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Gender Differences in Neurological Disease

Role of Estrogens and Cytokines

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Increasing evidence suggests that inflammatory response may be a critical component of different brain pathologies. However, the role played by this reaction is not fully understood. The present findings suggest that neuroinflammtory mediators such as cytokines may be involved in a number of key steps in the pathological cascade of events leading to neuronal injury. This hypothesis is strongly supported by experimental and clinical observations indicating that inhibition of the inflammatory reaction correlates with less neuronal damage. Estrogens are thought to play a role in the sex difference observed in many neurological diseases with inflammatory components including stroke, Alzheimer's and Parkinson's diseases, multiple sclerosis, or amyotrophic lateral sclerosis. Clinical and experimental studies have established estrogen as a neuroprotective hormone in these diseases. However, the exact mechanisms involved in the neuroprotective effects of estrogens are still unclear. It is possible that the beneficial effects of these hormones may be dependent on their inhibitory activity on the inflammatory reaction associated with the above-mentioned brain pathologies. Here, we review the current clinical and experimental evidence with respect to the inflammation-modulating effects of estrogens as one potential explanatory factor for sexual dimorzphism in the prevalence of numerous neurological diseases.

Key Words: Estrogen; inflammatory reaction; cytokines; neurological disorders.

Introduction

A large body of evidence has shown that the brain is capable of sustaining an immune response to injury and pathology *(1–3)*. The inflammatory reaction in the central nervous system (CNS) varies depending on the brain region and nature of injury; however, glial activation and infiltration of circulating immune cells is typically observed. Inflammatory processes within the CNS are stimulated and enhanced by inflammatory mediators—mainly by pro-inflammatory cytokines *(4–7)*. In response to neuronal insults, cytokines are produced mainly by activated glial cells and function to stimulate inflammation and may induce neuronal death or possibly act as trophic factors. Depending on the phase and extent of injury, a wide range of cytokines is produced and, what is more important, various concentrations of the same cytokine may have detrimental and beneficial effect on neurons *(8–10)*.

The precise role of the neuroinflammatory reaction in the brain is not fully clear. An important feature of this reaction is the first line of defense providing rapid detection and elimination of pathological changes in the CNS. However, an excessive or chronic inflammatory response can also be a source of additional neuronal injury *(11,12)*. Several lines of evidence suggest that neuroinflammation might contribute to the cascade of events leading to neuronal dysfunction and loss and that this immune phenomenon could be integral to mechanisms of different neuropathology progression *(13–15)*.

It has been well established that the increased levels of inflammatory mediators, such as cytokines, have been synthesized at the sites of neurodegeneration in several disorders including cerebrovascular stroke *(3,16)*, Alzheimer's and Parkinson's diseases (AD, PD) *(1,7,17,18)*, multiple sclerosis (MS) *(19,20)*, or amyotrophic lateral sclerosis (ALS) *(21)*. It is now generally believed that neuroinflammatory processes, including excessive pro-inflammatory cytokine production, may contribute to the pathogenesis, clinical onset, and progression of these devastating neurological disorders. This hypothesis is supported by clinical and experimental observations indicating that inhibition of the neuroinflammation correlates with less neuronal degeneration *(22,23)*. Therefore, steroidal and nonsteroidal anti-inflammatory drugs are considered promising therapeutic interventions for a number of neurodegenerative disorders, such as AD and PD *(7,24,25)*.

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It is well known that the immune response is under the control of numerous endogenous factors including specific sex hormones, such as estrogens (principally 17β-estradiol) *(26,27)*. Both in vivo and in vitro studies have confirmed the influence of these sex steroids on immune function and inflammatory processes in the brain, but the mechanisms involved are not completely understood. Several studies have established that estrogens decrease production of both cellular and molecular factors of neuroinflammatory reaction *(28,29)* thus blocking two crucial events that sustain the progression of the neurodegenerative diseases.

A large number of observations indicate that this hormonal factor provides a plausible candidate to explain a naturally occurring sexual dimorphism in the prevalence of the above-mentioned CNS disorders with neuroinflammatory components *(30–33)*. These findings have led to the proposal that the anti-inflammatory action of estrogen may represent an important mechanism underlying the neuroprotective effects exerted by estrogens in so many diverse neurological diseases. Thus better understanding the influence of estrogens on immune-mediator expression, among which cytokines play a prominent role, may clarify the gender difference in the susceptibility to these diseases.

Potential Mechanisms of Estrogen–Cytokine Interactions

Accumulating evidence from experimental and clinical studies has indicated that estrogens might influence the production or bioactivity of pro-inflammatory cytokines, such as TNFα, IL-1, and IL-6 *(34–36)*. It has been shown that estrogen depletion either after menopause in women or ovariectomy in female rats induces the pro-inflammatory cytokine production from peripheral blood monocytes and bone marrow cells *(37,38)*. Estrogen treatment conversely suppresses such cytokine synthesis *(39)*. Furthermore, in vitro, estrogens directly attenuate endotoxin-induced TNFα expression in cortical glial cultures *(40)*. Another example indicates that estrogens also increase IL-10 while decreasing TNFα and IFNγ release from resting and LPS-stimulated N9 murine microglia cells *(41)*. However, the ability of estrogens to repress cytokine gene induction may vary with the tissue.

Many of these immunosuppressive effects of estrogens are generally dependent on the presence of "classical" estrogen receptors (ERs) in the nuclei or cytoplasm, through which estrogens may alter the expression of estrogen-responsive genes. However, it is likely that estrogens use diverse signaling pathways to produce their anti-inflammatory properties. Thus, the nature of the estrogen–cytokine interaction mechanisms is highly complicated and not well understood.

Estrogen receptors have been detected not only in classical reproductive tissue, but also in immune cell populations, including lymphocytes, monocytes, and macrophages and even within brain glial cells *(42)*. Indeed, several experimental reports have demonstrated the presence of ERs in various populations of microglia and astrocytes *(43,44)*. These receptors are classified into two forms, ERα and ERβ, transcribed from two distinct genes. However, it is still unclear which receptor subtype is directly involved in estrogen-mediated neuroimmunosuppression.

The ERs belong to a large family of proteins—ligand-activated transcription factors—which regulate gene expression in target cells *(45,46)*. Upon binding to the ligand, ERs dissociate from the complexes with Hsp and the receptors dimerize. The activated ER dimer binds to the specific DNA sequences known as estrogen response elements (ERE) present in the promoter regions of estrogen-responsive genes, and influence the rate of gene transcription. The ERE have been identified in the promoter region of multiple genes.

Putative Estrogen Receptor–Meditated Signaling Pathways Involved in Pro-inflammatory Cytokine Gene Expression

It is known that the promoters of the pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, lack a classical ERE; thus, it was postulated that estrogen-dependent inhibition of transcription occurs without a physical interaction of the ERs with the pro-inflammatory gene promoters *(47)*. However, ERE has been mapped to the 5' flanking sequence of IFNγ *(48)*. The exact molecular mechanisms by which estrogens interfere with cytokine gene activity are still not completely clear; however, they likely include interactions of the ERs with other transcription factors.

Mutational analysis revealed the existence of the AP-1 (activator protein-1) binding sites in the promoter of TNFα gene, suggesting that AP-1 is a critical regulator of expression this cytokine *(49,50)*. However, it has been speculated that TNFα gene expression may also be regulated by NF-κB nuclear factor *(51)*.

It was recently reported that estrogens modulate AP-1–dependent gene expression without binding to either DNA or AP-1 proteins. In this regard, much evidence suggests that estrogens decrease upstream regulators of AP-1 expression. Indeed, the sex steroids decrease Jun NH2-terminal kinase (JNK) activity and diminish the phosphorylation rate of c-Jun and JunD at their NH2 termini. Thus, a consecutive decline is evident in the ability of these nuclear proteins to autostimulate the expression of c-Jun and JunD genes. This downregulation in the nuclear levels of c-Jun and JunD leads to decreased binding of c-Jun/cJun and JunD/cFos heterodimers to the AP-1 consensus sequence in the TNFα promoter and subsequent decreased transactivation of this cytokine gene *(49)*. It may also be speculated that estrogens could also repress the TNF-α promoter by disrupting the interaction between AP-1 and NF-κB.

ERβ seems to be more potent than ERα at repressing the TNFα gene expression. While it seems likely that ERβ may function predominantly as a transcriptional repressor, ERα is more effective at activating ERE *(52)*. Selective

transcriptional activity by ERα and ERβ together with interactions between the ER/coactivator complex and transcription factors may explain the tissue-specific effects of estrogen on cytokine expression.

It has been well documented that estrogens can modulate IL-6 gene expression and that this effect is largely controlled by NF-κB *(53–55)*. NF-κB normally exists in the cytoplasm, complexed to inhibitory binding proteins (IκBs) and remains inactive. Numerous signals, including cytokines, lead to activation of IκBs. Upon stimulation, IκBs are phosphorylated by cytokine-responsive IκB kinases or other protein kinases. Phosphorylation of IκBs leads to its proteasome-mediated degradation, which in turn enables dimeric NF-κB transcription factors to translocate to the nucleus where they bind to specific DNA sequences in target genes *(56)*.

Many groups have shown that both ERα and ERβ have an inhibitory effect on NF-κB activity, by various mechanisms, which generally depend on the specific stimulus or cell types. Although some studies have suggested a direct binding interaction between ER and NF-κB, recent studies also suggest multiple indirect interactions *(57)*. ERs could interact directly with NF-κB to inhibit its ability to bind to the IL-6 promoter *(53)*. The association between ER and NF-κB may introduce conformational changes in both proteins that lead to the inability to bind DNA. Alternatively, this cooperation may result in the formation of inactive complexes on the DNA by preventing the interaction with essential cofactors or the basal transcriptional machinery. Inhibition of NF-κB activity by estrogens has been also attributed to stabilization of IκBα *(58)*. For example, IκBα levels are increased by estrogens in HeLa cells overexpressing ERβ *(59)*.

Estrogen-mediated inhibition of IL-6 expression has been independently observed in cells that only express ERα or ERβ, suggesting that both ERs are capable of down-regulating NF-κB signaling to the IL-6 gene upon activation *(60)*.

At present, there are no data to support any specific molecular mechanism for the direct suppressive effect of estrogens on IL-1 gene expression. NF-κB is a potent regulator of IL-1 gene transcription, which raises the possibility that suppression of IL-1 gene expression by the sex hormone may be executed by a similar mechanism to that implied in the suppression of IL-6 gene expression *(61)*. Interestingly, it has been demonstrated that estrogens specifically regulate IL-1 receptor subtype expression in human osteoclast-like cells (hOCL cells) in such a way as to diminish their biologic responsiveness to IL-1 *(62)*. Two distinct IL-1 receptors are known: type I (IL-1RI) that mediates cellular responses to IL-1, and the type II or decoy (IL-1RII) that also binds IL-1, but lacks an intracellular domain and does not initiate signal transduction. It is known that the promoter of IL-1RI also contains an NF-κB response element that may mediate IL-1RI mRNA downregulation by estrogens *(63)*. Less is known about regulatory elements, including potential ER responsive sites, within the IL-1RII promoter *(64)*. It is evident that soluble IL-1RII is elevated in the serum of patients with inflammatory diseases and binds IL-1β with high affinity, thereby functioning as an endogenous anti-inflammatory suppressor of IL-1 action *(62)*. It is established that soluble IL-1RII protein released by hOCL cells declines as the age of the female donor increases and rises in response to estrogens. Moreover, increased IL-1RII levels correlated with decreased responsiveness of hOCL cells to IL-1 *(62)*. Therefore, by this novel pathway, estrogens may help inhibit inflammatory responses by modulating IL-1RII levels.

Recent evidence also suggests that estrogens might restrict the production of pro-inflammatory cytokines by decreased microglial expression of costimulatory molecules, such as CD40. This molecule, belonging to the TNF/nerve growth factor superfamily of receptors, is expressed by a wide variety of cells including microglia, macrophages, and B-cells. It is well known that activation of the signaling pathway through CD40 in activated microglial cells stimulates the release of numerous pro-inflammatory cytokines, such as IL-1, IL-6, or TNF by these cells *(41)*.

It is also known that ERs interact with the basal transcriptional machinery through complex interactions with other transcription factors and with identified coactivators, which also remodel chromatin. ERs can also act through less well-characterized nongenomic mechanism-membrane, namely, by membrane-associated ERs (pmER). Estrogens bind to a pmER and signal second messenger cascade [i.e., mitogen-activated protein kinase (MAPK)], leading to phosphorylation of transcription factors *(65)*. For example, 17β-estradiol has been shown to activate several MAPK signaling, including p42/44 MAP kinase. It is evident that this pathway could be important for estrogen-mediated anti-inflammatory action in cultured microglia *(66)*. This type of action of estrogens enhances transcription of genomic DNA, and might explain how estrogen can modulate genes without known consensus EREs.

In conclusion, the mechanisms underlying estrogen–cytokine molecular interactions have been discussed but are only partially understood. Future research will be devoted toward understanding which of these molecular mechanisms holds true for the ER-related anti-inflammatory action of estrogen.

Cytokines and Gender in Neurological Diseases

Parkinson's Disease

The major pathologic feature of PD is a progressive loss of dopamine (DA)–producing neurons of the substantia nigra (SN), resulting in a reduction of the DA level in the striatum *(67)*.

Both epidemiological and clinical reports indicate a positive role for estrogen use in PD. The potential for estrogens to act as a neuroprotectant of the dopaminergic system may be related to the epidemiological reports that show a sex difference in PD. Parkinson's disease demonstrates a

male predominance, the average standardized ratios of female to male are 1:3.5 for prevalence studies *(30)*. Epidemiological evidence suggests that postmenopausal estrogen therapy may be associated with reduced risk of PD in women *(68)*. Withdrawal of estrogen supplementation in postmenopausal women exacerbates Parkinson-related symptoms in the case of early onset PD, which is ameliorated by estrogen replacement *(69,70)*. Estrogen therapy has been associated with improved cognitive functioning and a reduced risk of dementia in women with PD *(68)*. PD was also significantly associated with a fertile life length shorter than 36 years *(71)*.

Sex-specific effects have been also described in animal models of PD. Male mice have shown stronger DA depletion post 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication than female mice *(72)*. Furthermore, estrogen exposure yields a reduction of the degree of the DA depletion resulting from treatment with the neurotoxin MPTP *(73,74)*.

Thus, these findings support the hypothesis that endogenous and exogenous estrogens play an important role in the predisposition to PD and in the development of this disease, as well as in neurotoxin-induced dopaminergic neurodegeneration. However, the role of estrogens in this neuropathological process remains unresolved.

Although the cause of death of the dopaminergic neurons is still unknown, cytokines are thought to contribute to the development of the disease. Increased number of glial cells expressing the pro-inflammatory cytokines have been reported within the nigrostratial regions of patients with PD *(75,76)*. It seems likely that the chronic excessive production of these pro-inflammatory cytokines may have deleterious effects upon dopaminergic functioning and subsequent motor behaviors. However, the possibility that these cytokines have a neuroprotective effect cannot be excluded. For example, elevated levels of IL-6 were found in the cerebrospinal fluid (CSF) and had an inverse correlation between severity of PD *(77)*. Thus, it was suggested that, at early stages of the degenerative process, an upregulation of endogenous IL-6 synthesis occurs in order to regenerate lesioned neurons *(77)*. Such a proposition is certainly consistent with the high degree of plasticity attributed to dopaminergic neurons and the neurotrophic actions often promoted by IL-6.

Immunohistochemical studies showed an increase of IFNγ expression in nigral astrocytes of patients with PD *(78)*. IFNγ is mainly derived from T cells. However, the data from the last decade suggest that under certain conditions IFNγ can be produced in the brain. IFNγ mRNA expression has been detected in human neurons as well as in microglia and astrocytes. Expression of IFNγ in PD is an interesting subject, because this cytokine might play an important role in both neural tissue repair and injury *(79,80)*. Epidemiologic evidence suggests that an increase of IFNγ concentration in the brain of patients with PD might be a compensatory response *(81,82)*.

Similar to clinical observations, mice subjected to the dopaminergic toxin MPTP displayed inflammatory alterations within the CNS including increased levels of pro-inflammatory cytokines—TNFα, IFNγ, IL-6, or IL1β *(83)*. In 6-hydroxydopamine (6-OHDA) lesioned rats, another well-established animal model of PD, pro-inflammatory cytokines were also found to be enhanced both in the striatum and in the SN *(84)*.

As mentioned earlier, PD demonstrates a male predominance, although the reason for gender bias in PD and MPTP-induced nigrostriatal degeneration is still unclear. Focusing on inflammatory reactions, one of the hypotheses to explain the significance of gender-induced effects on degenerative processes in the nigrostriatal pathways may result from a link between this risk factor and the inflammatory processes.

However, the clinical evidence for gender-related difference in the immune reaction observed in PD is still unknown. Therefore, for the first time, in our study, we aimed to determine whether gender and age could influence pro-cytokine IL-6, TNFα, IFNγ, IL-1β mRNA expression in a murine model of PD *(85)*. The levels of these cytokine mRNAs were measured by RT-PCR in the striatum of male and female C57BL/6 mice (3 and 12 mo old) after 6 h as well as 1, 3, 7, 14, 21 d post-MPTP intoxication.

In the case of TNFα, in young and aged male mice, enhanced gene expression for this cytokine was observed as early as 6 h with peak at 24 h post-MPTP intoxication (Fig. 1A). In contrast, in female mice we did not observe a significant increase in the TNFα mRNA expression at 6 h post-intoxication. However, similar to what is seen in male mice, the maximum TNFα expression occurred the first day post-intoxication in both ages of female mice (Fig. 1B). In the case of IL-1β in male mice, the expression of mRNA for this cytokine reached its peak 24 h post-intoxication (Fig. 2A), whereas in female mice at 3 d time point (Fig. 2B). We noted that the enhancement of TNFα and IL-1β mRNA expression was higher in old than in young intoxicated male as well as female mice. Our data demonstrate that the early increase in the transcriptional activity of TNFα and IL-1β genes coincided with the cascade of dopamine depletion after MPTP intoxication both in young and aged male as well as in female mice. These results are consistent with previous studies indicating a crucial role of TNFα and IL-1β in setting the cytokine network and development of the neurodegenerative processes in nigrostriatal pathway post-intoxication *(83,86,87)*. We demonstrated that induction of mRNA TNFα and IL-1β in male preceded the induction of the expression for these cytokines in females. Although the genesis of the delay in IL-1β peak and the late induction of mRNA for TNFα in female vs male mice remains unclear, these gender-related differences could be explained by the influence of estrogens.

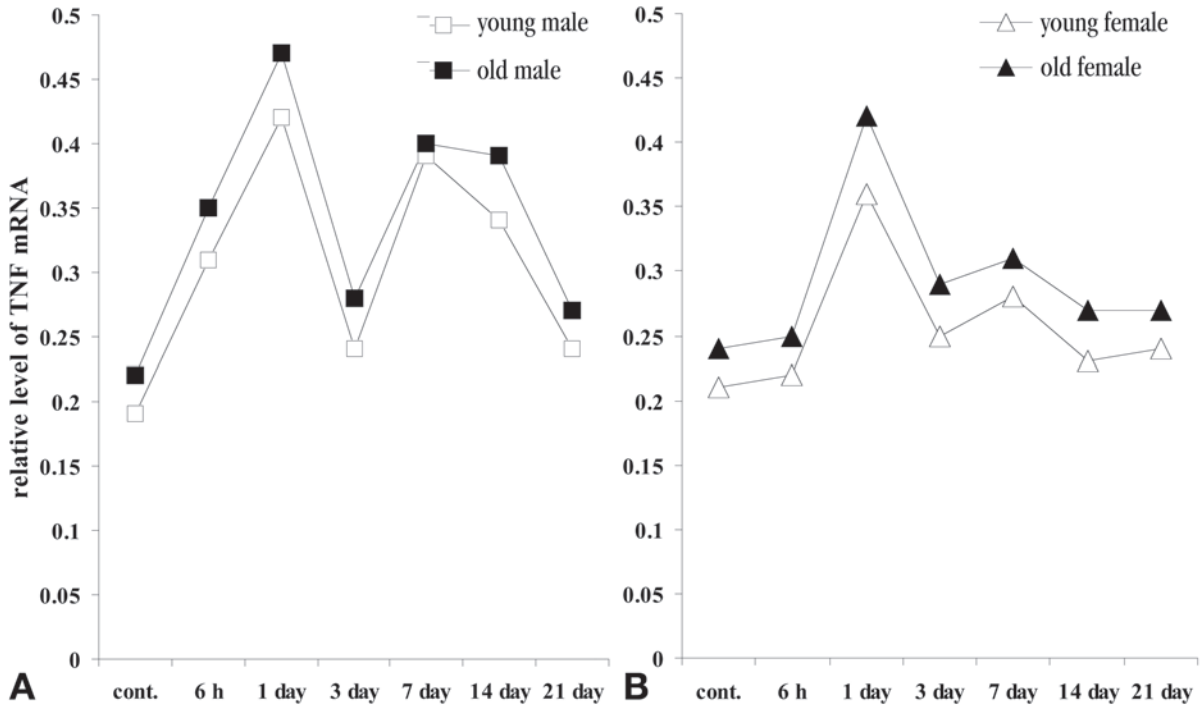

Fig. 1. TNF-α mRNA levels in the striatum of C57BL/6 young and old male (**A**) and young and old female (**B**) mice at control and 6 h; 1, 3, 7, 14, 21 d after MPTP intoxication.

Similar to TNFα and IL-1β, the kinetics of IL-6 gene expression after MPTP intoxication were gender-dependent. Specifically, although IL-6 mRNA was increased in both sexes, the effect was modestly greater in aged females than in aged males. Likewise, advanced age of mice of both sexes was associated with higher IL-6 mRNA expression following MPTP. However, the age-dependent increase was much more marked in female animals than males and this gender effect persisted across the entire 21-d sampling period (Figs. 3A,B). Recent studies indicate that this cytokine may have neuroprotective effects on nigrostriatal dopaminergic regions. For instance, Bolin et al. reported increased sensitivity of dopaminergic neurons to neurotoxic agents in the absence of IL-6 *(88)*, indicating a neuroprotective role for endogenous levels of the cytokine in the injured nigrostriatal system. It is possible that IL-6 expression in the aged brain still maintains neuroprotective functions, thus the endogenous upregulation of this cytokine in oldest mice suggests that the brain may have compensatory mechanisms that protect against neurodegenerative process in nigrostriatal system. Thus, this gender skewing in IL-6 expression observed in aged mice could offer an intriguing way to explain the marked gender differences in neurodegenerative responses to MPTP and other neurotoxins.

In our study we also observed significant MPTP-induced rapid increase in IFNγ mRNA expression with peak levels at 24 h after intoxication in aged and young male mice. However IFNγ increase was more prominent in young male mice at the 6 h time-point (Fig. 4A). Surprisingly, at the seventh day post-intoxication we again observed upregulation of the mRNA transcripts for IFNγ but only in aged male mice. In contrast to male mice, where increased IFNγ mRNA levels were already detected at early time-points, in female mice this was observed at later time-points (Fig. 4B). In young and aged female mice, the mRNA level for IFNγ was significantly elevated at d 7 post-intoxication, and it was sustained in young mice, whereas it was reduced in old female mice. The genesis of the late expression of IFNγ mRNA in female and aged male remained unclear, but previous studies could offer an explanation. Kurkowska-Jastrzębska et al. *(4)* have observed an influx of CD4+ and CD8+ T-cells into the SN and striatum between 3 and 14 d, with peak at d 7 post-MPTP intoxication. These results suggest that the second wave of IFNγ expression in aged male and increased levels of IFNγ mRNA during the later stages in female mice may be derived from these infiltrated T lymphocytes. Although not examined here, sex-related and age-related differences in recruitment and infiltration of peripheral T lymphocytes into nigrostriatal system may exist. The role of this cytokine in MPTP-induced degeneration is poorly understood and requires further investigation. IFNγ has been established as a strong immunological activator of microglia both in vitro and in vivo *(89)*. Thus, the early induction of IFNγ mRNA post-MPTP intoxication in male mice is probably related to activation of microglial cells. On the other hand, it cannot be excluded that the

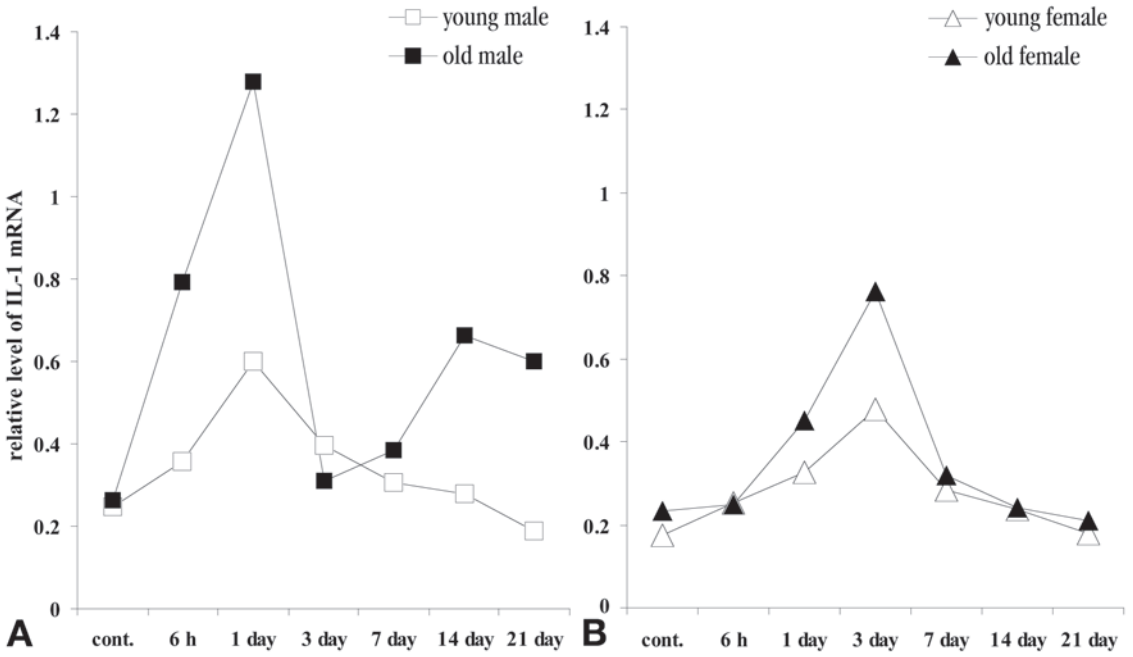

Fig. 2. IL-1β mRNA levels in the striatum of C57BL/6 young and old male (**A**) and young and old female (**B**) mice at control and 6 h; 1, 3, 7, 14, 21 d after MPTP intoxication.

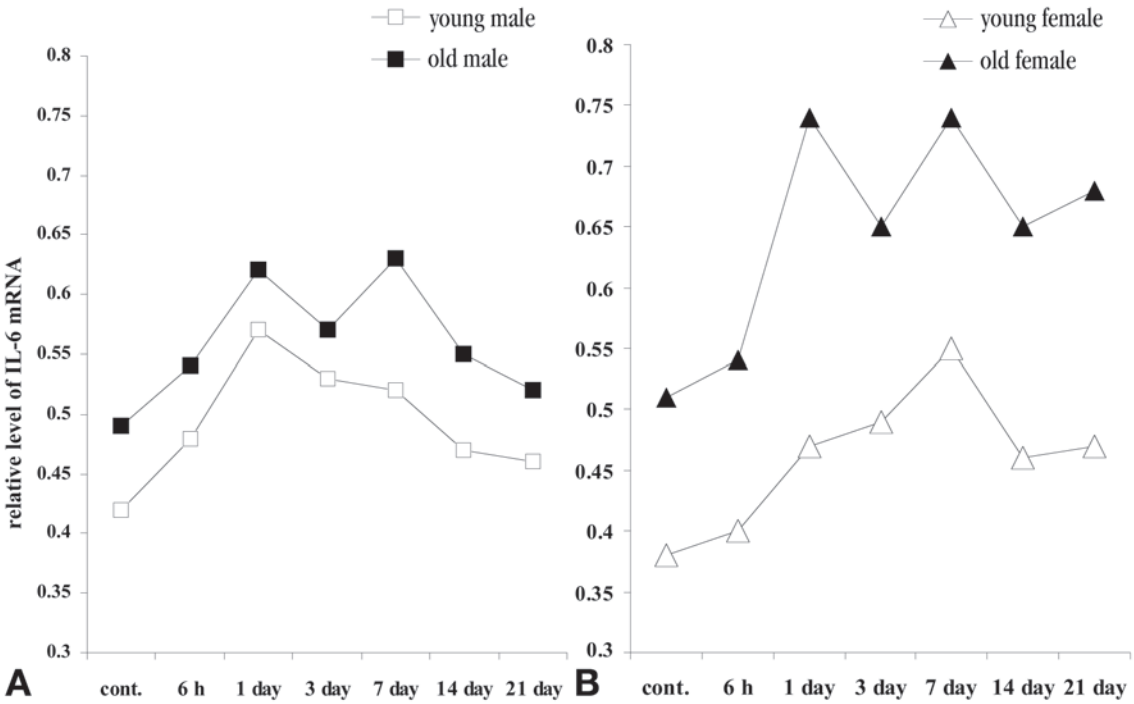

Fig. 3. IL-6 mRNA levels in the striatum of C57BL/6 young and old male (**A**) and young and old female (**B**) mice at control and 6 h; 1, 3, 7, 14, 21 d after MPTP intoxication.

extended production of IFNγ mRNA post-MPTP intoxication observed in female mice could be a beneficial mediator of neuroprotective mechanisms.

Taken together our data suggest a gender skewing of the investigated pro-inflammatory gene expression profiles in striatum by MPTP injection. These observations suggest that estrogens may be responsible, in part, for the altered production of these cytokines in female and male animals. Although it is tempting to suggest that a gender bias toward inflammatory-type responses may explain the marked gender differences in neurodegenerative responses to neurotoxins, it remains to be determined if differences of TNFα, IL-6, IFNγ, and IL-1β mRNA expression underlie such effects. Future research will define more clearly the role of inflam-

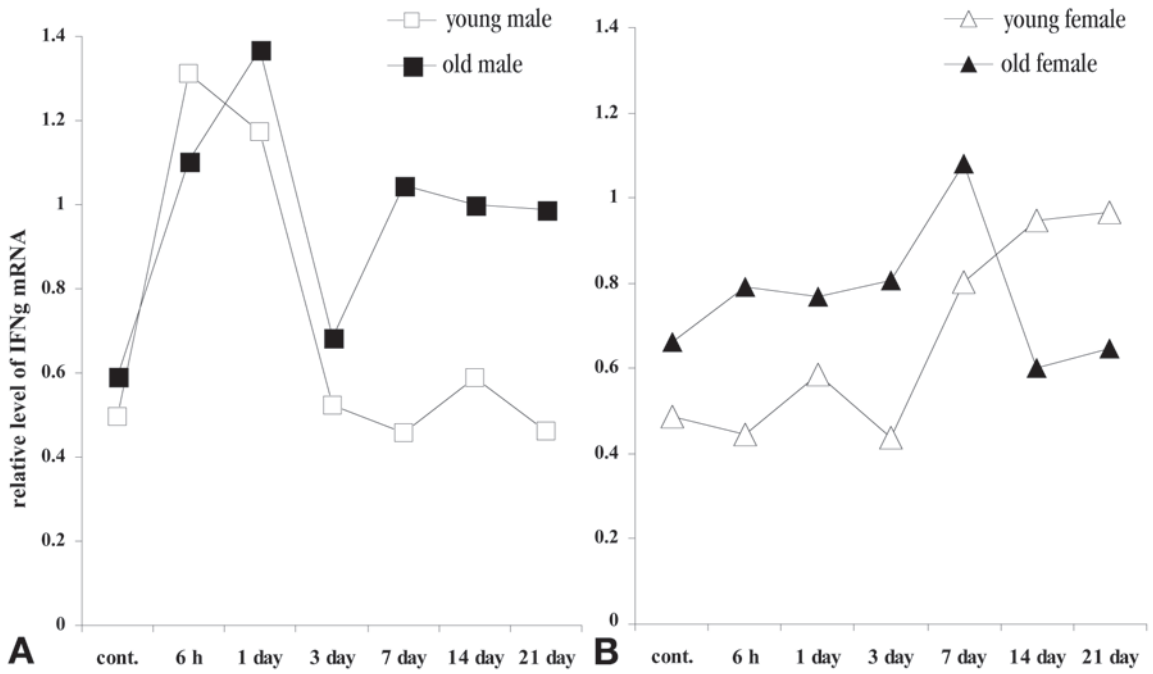

Fig. 4. IFNγ mRNA levels in the striatum of C57BL/6 young and old male (**A**) and young and old female (**B**) mice at control and 6 h; 1, 3, 7, 14, 21 d after MPTP intoxication.

matory cytokines in gender differences in Parkinson's disease and the role of the sex hormones in immune response regulation observed in this disease.

Alzheimer's Disease

Epidemiologic data show a predisposition of women to develop AD. Women suffer 1.6–3 times more than men *(90)*. One hypothesis used to explain the gender bias of a higher risk of AD in women is that women are generally living longer than men. It has been calculated that women older than 50 yr of age comprise a major portion of the total population of any country in the modern western world. Because 90% of cases of AD develop after 65 yr of age, it may be the clue for women's increased risk of the disease.

However, it was shown that among patients with diagnosed AD women have more pathological changes (e.g., neurofibrillary tangles) in the brain than men, although no sex difference in clinical manifestations of the disease was found. Additionally, similar AD changes in the brain were more clinically expressed as dementia in women than in men *(91)*.

The risk of AD increases with age for both sexes, but a decline of estrogen is related to increased risk of the disease in post-menopausal women *(90)*. In the Baltimore longitudinal study of aging, woman taking estrogen replacement therapy (ERT) were less likely to develop AD compared to those who did not have hormonal replacement *(92)*. Additionally, women taking replacement therapy showed improved performance in psychometric tests *(93)*. A prospective study of aging and health in a New York City community showed also that a use of estrogen in postmenopausal woman may delay the onset and decreased the risk of AD *(90)*. Several studies indicated that ERT increases performance in some memory/cognition tests in AD patients *(94–96)* but also showed that did not prevent cognitive decline in women with mild to moderate AD *(97,98)*. Unexpectedly, the Women's Health Initiative (WHI) Memory Study reported an increase in risk for probable dementia and impaired cognitive performance in postmenopausal women older than 65 yr treated with estrogen alone or estrogen with progestin *(99)*. The controversial results regarding estrogen replacement therapy have lead to investigation of other hormones of the hypothalamus–pituitary–gonadal axis. Gonadotropins, which are increased in the postmenopausal period and capable of crossing the blood–brain barrier, are proposed to play a crucial role in AD pathogenesis *(100)*.

The probable mechanism for steroid hormones in AD is postulated to influence the inflammatory reaction, which is extremely important in AD pathology. The involvement of immune mechanisms in the pathogenesis of AD has been largely based on laboratory evidence of local up-regulation of inflammatory cytokines (e.g., IL-1, IL-6, TNF), acute phase proteins (e.g., α1-antitrypsin), activation of the complement cascade and of accumulation of microglia in brain regions damaged in AD *(101)*. The pathogenesis of AD is strictly connected with β-amyloid formation associated with the deposition of plaques and neurofibrillary tangles. The main cytokine involved in such β-amyloid and plaque formation is believed to be interleukin-1 (IL-1), which promotes microglial and astrocyte activation as well as amyloid precursor protein production. IL-1 is highly expressed in

amyloid plaques and in regions of neurodegeneration in AD, such as the cortex and hippocampus. IL-1 also promotes an astrocytic overexpression of the S100 cytokine-like protein, the expression of which correlates with the degree of neuritic pathology *(102)*. Thus, it was suggested that IL-1 and S100 together contribute to β-amyloid plaque formation and evolution of neural lesions in AD *(102)*. It is interesting to note that IL-1 expression is induced in the brain by head trauma and advanced age, which constitute important risk factors of AD *(103,104)*. Other cytokines promoting inflammatory response, such as IL-6 and TNF-α, are also elevated in the various brain structures of AD patients as well as in the cerebrospinal fluid *(101)*. In an animal model of AD (transgenic mice carrying the mutation of APP and of presenilin-1, A246E), it was shown that female mice accumulate more amyloid deposits and at an earlier age than male mice *(105)*. This suggests that some dissimilarity in inflammatory response in man and woman may thus play a role in gender differences in prevalence of AD. However, until now, no studies concerning the variation in cytokine production and inflammatory response between male and female in AD, have been performed.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis demonstrates a clear male predominance (the ratio of man/woman is at every age more than 1.6), although this difference in frequency between genders diminishes with increasing age *(106)*. The onset of the disease is also earlier for men than for woman *(106)*.

The pathogenesis of the disease is obscure, but as in other neurodegenerative conditions the possible role of inflammation is considered. Indeed, pro-inflammatory cytokines are elevated in experimental models of ALS and in patients suffering from the disease. For example, skin, serum, and CSF levels of IL-6 were increased in the ALS patients *(107, 108)*. Mice expressing mutant superoxide dismutase 1 (SOD-1), the animal model for ALS, display a chronic expression of IL-1 mRNA as well as increased expression of TNFα, IL-6, IFNγ, and chemokine mRNA and proteins in the spinal cord *(109)*. Surprisingly, however, the lack of IL-1β in knockout mice did not improve the extent of motor neuron degeneration in this model *(109)*. Several pro-inflammatory cytokine mRNAs are also upregulated in the spinal cord of mutant SOD-1 rat, a novel model of ALS *(110)*. Additionally, microglia from SOD-1 transgenic mice have increased cytotoxic activity during activation (e.g., release more TNFα and less IL-6) as compared to microglia from nontransgenic animals. Authors indicate that more cytotoxic microglia may be responsible for neuronal death in this model *(111)*.

The risk of ALS increases in women following menopause, suggesting a clear role for sex hormones, such as estrogen. Animal studies with transgenic mice overexpressing the mutant human SOD-1 gene showed that female mice have the onset of the disease later, and better respond to exercise than males *(112)*. Administration of genistein, a phytoestrogen therapy, to male SOD-1 transgenic mice eliminated the sexual dimorphism of the disease, indicating its dependence on sex hormones *(113)*. Moreover, experimental studies showed that estradiol protects spinal motor neurons from excitotoxic insults in vitro *(114)*. However, the replacement therapy did not show any positive effect in women suffering from ALS. In fact, women who used estrogen had an earlier onset of the disease compared to those not taking hormonal replacement. There was no difference in survival time of those patients taking estrogen compared to those not on the medication *(115)*. Therefore, no clear evidence of estrogen neuroprotection in ALS was found.

Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the CNS. MS is more common among woman than man (female:male ratio about 2–3:1). The mechanism underlying gender differences is unknown. However, the dissimilarity in genetic predisposition between sexes and gonadal hormone influence are involved in the increased susceptibility of women to MS. There is evidence of genetic predisposition of the disease and HLA-DR is a risk factor of developing MS in the Caucasian population *(116–118)*. HLA-DR2 and HLA-DR15 were shown to be more frequent among female than male patients suffering from MS, and the frequency decreases with age of diagnosis *(119)*. MS, besides more female frequency, shares many features with other autoimmune diseases and clusters with Hashimoto's thyroiditis and rheumatoid arthritis, diseases connected with cell-mediated autoimmunity.

Many observations support the view that sex hormones influence course and severity of the disease. For example, pregnancy influences multiple sclerosis symptoms, with remission in the third trimester of gestation, followed by exacerbation in the postpartum period *(120)*. In addition, oral contraceptives containing female sex hormones have been associated with a lower risk of developing MS and decreased disability *(121)*.

MS, as its animal model, an experimental autoimmune encephalomyelitis (EAE), is postulated to be partially induced by Th1 autoimmune cells infiltrating the CNS. Many studies showed that pregnancy protects animals from EAE and estrogen administration suppress clinical EAE in mice and rats *(122)*. Recent studies have suggested that female hormones may modulate EAE through direct effects on such infiltrating T cells. It was reported that not only female mice are more susceptible to EAE than their male counterparts, but also that the transfer of T cells from female mice produce more severe disease in male recipients than transfer of T cells from males *(123)*.

The basis for a female predominance in autoimmune diseases, such as MS, is likely related to the fact that relative to men, women have more robust immune response and higher absolute number of CD4+ lymphocytes during their repro-

ductive years *(124,125)*. Indeed, distinct immune activity and responsiveness in males and females underlies many gender differences in autoimmunity. For instance, rodent studies provided evidence that after immunization, female mice produce more antibodies and show more vigorous T-cell activation than male mice. Under resting conditions, females also produce more Th1-derived cytokines than men (e.g., IFNγ, IL-12). Females are also more likely to develop preferential Th1 responses to challenges with infectious agents or antigens; however, during pregnancy, progesterone typically promotes Th2 responses. Th1 cells secrete pro-inflammatory cytokines and promote cell-mediated immune response, whereas Th2 cells trigger antibody production.

IFN-γ and TNFα, cytokines that are characteristic of Th1-type immune activation, have been shown to play an important role in the pathogenesis of MS. Increased production of IFNγ precedes clinical attacks of MS *(126)* and there is evidence for differential IFNγ responses between males and females. Physiological levels of sex hormones can regulate the IFNγ promoter in lymphoid cells and can significantly augment production of the cytokine from Con A–stimulated spleen cells. This may account for the observation that female mice produce higher levels of IFNγ after immune stimulation than males *(126,127)*. These gender discrepancies are poorly understood. However, it seems likely that the underlying sex differences in IFNγ secretion may be a downstream consequence in IL-12 expression between the male and female *(127)*. In this respect, there is increasing evidence supporting the role of IL-12 in pathogenesis of MS and that this occurs through one of many mechanisms, including induction of IFNγ.

The influence of estrogen upon regulation of APC (antigen presenting cells)–dependent T-cell activation may be of great importance in the development of MS *(127)*. Indeed, a link was demonstrated between sex steroids and APC-dependent secretion of IL-12 and IL-10. APC derived from males secreted IL-10, but not IL-12, during T-cell activation. However, female APCs displayed the reverse pattern of cytokine secretion. Inhibition of IL-10 or castration of male mice resulted in APC that preferentially secreted IL-12 during T-cell activation *(127)*. Furthermore, recent clinical studies indicate that the percentage of TNFα-producing CD3 positive cells was significantly higher in male than female MS patients *(126)*. These findings raise the possibility that gender differences in production of the pro-inflammatory cytokines, IL-12 and TNF-α and anti-inflammatory cytokine IL-10, in response to T-cell activation may be relevant in MS patients *(128)*. However, in vitro studies indicate that although sex hormones, such as estrogen, shift T cells toward the Th2 phenotype, they do not increase Th2 cytokines production in the CNS and suppress EAE even in IL-4– or IL-10–deficient mice *(126,127)*.

Our studies failed to detect any effect of gender upon cytokine secretion among MS patients. We studied 20 outpatients (11 women and 9 men) with clinically definite relapsing–remitting MS and a mean age of 37.9 yr (women, 36.8 yr; men, 39.2 yr). All patients received an intramuscular injection of IFN-beta-1a once a week over the period of 2 yr. Cytokine-producing cells (monocytes, lymphocytes), which were assayed before immunotherapy, displayed no difference between male and female patients in the basal expression of IL-4, IL-10, IL-12, and IFNγ. Correspondingly, at 12 and 24 mo following initiation of immunotherapy with IFNβ, no statistically significant differences between female and male populations were found *(129)*.

One mechanism through which estrogen may influence EAE or MS is downregulation of TNFα production *(130)*. Indeed, TNFα is considered a main deleterious cytokine secreted by macrophages/microglia during early development of sclerotic plaques. In particular, TNFα is toxic to oligodendrocytes and neurons depending on the dose administered. It was shown that estrogen decreased macrophage-derived production of this cytokine and of the oxidative radical, nitric oxide. In addition to TNFα, 17β-estradiol inhibited mRNA expression of the chemokines, RANTES, MIP-1α, MIP-2, IP-10, and MCP-1 and chemokine receptors CCR1, CCR2, and CCR5 during the acute phase and recovery during EAE. In contrast, ovariectomy, which diminishes basal 17β-estradiol levels, increased severity of EAE and chemokine expression. 17β-estradiol also diminishes TNFα and IFNγ expression in the spinal cords of mice subjected to EAE *(131)*. In conclusion, future investigations are required to more clearly define the cytokine network responsible for gender differences in MS pathology. Such information would undoubtedly aid in understanding the role of sex hormones in autoimmunological disease.

Ischemic Stroke (IS)

Gender Differences in IS Incidence

In general, stroke occurs about 5–10 yr later in women than in men *(132–136)*. IS incidence increases with age in both sexes, with a doubling of stroke rates for each decade after the age of 55 yr *(136)*. Some studies addressing the issue of gender in IS incidence revealed higher stroke incidence in men than in women *(133,136–140)*. Also, 5-yr stroke recurrence rates for men were almost twice than for women *(140)*. Although in the American Heart Association (AHA) study men had higher first stroke event than women, even after adjustment for age *(133)*, in some studies gender effects on IS incidence depended on women's age and hormonal status *(141,142)*. Postmenopausal women up to age 55 had increased risk of stroke compared with premenopausal women at the same age *(141)*. Among persons aged above 85 yr, women had even higher IS incidence than men *(143)*. Concluding, women seem to be protected from IS incidence but this effect is evident predominantly at the premenopausal stage. So, possibly estrogen (E) action prevents vascular events resulting in acute brain ischemia.

Ischemic cerebrovascular disease can be due to either thromboembolism or the cerebral manifestation of gener-

alized atherosclerosis. Thus, understanding why premenopausal women are protected from IS requires an understanding of E actions on atherogenesis and thrombogenesis. Known mechanisms for E action on these processes include lipid lowering, preventing lipid peroxidation, vasodilatation, and reduction of inflammation *(144,145)*. An important mechanism mediating estrogen action, preventing both athero- and thrombogenesis, may be limitation of proinflammatory cytokine (PC) activities.

PC have potent actions upon pathology during athero- and thrombogenesis. During atherogenesis, PC activities mainly mediate the recruitment of blood monocytes to the site of vascular injury, stimulate migration of smooth muscle cells to the intima layer of the vessel wall, and, at more advanced stages of atherosclerosis, increase possibility of plaque rupture *(146,147)*. PC are also able to stimulate intravascular coagulation *(146–148)*.

Estrogen–Cytokines–Atherogenesis. Until now only few studies have been published aimed at establishing estrogen influence on cytokine response during atherogenesis. Sukovich et al. demonstrated that interleukin-6 (IL-6) mRNA expression and protein production in the aortic rings of *IL-6*$^{+/+}$/*APOE*$^{-/-}$ male mice positively correlated with a total lesion area. Estradiol treatment in these animals resulted in a 50% reduction of IL-6 secretion from aortic tissue segments. The authors concluded that possibly the suppression of IL-6 by E may contribute to the antiatherosclerotic mechanism of this ovarian steroid hormone *(149)*. However, in another study E supplementation was associated with significantly reduced fatty streak formation both in wild-type *IL-6*$^{+/+}$ and in *IL-6*-knockout animals *(150)*. So, possibly *IL-6* gene expression does not directly mediate the prevention of fatty streak formation induced by E. Another study revealed that interferon-γ (IFN-γ) deficiency, evoked by the *IFN*-γ gene knockout, resulted in markedly decreased atherosclerocitc lesion formation in male apolipoprotein E-deficient (*APOE*$^{-/-}$) mice. These animals had also significantly reduced the number of T lymphocytes and MHC class II–positive cells in atherosclerotic lesions *(151)*. The lack of similar effect in females indirectly suggests E atheroprotective action mediated by downregulating immune response mediated through the suppression of *IFN*-γ production.

Concluding, studies that have addressed the action of E on PC production in atherogenesis are still extremely limited, precluding a more definitive conclusion on the relevance of these effects.

Gender Differences in Stroke Course and Prognosis

Brain Pathology. Gender effects on stroke pathology have been clearly established in animals subjected to transient or permanent, global or focal cerebral ischemia (for review, see ref. *135*). Premenopausal female animals had lower incidence and less severe brain lesions after middle cerebral artery occlusion (MCAO) compared with males and with postmenopausal females. Ovariectomized females sustained a similar infarct size as males. Female rats at proestrus had smaller infarct volumes than at metestrus. Further studies, examining the effects of exogenous E administration on cerebral ischemic injury, provided additional evidence for the protective role of E in brain ischemia. In almost all studies, E significantly reduced brain damage and cerebral edema, and attenuated neurological abnormalities.

Mortality and Functional Outcome. Female animals subjected to MCAO had greater survival rates and enhanced functional recovery after an ischemic insult, than males *(135)*. Gender effects on stroke mortality have also been described in clinical studies *(134–136,152–154)*. In general, younger women (<64 yr) had a lower mortality than men; a reverse relationship was reported among older patients (≥65 yr of age) *(133,152)*. Gender differences in terms of functional outcome after IS, are still under exploration. In papers published so far, women had more severe disturbances of consciousness than men; they also presented more profound neurological symptoms and had a poorer functional outcome assessed at 3 and 6 mo following the stroke *(134,135, 154)*. However, it has to be underlined, that women included into these studies were about 5 yr older than men, and most of them were postmenopause.

Concluding, results of clinical observations, and much more clear results of experimental investigations, suggest that circulating E may maintain less severe brain damage and better prognosis. So, investigations were taken up, addressed to recognize E mechanisms of protection for brain after ischemia.

Cerebral ischemia triggers a complex series of events including excitotoxicity, edema formation, apoptosis, and necrosis. An early event evoked by brain ischemia is also cerebrovascular inflammation *(155–157)*. The inflammatory response to brain ischemia involves the recruitment of polymorphonuclear leukocytes and mononuclear phagocytes (macrophages, microglia), and astrogliosis. Cerebrovascular inflammation is believed to contribute to secondary brain damage by promoting blood–brain barrier injury, hyperemia, vasogenic edema, and increased intracranial pressure. Important regulators of inflammation are proinflammatory cytokines.

Pro-inflammatory Cytokines in Stroke Pathology. Strong evidence for a causal role of PC in the clinical course of ischemic stroke (IS) is derived from experimental and clinical studies. Upregulation of PC occurs rapidly in the brain following ischemia *(158–162)*. Injections of PC [IL-1, tumor necrosis factor (TNF), IL-6] resulted in increased ischemic infarct size, more intensive leukocyte recruitment, enhanced apoptosis of neurons, increased neurological deficits, and higher mortality rate in experimental stroke models *(163,164)*. Blocking PC activity by anti-cytokine monoclonal antibodies or soluble forms of PC receptors diminished post-ischemic injury *(165–172)*. In clinical studies, the in-

creased PC (IL-1β, TNF, IL-6, IL-8) concentration in cerebrospinal fluid and serum of IS patients was associated with poorer progression and prognosis of the disease *(173–178)*.

Estrogen–Cytokines–IS Course and Prognosis. Very few studies were published focused on the issue of E and cytokine responses during IS. It has been reported that in normal cycling female rats, lower levels of circulating E were accompanied by more severe post-ischemic changes *(179)*. The same investigators documented that in female rats undergoing transient focal cerebral ischemia, low levels of circulating E were accompanied by higher expression of TNF-alpha mRNA in ischemic cortex. This findings suggest that the presence of circulating E might attenuate ischemia-induced TNF expression and diminish potentially neurotoxic effects of this cytokine.

Results of other study suggest that E could exert protective effect in cerebral ischemia not only by reducing PC synthesis, but also by modulating adverse cytokine effects. Chronic in vivo 17β-estradiol treatment of ovariectomized rats prevented dramatic decrease in vascular tone induced by injection of IL-1β *(180)*. This effect was not mediated through the influence on metabolism of circulating IL-1β (serum levels of IL-1β were not changed by E treatment), but was mediated by attenuation of activation of nuclear factor-κB–dependent activation of COX-2 transcription by IL-1β. Taking these results together with previous observations of substantial reduction in infarct volume and neurological deficits after MCAO by selective COX-2 inhibitors *(181)*, it may be hypothesized that suppression of cerebrovascular COX-2 pathway activation, stimulated with PC, can be one of the mechanisms by which E exerts neuroprotective action in cerebral ischemia.

Until now no clinical study had been published that establishes a potential association among gender, cytokines, and the course of ischemic stroke. We analyzed TNF and IL-10 mRNA levels in peripheral blood mononuclear cells and serum concentration of these cytokines at the first 12 and 24 h, and at the seventh day after IS onset. We observed no gender difference in mRNA expression for TNF-alpha and IL-10, and in serum concentrations of these cytokines. However, it has to be mentioned that the mean age of women included in this study was 73 ± 13 yr and most of them were postmenopausal.

We also tried to analyze if gender differences exist in the effect of functionally significant polymorphisms in genes encoding PC on the clinical course of ischemic stroke. We genotyped the following polymorphisms: variable number tandem repeats (VNTR) IL-1RN polymorphism, IL1-β C-511T polymorphism, IL1-α G+4845T polymorphism, IL-6 and G-174C polymorphism. We did not note gender differences in the effect of polymorphisms in the genes for IL-1 receptor antagonist (IL-1ra), IL-1β, and IL-1α on 30-d stroke mortality and disability. However, we detected gender-specific effect of G-174C polymorphism in the gene for IL-6 on early outcome in stroke. It has been established that allele C at position –174 of the *IL-6* gene creates a binding site for the transcription factor NF-1 that acts as a repressor of gene expression *(182)*. Genotype homozygotic for allele –174C was associated with decreased production of IL-6 by blood mononuclear cells *(183)*. In our study, patients homozygotic for the *IL-6*–174G allele had significantly poorer functional outcome following IS, as measured using the Barthel Stroke Scale and Oxford Handicap Scale (Rankin Scale), as compared with carriers of the *IL-6*–174G/C and *IL-6*–174C/C genotypes *(184)*. However, this observation was restricted to the male population. Such sex-dependent effects of the *IL-6*–174C allele may be partially explained by a recent study demonstrating that this allele was associated with diminished serum IL-6 concentrations in male, but not in female population *(185)*.

Concluding, studies that have addressed the action of estrogen on proinflammatory cytokine production during cerebral ischemia are very limited. As in the case of atherosclerosis, existing results preclude a definitive conclusion on the relevance of estrogen modulating effects on cytokines synthesis during ischemic stroke.

Conclusion

Although naturally occurring sexual dimorphism in the prevalence of numerous traumatic or chronic neurological disorders associated with inflammatory response are well recognized, the precise mechanism by which sex hormones influence differences between men and women remains unclear and needs further study. These studies will be required to determine, for example, whether elevation in estrogen is beneficial or deleterious to the immune system and if observed sex-hormone modulation of cytokine production could be responsible for the gender differences in immune responses under pathological conditions. Taken together, these observations suggest that sex-hormonal regulation of immune processes in neuropathological conditions is very complex and that in the future gender-based therapies may not be excluded.

References

1. Benveniste, E. N., Nguyen, V. T., and O'Keefe, G. M. (2001). *Neurochem. Int.* **39,** 381–391.
2. Kohutnicka, M., Lewandowska, E., Kurkowska-Jastrzębska, I., Czlonkowski, A., and Członkowska, A. (1999). *Immunopharmacol.* **39,** 167–180.
3. De Simoni, M. G., Milia, P., Barba, M., De Luigi, A., Parnetti, L., and Gallai, V. (2002). *Clin. Exp. Hypertens.* **24,** 535–542.
4. Kurkowska-Jastrzębska, I., Wronska, A., Kohutnicka, M., Czlonkowski, A., and Członkowska, A. (1999). *Exp. Neurol.* **156,** 50–61.
5. Collawn, J. F. and Benveniste, E. N. (1999). *Microbes Infect.* **11,** 893–902.
6. Mrak, R. E. and Griffin, W. S. (2005). *Neurobiol. Aging* **26,** 349–354.

7. Moore, A. H. and O'Banion, M. K. (2002). *Adv. Drug Deliv. Rev.* **54,** 1627–1656.
8. Bodles, A. M. and Barger, S. W. (2004). *Trends Neurosci.* **27,** 621–626.
9. Quan, N. and Herkenham, M. (2002). *Histol. Histopathol.* **17,** 273–288.
10. Szelenyi, J. (2001). *Brain Res. Bull.* **54,** 329–338.
11. Perry, V. H. (2004). *Brain Behav. Immun.* **18,** 407–413.
12. Marklund, N., Keck, C., Hoover, R., et al. (2005). *Restor. Neurol. Neurosci.* **23,** 31–42.
13. Hailer, N. P., Vogt, C., Korf, H. W., and Dehghani, F. (2005). *Eur. J. Neurosci.* **21,** 2347–2360.
14. Gomes-Leal, W., Corkill, D. J., Freire, M. A., Picanco-Diniz, C. W., and Perry, V. H. (2004). *Exp. Neurol.* **190,** 456–467.
15. Craft, J. M., Watterson, D. M., Hirsch, E., and Van Eldik, L. J. (2005). *J. Neuroinflammation* **2,** 15.
16. Marquardt, L., Ruf, A., Mansmann, U., et al. (2005). *J. Neurol. Sci.* **15,** 65–71.
17. Grammas, P. and Ovase, R. (2001). *Neurobiol. Aging* **22,** 837–842.
18. Hirsch, E. C., Hunot, S., and Hartmann, A. (2005). *Parkinsonism Relat. Disord.* **11(Suppl. 1),** S9–S15.
19. Silberberg, D. (2001). *Mult. Scler.* **7,** 1.
20. Al-Shammri, S., Rawoot, P., Azizieh, F., et al. (2004). *J. Neurol. Sci.* **15,** 21–27.
21. Barbeito, L. H., Pehar, M., Cassina, P., et al. (2004). *Brain Res. Brain Res. Rev.* **47,** 263–274.
22. Kurkowska-Jastrzębska, I., Litwin, T., Joniec, I., et al. (2004). *Int. Immunopharmacol.* **4,** 1307–1318.
23. Fahrig, T., Gerlach, I., and Horvath, E. (2005). *Mol. Pharmacol.* **67,** 1544–1555.
24. Gao, H. M., Liu, B., Zhang, W., and Hong, J. S. (2003). *Trends Pharmacol. Sci.* **24,** 395–401.
25. Youdim, M. B. and Buccafusco, J. J. (2005). *J. Neural. Transm.* **112,** 519–537.
26. Lei, D. L., Long, J. M., Hengemihle, J., et al. (2003). *Neuroscience* **121,** 659–666.
27. Ospina, J. A., Brevig, H. N., Krause, D. N., and Duckles, S. P. (2003). *Am. J. Physiol. Heart Circ. Physiol.* **286,** H2010–2019.
28. Baker, A. E., Brautigam, V. M., and Watters, J. J. (2004). *Endocrinology* **145,** 5021–5032.
29. Mor, G., Nilsen, J., Horvath, T., et al. (1999). *J. Neurobiol.* **40,** 484–496.
30. Wooten, G. F., Currie, L. J., Bovbjerg, V. E., Lee, J. K., and Patrie, J. (2004). *J. Neurol. Neurosurg. Psychiatry* **75,** 637–639.
31. Beeson, P. B. (1994). *Am. J. Med.* **96,** 457–462.
32. Hurn, P. and Brass, L. (2003). *Stroke* **34,** 338–343.
33. Dluzen, D. E. and McDermott, J. L. (2000). *J. Gend. Specif. Med.* **3,** 36–42.
34. Nordell, V. L., Scarborough, M. M., Buchanan, A. K., and Sohrabji, F. (2003). *Neurobiol. Aging* **24,** 733–743.
35. Mikkola, T. S. and St Clair, R. W. (2002). *Maturitas* **41,** 313–319.
36. Suuronen, T., Nuutinen, T., Huuskonen, J., Ojala, J., Thornell, A., and Salminen, A. (2005). *Inflamm. Res.* **54,** 194–203.
37. Sato, F., Ouchi, Y., Masuyama, A., et al. (1993). *Calcif. Tissue Int.* **53,** 111–116.
38. Bismar, H., Diel, I., Ziegler, R., and Pfeilschifter, J. (1995). *J. Clin. Endocrinol. Metab.* **80,** 3351–3355.
39. Cantatore, F. P., Loverro, G., Ingrosso, A. M., Lacanna, R., Sassanelli, E., and Selvaggi, L. (1995). *Clin. Rheumatol.* **14,** 157–160.
40. Liao, S. L., Chen, W. Y., and Chen, C. J. (2002). *Neurosci. Lett.* **330,** 159–162.
41. Dimayuga, F. O., Reed, J. L., Carnero, G. A., et al. (2005). *J. Neuroimmunol.* **161,** 123–136.
42. Danel, L., Souweine, G., Monier, J. C., and Saez, S. (1983). *J. Steroid. Biochem.* **18,** 559–563.
43. Vegeto, E., Belcredito, S., Etteri, S., et al. (2003). *Proc. Natl. Acad. Sci. USA* **100,** 9614–9619.
44. Liu, X., Fan, X. L., Zhao, Y., et al. (2005). *J. Neurosci. Res.* **81,** 653–665.
45. Hall, J. M. and McDonnell, D. P. (1999). *Endocrinology* **140,** 5566–5578.
46. Beato, M. and Klug, J. (2000). *Hum. Reprod. Update* **6,** 225–236.
47. Pottratz, S. T., Bellido, T., Mocharla, H., Crabb, D., and Manolagas, S. C. (1994). *J. Clin. Invest.* **93,** 944–950.
48. Fox, H. S., Bond, B. L., and Parslow, T. G. (1991). *J. Immunol.* **146,** 4362–4367.
49. Srivastava, S., Weitzmann, M. N., Cenci, S., Ross, F. P., Adler, S., and Pacifici, R. (1999). *J. Clin. Invest.* **104,** 503–513.
50. Mishra, J. P., Mishra, S., Gee, K., and Kumar, A. (2005). *J. Biol. Chem.* **280,** 26825–26837.
51. Scott, B. B., Zaratin, P. F., Gilmartin, A. G., et al. (2005). *Biochem. Biophys. Res. Commun.* **328,** 409–414.
52. An, J., Ribeiro, R. C., Webb, P., Gustafsson, J. A., Kushner, P. J., and Baxter, J. D. (1999). *Proc. Natl. Acad. Sci. USA* **96,** 15161–15166.
53. Galien, R. and Garcia, T. (1997). *Nucleic Acids Res.* **25,** 2424–2429.
54. Ghosh, S., May, M. J., and Kopp, E. B. (1998). *Annu. Rev. Immunol.* **16,** 225–260.
55. Boyce, B. F., Xing, L., Franzoso, G., and Siebenlist, U. (1999). *Bone* **25,** 137–139.
56. Wen, Y., Yang, S., Liu, R., et al. (2004). *Brain Res.* **1008,** 147–154.
57. Kalaitzidis, D. and Gilmore, T. D. (2005). *Trends Endocrinol. Metab.* **16,** 46–52.
58. Quaedackers, M. E., Van Den Brink, C. E., Wissink, S., and Schreurs, R. H. (2001). *Endocrinology* **142,** 1156–1166.
59. Sun, W. H., Keller, E. T., Stebler, B. S., and Ershler, W. B. (1998). *Biochem. Biophys. Res. Commun.* **244,** 691–695.
60. Waters, K. M., Rickard, D. J., Riggs, B. L., et al. (2001). *J. Cell Biochem.* **83,** 448–462.
61. Feldmann, M., Brennan, F. M., and Maini, R. (1996). *Cell* **85,** 307–310.
62. Sunyer, T., Lewis, J., Collin-Osdoby, P., and Osdoby, P. (1999). *J. Clin. Invest.* **103,** 1409–1418.
63. Ye, K., Dinarello, C. A., and Clark, B. D. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 2295–2299.
64. Sims, J. E., Painter, S. L., and Gow, I. R. (1995). *Cytokine* **7,** 483–490.
65. Zhang, Z., Maier, B., Santen, R. J., and Song, R. X. (2002). *Biochem. Biophys. Res. Commun.* **294,** 926–933.
66. Bruce-Keller, A. J., Keeling, J. L., Keller, J. N., Huang, F. F., Camondola, S., and Mattson, M. P. (2000). *Endocrinology* **141,** 3646–3656.
67. Marsden, C. D. and Olanow, C. W. (1998). *Ann. Neurol.* **44,** S189–196.
68. Currie, L. J., Harrison, M. B., Trugman, J. M., Bennett, J. P., and Wooten, G. F. (2004). *Arch. Neurol.* **61,** 886–888.
69. Sandyk, R. (1989). *Int. J. Neurosci.* **45,** 119–122.
70. Saunders-Pullman, R., Gordon-Elliott, J., Parides, M., Fahn, S., Saunders, H. R., and Bressman, S. (1999). *Neurology* **52,** 1417–1421.
71. Ragonese, P., D'Amelio, M., Salemi, G., et al. (2004). *Neurology* **62,** 2010–2014.
72. Brooks, W. J., Jarvis, M. F., and Wagner, G. C. (1989). *Res. Commmmun. Subst. Abuse* **10,** 181–184.
73. Dluzen, D. E., McDermott, J. L., and Liu, B. (1996). *Neurotoxicol. Teratol.* **18,** 603–606.
74. Dluzen, D. E., McDermott, J. L., and Liu, B. (1996). *J. Neurochem.* **66,** 658–666.

75. Nagatsu, T. (2002). *J. Neural. Transm.* **109,** 731–745.
76. Nagatsu, T., Mogi, M., Ichinose, H., and Togari, A. (2000). *J. Neural. Transm. Suppl.* **58,** 143–151.
77. Muller, T., Blum-Degen, D., Przuntek, H., and Kuhn, W. (1998). *Acta Neurol. Scand.* **98,** 142–144.
78. Hunot, S., Dugas, N., Faucheux, B., et al. (1999). *J. Neurosci.* **19,** 3440–3447.
79. Chang, J. Y., Martin, D. P., and Johnson, E. M. (1990). *J. Neurochem.* **55,** 436–445.
80. Hammarberg, H., Lidman, O., Lundberg, C., et al. (2000). *J. Neurosci.* **20,** 5283–5291.
81. Wandinger, K. P., Hagenah, J. M., Kluter, H., Rothermundt, M., Peters, M., and Vieregge, P. (1999). *J. Neuroimmunol.* **98,** 214–220.
82. Mizuta, I., Nishimura, M., Mizuta, E., Yamasaki, S., Ohta, M., and Kuno, S. (2001). *J. Neurol. Neurosurg. Psychiatry* **71,** 818–819.
83. Ciesielska, A., Joniec, I., Przybyłkowski, A., et al. (2003). *Acta Neurobiolog. Exp.* **63,** 117–126.
84. Mandel, S., Grunblatt, E., and Youdim, M. (2000). *J. Neural. Transm. Suppl.* **60,** 117–124.
85. Ciesielska, A. and Czlonkowski, A. (2005). *Pharmacol. Rep.* **57,** 4150.
86. Lawrence, C. B., Allan, S. M., and Rothwell, N. J. (1998). *Eur. J. Neurosci.* **10,** 1188–1195.
87. Hebert, G., Arsaut, J., Dantzer, R., and Demotes-Mainard, J. (2003). *Neurosci. Lett.* **349,** 191–195.
88. Bolin, L. M., Strycharska-Orczyk, I., Murray, R., Langston, J. W., and Di Monte, D. (2002). *J. Neurochem.* **83,** 167–175.
89. Hinkerohe, D., Smikalla, D., Haghikia, A., et al. (2005). *Glia* **52,** 85–97.
90. Tang, M. X., Jacobs, D., Stern, Y., et al. (1996). *Lancet* **348,** 429–432.
91. Barness, L. L., Wilson, R. S., Bienias, J. L., Schneider, J. A., Evans, D. A., and Bennett, D. A. (2005). *Arch. Gen. Psychiatry* **62,** 685–691.
92. Kawas, C., Resnick, S., Morrison, A., et al. (1997). *Neurology* **48,** 1517–1521.
93. Doraiswamy, P. M., Bieber, F., Kaiser, L., et al. (1997). *Neurology* **48,** 1511–1517.
94. Henderson, A. S., Easteal, S., Jorm, A. F., et al. (1995). *Lancet* **346,** 1387–1390.
95. Yaffe, K., Sawaya, G., Lieberburg, I., and Grady, D. (1998). *JAMA* **279,** 688–695.
96. Costa, M. M., Reus, V. I., Wolkowitz, O. M., Manfredi, F., and Lieberman, M. (1999). *Biol. Psychiatry* **46,** 182–188.
97. Henderson, V. W., Paganini-Hill, A., Miller, B. L., et al. (2000). *Neurology* **54,** 295–301.
98. Mulnard, R. A., Cotman, C. W., Kawas, C., et al. (2000). *JAMA* **283,** 1007–1015.
99. Craig, M. C., Maki, P. M., and Murphy, D. G. M. (2005). *Lancet Neurol.* **4,** 190–194.
100. Webber, K. M., Casadesus, G., Marlatt, M. W., et al. (2005). *Ann. NY Acad. Sci.* **1052,** 201–209.
101. McGeer, E. G. and McGeer, P. L. (1997). *Mov. Disord.* **12,** 855–860.
102. Mrak, R. E. and Griffinbe, W. S. (2001). *Neurobiol. Aging* **22,** 915–922.
103. Rothwell, N. (2003). *Brain Behav. Immun.* **17,** 152–157.
104. Ciallella, J. R., Ikonomovic, M. D., Paljug, W. R., et al. (2002). *J. Neurotrauma* **19,** 1555–1567.
105. Wang, J., Tanila, H., Puolivali, J., Kadish, I., and Groen, T. (2003). *Neurobiol. Dis.* **14,** 318–327.
106. Morris, F., Shepherd, R., Denys, E., et al. (1993). *J. Neurol. Sci.* **118,** 48–55.
107. Ono, S., Hu, J., Shimizu, N., Imai, T., and Nakagawa, H. (2001). *J. Neurol. Sci.* **187,** 27–34.
108. Sekizawa, T., Openshaw, H., Ohbo, K., Sugamura, K., Itoyama, Y., and Nilan, J. C. (1998). *J. Neurol. Sci.* **154,** 194–199.
109. Hensley, K., Fedynyshyn, J., Ferrell, S., et al. (2003). *Neurobiol. Dis.* **14,** 74–80.
110. Xie, Y., Weydt, P., Howland, D. S., Kliot, M., and Moller, T. (2004). *Neuroreport* **15,** 2513–2516.
111. Weydt, P., Yuen, E. C., Ransom, B. R., and Moller, T. (2004). *Glia* **48,** 179–182.
112. Veldink, J. H., Bar, P. R., Joosten, E. A., Otten, M., Wokke, J. H., and van den Berg, L. H. (2003). *Neuromuscul. Disord.* **13,** 737–743.
113. Trieu, V. N. and Uskun, F. M. (1999). *Biochem. Biophys. Res. Commun.* **258,** 685–688.
114. Nakamizo, T., Urushitani, M., Inoue, R., et al. (2000). *Neuroreport* **11,** 3493–3497.
115. Rudnicki, S. A. (1999). *J. Neurol. Sci.* **169,** 126–127.
116. Runmarker, B., Martinsson, T., Wahlstrom, J., and Andersen, O. (1994). *J. Neurol.* **241,** 385–390.
117. Hensiek, A., Sawcer, S., Feakes, R., et al. (2002). *J. Neurol. Neurosurg.* **72,** 184–187.
118. Hensiek, A., Roxburgh, R., Smilie, B., et al. (2003). *J. Neuroimmunol.* **143,** 25–30.
119. Cedlius, E. G., Harbo, H. F., Egeland, T., Vartal, F., Vandvik, B., and Spurkiand, A. (2000). *J. Neurol. Sci.* **178,** 132–135.
120. Confavreux, C., Hutchinson, M., Heurs, M. M., Cortinivis-Tourniare, P., and Moreau, T. (1998). *N. Engl. J. Med.* **39,** 285–291.
121. Villard-Mackintosh, L. and Vessey, M. P. (1993). *Contraception* **47,** 161–168.
122. Offner, H. (2004). *J. Neurosci. Res.* **78,** 603–624.
123. Dalal, M., Kim, S., and Voskuhl, R. R. (1997). *J. Immunol.* **159,** 3–6.
124. Vranckx, R., Muylle, L., Cole, J., Moldenhaser, R., and Peetermans, M. (1986). *Vox Sang* **50,** 220–222.
125. Amadori, A., Zamarchi, R., De Silvestro, G., et al. (1995). *Nat. Med.* **1,** 1279–1283.
126. Pelfrey, C. M., Cotleur, A. C., Lee, J. C., and Rudick, R. A. (2002). *J. Neuroimmunol.* **130,** 211–223.
127. Wilcoxen, S. C., Kirkman, E., Dowdell, K. C., and Stohlman, S. A. (2000). *J. Immunol.* **164,** 6237–6243.
128. Nguyen, L. T., Ramanathan, M., Weinstock-Guttman, B., Baier, M., Brownscheidle, C., and Jacobs, L. D. (2003). *J. Neurol. Sci.* **209,** 93–99.
129. Mirowska, D., Skierski, J., Paź, A., et al. (2003). *J. Neurol.* **250,** 1229–1236.
130. Ito, A., Bebo, B. F. Jr., Matejuk, A., et al. (2001). *J. Immunol.* **1,** 542–552.
131. Matejuk, A., Adlard, K., Zamora, A., Silverman, M., Vandenbark, A. A., and Offner, H. (2001). *J. Neurosci. Res.* **65,** 529–542.
132. Bonita, R. (1992). *Lancet* **339,** 342–344.
133. 2002 Heart and Stroke Statistical Update, American Heart Association; http://www.americanheart.org/statistics.
134. Niewada, M., Kobayashi, A., Sandercock, P. A., Kaminski, B., and Członkowska, A. (2005). *Neuroepidemiology* **24,** 123–138.
135. Di Carlo, A., Lamassa, M., Baldereschi, M., et al. (2003). *Stroke* **34,** 1114–1119.
136. Murphy, S. J., McCullough, L. D., and Smith, J. M. (2004). *ILAR J.* **45,** 147–159.
137. Sudlow, C. L. M. and Warlow, C. P. (1997). *Stroke* **28,** 491–499.
138. Jerntorp, P. and Berglund, G. (1992). *Stroke* **23,** 357–361.
139. Ricci, S., Celani, M. G., La Rosa, F., et al. (1991). *J. Neurol. Neurosurg. Psychiatry* **54,** 695–698.
140. Sacco, R. L., Wolf, P. A., Kannel, W. B., and McNamara, P. M. (1982). *Stroke* **13,** 290–295.
141. Kannel, W. B., Hjortland, M. C., McNamara, P. M., and Gordon, T. (1976). *Ann. Intern. Med.* **85,** 447–452.

142. Wolf, P. A. (1990). *Stroke* **21(9 Suppl.),** II4–6.
143. Barker, W. H. and Mullooly, J. P. (1997). *Stroke* **28,** 284–290.
144. Mikkola, T. S. and Clarkson, T. B. (2002). *Cardiovasc. Res.* **53,** 605–619.
145. Sader, M. A. and Celermajer, D. S. (2002). *Cardiovasc. Res.* **53,** 597–604.
146. Cusa, C. and Mazzone, A. (2002). *Drugs Today* **38,** 699–712.
147. Young, J. L., Libby, P., and Schonbeck, U. (2002). *Thromb. Haemost.* **88,** 554–567.
148. Chamorro, A. (2004). *Cerebrovasc. Dis.* **17(Suppl. 3),** 1–5.
149. Sukovich, D. A., Kauser, K., Shirley, F. D., DelVecchio, V., Halks-Miller, M., and Rubanyi, G. M. (1998). *Arterioscler. Thromb. Vasc. Biol.* **18,** 1498–1505.
150. Elhage, R., Clamens, S., Besnard, S., et al. (2001). *Atherosclerosis* **156,** 315–320.
151. Whitman, S. C., Ravisankar, P., and Daugherty, A. (2002). *J. Interferon Cytokine Res.* **22,** 661–670.
152. Ayala, C., Croft, J. B., Greenlund, K. J., et al. (2002). *Stroke* **33,** 1197–1201.
153. Glader, E. L., Stegmayr, B., Norrving, B., et al. (2003). *Stroke* **34,** 1970–1975.
154. Wyller, T. B., Sodring, K. M., Sveen, U., Ljunggren, A. E., and Bautz-Holter, E. (1997). *Clin. Rehabil.* **11,** 171–179.
155. Iadecola, C. and Alexander, M. (2001). *Curr. Opin. Neurol.* **14,** 89–94.
156. del Zoppo, G., Ginis, I., Hallenbeck, J. M., Iadecola, C., Wang, X., and Feuerstein, G. Z. (2000). *Brain Pathol.* **10,** 92–112.
157. Zheng, Z. and Yenari, M. A. (2004). *Neurol. Res.* **26,** 884–892.
158. Zhai, Q. H., Futrell, N., and Chen, F. J. (1997). *J. Neurol. Sci.* **152,** 119–124.
159. Buttini, M., Appel, K., Sauter, A., Gebicke-Haerter, P. J., and Boddeke, H. W. (1996). *Neuroscience* **71,** 1–16.
160. Li, H. L., Kostulas, N., Huang, Y. M., et al. (2001). *J. Neuroimmunol.* **116,** 5–14.
161. Wang, X., Yue, T. L., Young, P. R., Barone, F. C., and Feuerstein, G. Z. (1995). *J. Cereb. Blood Flow. Metab.* **15,** 166–171.
162. Hill, J. K., Gunion-Rinker, L., Kulhanek, D., et al. (1999). *Brain Res.* **820,** 45–54.
163. Barone, F. C., Arvin, B., White, R. F., et al. (1997). *Stroke* **28,** 1233–1244.
164. Yamasaki, Y., Matsuura, N., Shozuhara, H., Onodera, H., Itoyama, Y., and Kogure, K. (1995). *Stroke* **26,** 676–681.
165. Mulcahy, N. J., Ross, J., and Rothwell, N. J. (2003). *Br. J. Pharmacol.* **140,** 471–476.
166. Relton, J. K., Martin, D., Thompson, R. C., and Russel, D. A. (1996). *Exp. Neurol.* **138,** 206–213.
167. Nawashiro, H., Martin, D., and Hallenbeck, J. M. (1997). *J. Cereb. Blood Flow. Metab.* **17,** 229–232.
168. Martin-Villalba, A., Hahne, M., Kleber, S., et al. (2001). *Cell. Death Differ.* **8,** 679–686.
169. Yang, G. Y., Gong, C., Qin, Z., Ye, W., Mao, Y., and Bertz, A. L. (1998). *Neuroreport* **9,** 2131–2134.
170. Dawson, D. A., Martin, D., and Hallenbeck, J. M. (1996). *Neurosci. Lett.* **218,** 41–44.
171. Loddick, S. A. and Rothwell, N. J. (1996). *J. Cereb. Blood. Flow Metab.* **16,** 932–940.
172. Martin, D., Chinookoswong, N., and Miller, G. (1994). *Exp. Neurol.* **130,** 362–367.
173. Vila, N., Castillo, J., Davalos, A., and Chamorro, A. (2000). *Stroke* **31,** 2325–2329.
174. Vila, N., Reverter, J. C., Yague, J., and Chamorro, A. (2000). *J. Interferon. Cytokine Res.* **20,** 325–329.
175. Zaremba, J. and Losy, J. (2002). *Acta Neurol. Scand.* **104,** 288–295.
176. Kim, H. M., Shin, H. Y., Jeong, H. J., et al. (2000). *J. Mol. Neurosci.* **14,** 191–196.
177. Grau, A. J., Reis, A., and Buggle, F. (2001). *J. Neurol. Sci.* **15,** 41–47.
178. Smith, C. J., Emsley, H. C., Gavin, C. M., et al. (2004). *BMC Neurol.* **4,** 2–9.
179. Su-Lan, L., Wen-Yin, Ch., and Chun-Jung, Ch. (2002). *Neurosci. Lett.* **330,** 159–162.
180. Ospina, J. A., Brevig, H. N., Krause, D. N., and Duckles, S. P. (2004). *Am. J. Physiol. Heart Circ. Physiol.* **286,** H2010–H2019.
181. Nogawa, S., Zhang, F., Ross, M. E., and Iadecola, C. (1997). *J. Neurosci.* **17,** 2746–2755.
182. Terry, C. F., Loukaci, V., and Green, F. R. (2000). *J. Biol. Chem.* **275,** 18138–18144.
183. Olivieri, F., Bonafe, M., Cavallone, L., et al. (2002). *Exp. Gerontol.* **37,** 309–314.
184. Gromadzka, G., Sarzyńska-Dlugosz, I., Ciesielska, A., and Członkowska, A. (2002). *Eur. J. Neurol.* **9(Suppl. 2),** 25.
185. Bonafe, M., Olivieri, F., Cavallone, L., et al. (2001). *Eur. J. Immunol.* **31,** 2357–2361.