findings
	Males: 36.6% Females: 63.4% **Q3** (15.3-19.5 ng/mL) Males: 36.8% Females: 63.2% **Q4** (22.4-32.7 ng/mL) Males: 36.3% Females: 63.7%	Males and Females: 544 **Q3** Males and Females: 544 **Q4** Males and Females: 546	**Q2** Males and Females Physically active: 17.1% Self-reported Hypertension: 41.0% Current smoker: 20.4% **Q3** Males and Females Physically active: 14.7% Self-reported Hypertension: 52.2% Current smoker: 18.9% **Q4** Males and Females Physically active: 11.4% Self-reported Hypertension: 63.7% Current smoker: 16.7%	
Åkerblom et al.[108]	Quartiles (Q) of IL-18 in plasma **Q1** (<180.0 ng/L): Males: 2,613 Females: 1,563 **Q2** (180-237.0 ng/L) Males: 2,963 Females: 1,211 **Q3** (237.0-311.0 ng/L) Males: 3,099 Females: 1019 **Q4** (>311.0 ng/L) Males: 3,204 Females: 964	Quartiles (Q) of IL-18 in plasma **Q1** Males and Females: 63 **Q2** Males and Females: 62 **Q3** Males and Females: 61 **Q4** Males and Females: 61	Quartiles (Q) of IL-18 in plasma **Q1** Males and Females Smoker: 1338 Hypertension: 2749 Dyslipidemia: 2018 Diabetes: 866 **Q2** Males and Females Smoker: 1484 Hypertension: 2670 Dyslipidemia: 2030 Diabetes: 985 **Q3** Males and Females Smoker: 1526 Hypertension: 2725 Dyslipidemia: 1917 Diabetes: 1045 **Q4** Males and Females Smoker: 1561 Hypertension: 2734 Dyslipidemia: 1886 Diabetes: 1256	A positive correlation with circulating plasma levels of IL-18 in ACS men was an independent risk factor for ischemic stroke.
Stamova et al.[109]	**Vascular Risk Factor Controls:** Males: 12 Females: 11 **Embolic Stroke:** Males: 12 Females: 11	**Vascular Risk Factor Controls:** Males: 56.8 Females: 59 **Embolic Stroke:** Males: 72.1 Females: 71.3	**Vascular Risk Factor Controls:** Hyperlipidemia: Males: 9 Females: 7 Hypertension: Males: 9 Females: 7 Diabetes: Males: 3 Females: 2 Atrial fibrillation: Males: 0 Females: 0	Female cardioembolic stroke patients had significantly more differentially expressed genes in the blood linked to cell death and cell-cell inflammatory signaling. Circulatory neutrophil-specific genes were identified as early as 3 hours in females with cardioembolic stroke.

Study	Male/Female Ratio	Mean Age	Comorbidities	Key Findings
			Cardioembolic Stroke: Hyperlipidemia: Males: 3 Females: 3 Hypertension: Males: 8 Females: 8 Diabetes: Males: 2 Females: 2 Atrial fibrillation: Males: 3 Females: 6	
Nguyen et al.[110]	**Normal controls:** Males: 15 Females: 8 **Acute stroke:** Males: 3 Females: 2 **Liquefactive necrosis** Males: 8 Females: 8 **Encephalomalacia:** Males: 2 Females: 7	**Normal controls**: Males and Females: 77.4 **Acute stroke:** Males and Females: 76.2 **Liquefactive necrosis** Males and Females: 86.8 **Encephalomalacia**: Males and Females: 79.8	Not reported	A wide array of sex-based cytokines and chemokines were identified within the infarct at the liquefactive stage of necrosis post-stroke.
Stamova et al.[111]	**Controls:** Males: 41 Females: 68 **Ischemic Stroke** Males: 35 **Females: 26**	**Controls:** Males: 50.2 Females: 49 **Ischemic Stroke** Males: 67.1 Females: 67	Not reported	Distinct classes of X-chromosome genes were altered across male and female patients after stroke. Male-specific genes regulated pathways involved in cellular movement, development, cell-trafficking, and cell death while female-specific genes included post-translational modification, small-molecule biochemistry, and cell-cell signaling.
Tian et al.[112]	**Controls:** Males: 28 Females: 24 **Ischemic stroke:** Males: 27 Females: 24	**Controls:** Males: 60.3 Females: 60.6 **Ischemic stroke:** Males: 63.6 Females: 65.1	**Controls:** Hyperlipidemia: Males: 12 Females: 16 Hypertension: Males: 13 Females: 13 Diabetes: Males: 4 Females: 1 **Ischemic stroke:** Hyperlipidemia: Males: 10 Females: 6 Hypertension: Males: 17	Sex differences in gene patterns in the blood after ischemic stroke. These contributed to sexually dimorphic immune, inflammatory, and cell death responses after stroke.

Study	Male/Female Ratio	Mean Age	Comorbidities	Key Findings
			Females: 9 Diabetes: Males: 6 Females: 5	