CHAPTER II

Literature Reviews

Microglial Activation is a double-edged sword.

Resident microglia are ubiquitously distributed in non-overlapping territories in the brain. In the early stage of brain development, microglia show a macrophage-like morphology with a relatively large cell body and short processes and are generally known as "ameboid microglia"(Ling ,1979). Amoeboid microglia have been proposed to play a role in sustaining normal growth pattern in the developing CNS. The number of neurons produced in a mammalian brain far exceeds what is necessary and the majority of these neurons undergo programmed death. These amoeboid cells actively participate in cleaning the debris formed by the dying neurons. As the brain develops, ameboid microglia tramsform into ramified microglia, which have smaller cells bodies with long, branched processes. Ramified microglia are thought of being functionally inactive or in a resting state (Nakajima et al., 2001).

The basic function of microglia in the CNS is to maintain tissue integrity under all circumstances. When the tissue injury is mild, and the damage is subtle and not necessarily resulting in cell death, microglia play a protective role. Along with astrocytes, activated microglia isolate the intact cells from the lesion and support regenerative processes (Aldskogius, 2001). They are known to express and secrete growth factors that act on neurons and other glial cells. Included in this list are NGF, BDNF and NT-4/5 and their expression levels could be elevated by inflammatory stimulus as LPS (Miwa et al., 1997; Elkabes et al., 1996).

Activation of microglia appears to be a double-edged sword. Activated microglia are found to be performing cytotoxic as well as protective functions under

various pathological condition. When tissue is severely damaged and the neural tissue is dying, microglia act as scavengers and remove the tissue debris, including the bacterial or viral invaders. The cells in these situations are strongly cytotoxic by secreting free radicals, NO, and proteases that help in scavenger activity (Streit, 1988). An interesting point is whether activated microglia somehow kill neurons by producing cytotoxic molecules. As suggested by their resemblance to tissue macrophages, activated microglia in culture have been shown to produce several potentially cytotoxic molecules, including superoxide anion (Colton and Gilbert, 1987), nitric oxide (Boje and Arora, 1992) and proinflammatory cytokines. Lipopolysaccharide (LPS), interferon g (INF-γ), and β-amyloid (McDonald et al., 1997) are among the stimulators for the production of harmful factors from microglia. Reactive oxygen species (ROS) including superoxide anions, hydroxy radicals, and hydrogen peroxide are generally hazardous, particularly to myelin and its forming cells, oligodendrocytes, owing to their capability of inducing lipid peroxidation. LPS and phorbol-12-myristate-13-acetate (PMA) are stimulators of ROS production from cultured microglia. Nitrogen oxides such as NO˙ are highly reactive free radicals, of which nitrite peroxide is the strongest species. These radicals are believed to inhibit respiratory enzymes, oxidize the SH group of proteins, and enhance DNA injury, finally resulting in neuronal cell death (Nakajima and Kohsaka, 2001). In the presence of INF-γ, β-amyloid synergistically stimulates the production of NO and TNF-α in microglia (Meda et al., 1995). The targets of TNF-α in the CNS have been reported to be oligodendrocytes and myelin due to their susceptibility to this factor *in vitro*. Some neurons have also been reported to undergo apoptosis by treatment with this factor (Nakajima and Kohsaka, 2001). The producers of TNF-α in the CNS are microglia as well as astrocytes. Although activated microglia-derived TNF-α has been suspected to cause inflammation in MS and AIDS dementia, recent evaluation of TNF-α function by gene knockout has showed that TNF-α is not essential for the initiation and/or progression of inflammation (Liu et al., 1998), suggesting that TNF-α is not

necessarily neurotoxic. Additionally, microglia-derived eicosanoids, vasoactive histamine or an excitotoxic glutamate may possibly promote the degenerative processes and inflammation. It can be imagined that neurons in pathological states can be severely damaged if acted upon cooperatively by potentially cytotoxic molecules.

Microglial activation in acute brain injury and chronic brain inflammation

In acute injury such as axotomy of the facial nerve, microglia in the facial nucleus begin to change morphologically just after transection and proliferate 2-3 days later. Subsequently, the activated microglia increase in number over a period of 1-2 weeks and surround injured motoneuron cell bodies. After the persistent activation, they gradually regenerate and return to the morphology and cell density of the ramified type. The state of activated microglia in the axotomized facial nucleus is divided into two, based on phagocytic properties (Streit et al., 1988; Graeber et al., 1998). One state is activated ($OX42^+$, $Iba1^+$) but non-phagocytic ($ED1^-$). The other is activated ($OX42^-$, $Iba1^-$) and phagocytic ($ED1^-$). Whether or not they express antigen for ED1 antibody depends on the presence or absence of dying cells in the milieu. This activated microglia are derived from resident ramified microglia, because facial nerve transection at the stylomastoid foramaen does not injure the BBB of the axotomized facial nucleus and no peripheral monocyte-lineage cells infiltrate into the brain parenchyma.

Similarly, in case of chronic inflammatory diseases such as Alzheimer's disease, multiple sclerosis (MS) and acquired immunodeficiency syndrome (AIDS), microglia/macrophages in the activated state are observed in the affected sites. However, it is difficult to observe the responses of only resident microglia, because blood-derived cells including monocytes and macrophages infiltrate into the brain parenchyama, and these infiltrated cells and resident microglia can not be distinguished from each other due to their shared immunological markers. Presumably,

resident microglia as well as the infiltrated cells are activated in these chronic diseases and act as immune and/or inflammatory cells (Nakajima and Kohsaka, 2001).

Brain iron and Activated microglia

Although, iron is essential for normal brain growth and development, because it forms an important component of hemoproteins and enzymes involved in neuronal oxidative metabolism, neurotransmitter synthesis, and myelin synthesis (Dallman et al., 1986), too much iron may have even more devastating effects. This is due to the ability of iron to catalyze the generation of free radicals in biological systems is well known (Yagi et al., 1992). In general, iron is tightly complex with proteins, but can also be present in a soluble "pool" of low molecular weight complexes, such as ferric citrate and Fe (III) ATP (Weaver and Pollack, 1989). This latter soluble pool of iron reacts with hydrogen peroxide and superoxide, both normally produced in biological systems, to form hydroxyl radicals (Floyd, 1990). Hydroxyl radicals are extremely reactive causing lipid peroxidation, DNA strand breaks and degradation of biomolecules (Halliwell et al., 1984) and are suspected to play a major factor in AD (Sayre et al., 1997; Smith et al., 1997; Smith and Perry et al., 1995) and other CNS disorders (Floyd, 1990; Hall et al., 1993; Halliwell et al., 1992; Sayre and Perry, 1999). As a result, the distributions of iron binding and iron transport proteins have been extensively examined in AD brain tissues (Burdo et al., 2003; Connor et al., 1992a; Connor et al., 1992b).

Mostly studies concerning microglial activation have not been focused on cellular iron content of microglia. As seen in many neurodegenerative diseases such as AD, Parkinson's disease, AIDS-related dementia, multiple sclerosis (MS) and stroke, microglial activation is progressive and generally accompanied by intracellular iron accumulation of iron (Craelius et al., 1982; Levine et al., 1989; Kaneko et al; 1997). Previous studies indicated that accumulation of iron in microglia affected the

production of nitric oxide (NO), tumor necrosis factor (TNF), as well as the expression of a family of zinc-dependent endopeptidase known as matrix metalloproteinases (Cheepsunthorn et al., 2001b). Recent study shows that activity of brain MMPs can be modified by intracellular iron status (Cheepsunthorn et al., in preparation). First, the amount of MMP-9 secreted by activated microglia is increased, if the cells are loaded with iron. This finding is consistent with the observation that elevated levels of MMP-9 is localized predominantly in iron-rich amyloid plaques (Backstrom et al., 1996), as well as in the plasma of patients with Alzheimer's disease (Lorenzl et al., 2003). Therefore, little is known what role of iron plays in microglial activation.

Inhibition of Microglia activation

Microglia activation occurs in all diseases of the CNS. Because of their ability to react promptly to any kind of insults to the CNS, microglia have been described as the sensor of pathology (Giuliani et al., 2005). It is currently viewed that chronic stimulation of microglia may play an important role in the progressive neuronal death that occurs in neurodegenerative diseases such as Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease. Many stimuli can induce microglia activation, and these include viruses or bacteria when they invade the CNS. Lipopolysaccharide (LPS), a component of the wall of gram-negative bacteria, is a prominent stimulator of miceoglia activation through CD14 and Toll-like receptor interaction. LPS binding to the Toll-like receptor 4 (TLR4) of microglia activate a complex array of intracellular signaling pathways involving tyrosine kinases, mitogen-activated protein (MAP) kinases, and nuclear factor (NF)-κB mediated cytokine gene expression (Aderem et al., 2000). Mitogen-activated protein kinases (MAPKs) are among the most important molecules in the signaling pathways that control the synthesis and release of neurotoxic substances by activated microglia (Ozato et al., 2002). It is reported that MAPKs regulate the production of cytokines IL-1β, TNF-α

and reactive oxygen species (ROS) in LPS- or Aβ-activated microglia. (Koistinaho et al., 2002; Combs et al., 2001; Bhat et al., 1998; Garcia-Pineres et al., 2001; McDonald et al., 1997; Pyo et al., 1998). p38 MAPK has been implicated in the signal transduction pathways responsible for increased iNOS and TNF-α gene expression in glial cells (Bhat et al., 1998; Silva et al., 1997; Won et al., 2001; Xu et al., 2000). Inhibition of microglial activation is a promising target in the treatment of neurodegeneration. Not only protein kinase C (PKC), NF-κB (Fiebich et al., 1998) and p38 MAPK, but also p42/44 MAPK has shown to be involved in the LPS-induced signal transduction, leading to iNOS in microglia (Bhat et al., 1998) and the microglial related mononuclear cells (Chan et al., 2001). It has been further described in iNOS promoter studies that the activation of p42/44 leads to an activation of the AP-1 transcription factor complex (Kristof et al., 2001) and, therefore, plays a crucial role in iNOS signal transduction induced by LPS. Therefore, p42/44 MAPK is an interesting target to be inhibited by pharmacological compounds leading to a reduced iNOS and NO release in microglia and other cells. Several studies have suggested that minocycline, a second-generation tetracycline antibiotic that has recently been found to have numerous immonodulatory activities, prevent microglial activation and is neuroprotective in model of cerebral ischemia, Parkinson's disease, traumatic brain injury and glutamate-induced nerotoxicity (Ledeboer et al., 2005). In vitro study, the protective effect of minocycline was associated with reduced activation of iNOS and IL-1β-converting enzyme, which are mainly express in microglia (Ledeboer et al., 2005). Minocycline inhibited the NMDA-induced activation of p38 mitogen activated protein kinase (MAPK) in microglia cells and a specific p38 MAPK inhibitor but not a p40/42 MAPK inhibitor reduced the NMDA toxicity. Furthermore, minocycline can reduce the production of chemokines (MIP-1α) and chemokine receptors (CCR5, CXCR3) in response to LPS (Kremlrv et al., 2004).

Neuroprotective Effect of Estrogen

Recently, several studies demonstrated the neroprotective effects of estrogen in CNS and an anti-apoptotic effect of 17β-estradiol (E_2) in neural cells (Behl et al., 1995; Goodman et al., 1996; Green et al., 1996; McMillian et al., 1996; Toran-Allerand et al., 1996; Behl et al., 1997; Hashimoto et al., 1997; Garcia-Segura et al., 1998; Xu et al., 1998; Pike et al., 1999). The neuroprotective action has been claimed to be independent of genomic, receptor-mediated activity. Behl's and other groups showed that in immortalized hippocampal cells not expressing the ER, or in dissociated embryo neurons, E_2 protects from glutamate excitotoxicity, oxidative stress, or β-amyloid at a concentration of 100 nM–10 mM (Behl et al., 1995; Goodman et al., 1996; Regan et al., 1997). Several authors have suggested that the protective effect of E_2 is exerted via modulation of cytoplasmic transduction signals induced via nongenomic paths (Hashimoto et al., 1997). Others favor a genomic effect which results in the decreased synthesis of glutamate receptors, augmented expression of neurotrophic factors (McMillian et al., 1996; Toran-Allerand et al., 1996), or anti-apoptotic proteins (Garcia-Segura et al., 1998; Pike et al., 1999). However, other studies showed a lack of protection by low concentrations of E_2 in cells of neural origin deprived of ER (SK-N-BE) and a protection in the same cells stably transfected with ERα. This clearly indicates the involvement of genomic effects in E_2-dependent protection (Meda et al., 2000). The interpretation of protective effects of estrogen via a genomic mechanism is also supported by the observation that estradiol regulates the expression of several genes implicated in the apoptotic process. In particular, E_2 was described as increasing the synthesis of Bcl 2 in MCF-7 cells (Teixeira et al., 1995) and decreasing the expression of a novel pro-apoptotic gene, nip2, in neuroblastoma (Garnier et al., 1997) and in rat embryo neurons in primary culture (Meda et al., 2000).

Recent evidence demonstrates that glial cells (oligodendrocytes and astrocytes) may be a target of gonadal steroids since they express both ERα and β (Jung-Testas et al., 1992; Santagati et al., 1994; Buchanan et al., 2000). Recent studies show that E_2 treatment of resting microglia were isolated from culture of new born rat brains in vitro or in vivo prior to its activation with lipopolysaccharide or other inflammatory stimuli prevents the morphological differentiation associated with activation (Vegeto et al., 2000). In addition, they showed that estrogen treatment prevents the secretion of several inflammatory stimuli, such as metalloproteases (metalloproteinase-9 or MMP-9) and prostaglandins (PGE2), and blocks the LPS-induced increase of iNOS synthesis and activity (Vegeto et al., 2000). These effects seem to be mediated by the ERs because they are blocked by specific ER antagonists. Furthermore, immunocytochemical and RT–PCR studies showed the presence of ERα protein and ERα and ERβ mRNA in primary cultures of 2-day-old rat microglia. These findings pointed a potential anti-inflammatory effect of estrogens in the nervous system. This finding may have important repercussions in the understanding of the beneficial role of estrogens in neurodegenerative diseases.

With regard to the mechanism of action of estrogen on the inflammatory mediators, many studies are suggestive of a receptor-mediated genomic control. Estrogen exerts its activity at concentrations compatible with receptor activation. And estrogen activity is blocked by the receptor antagonist ICI 182,780, and when the time interval between hormone and LPS treatments. Furthermore, ERα and ERβ are expressed in microglia and monocyte-derived macrophages. It is well known that estrogen modulates target cell activity by binding to specific intracellular receptors, which are hormone-regulated transcription factors (Katzenellenbogen et al., 1996; Kuiper et al., 1996). Hormone-activated estrogen receptors (ERs) may modulate gene transcription by direct binding to promoters containing an estrogen responsive element (ERE) or via the interaction with other transcription factors (McKay et al., 1999; Webb

et al., 1999) or unidentified membrane receptor (Gu and Moss, 1996; Norfleet et al., 2000).

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