CHAPTER 1

INTRODUCTION

1.1 INFLAMMATION

Inflammation is the first reaction of the immune response, is a complex response of the host to tissue injury such as infection or physical insult (Nathan 2002). An inflammatory response promotes inactivation and removal of toxic agents and invading microorganisms such as a virus or bacteria, and it allows recovery from physical or hypoxic trauma. Inflammatory response is organized by the interaction of several cell types and signalling molecules producing an inflammatory response that is both local and systemic. Inflammation can also mount in response to threats generated within the organism itself. For example, accumulation of aggregated or abnormally modified proteins, aberrant signals emanating from damaged cells, or imbalances in immune signalling can each generate inflammatory response.

Interestingly, possible outcome of inflammation involves a chronic non-resolving immune response that generates secondary tissue injury (Wyss-Coray & Mucke 2002). This outcome is thought to be a common thread linking a variety of neurodegenerative diseases of the central nervous system (CNS), including Alzheimer’s and Parkinson’s diseases.
1.1.1 Inflammation in the CNS

Neuroinflammation is a defense mechanism the main aim to protect the central nervous system against from bacterial infection and injury. It may be triggered by various factors including immunological challenges (bacterial or viral infections), neuronal injury (brain trauma or stroke), and other factors such as chronic inflammatory syndromes (rheumatoid arthritis, arthrosclerosis, type 2 diabetes, Crohn's disease and multiple sclerosis) and environmental toxins (pesticides, particulate matter, etc.) (Block & Hong 2005). Although the exact molecular and cellular components of neuroinflammation are still unknown, activation of microglia and astrocytes play an important role in neuroinflammation (Bouchard et al 2007).

In the brain, inflammatory responses are driven by a multi-component system which includes microglia, the resident macrophage-like cells, and immune cells infiltrating from the periphery, such as T cells. Non-immune CNS cells such as astrocytes and neurons, can also contribute to and modulate immune responses in the CNS. However, while these cells can also influence the neuroinflammation. Neuroinflammation is a key event, although the cause and significance of this process is not clear (McGeer & McGeer 2002). Inflammatory processes occur in the CNS through mechanisms that differ from systemic inflammation, and with distinct cellular effects. There are multiple aspects of neuroinflammation, all working simultaneously. Following exposure to noxious stimuli, components of neuroinflammation include immune cell proliferation, activation of microglia, release of cytokines, and induction of tissue repair enzymes that together limit cellular damage and regenerate the CNS. However, these same inflammatory mediators are often the primary cause of tissue damage in both acute and chronic CNS pathology. Acute neuroinflammation following stroke or trauma may contribute to the initial extension of the lesion by increasing neuronal
loss in the penumbra, but may also promote subsequent functional recovery by enabling neuronal plasticity. Chronic neuroinflammatory processes are suspected to sustain neuronal loss in a number of pathological conditions such as autoimmune diseases including Alzheimer’s disease and multiple sclerosis.

1.1.2 Specific Aspects of CNS Inflammation

The CNS has protected from immune-mediated inflammation both anatomically and physiologically. The brain has several distinctive features and it can differ from other tissues in response to inflammatory insult. The inflamed brain does not show pain, redness and swelling due to lack of sensory nerve endings and lymphatic vessels. In neuroinflammation, there is little neutrophils recruitment and the major resident inflammatory cells are microglia and astrocytes and it can release many proinflammatory mediators including cytokines, nitrogen and oxygen species.

1.1.2.1 Cells Involved in Neuroinflammation

The endothelial layer present in the brain called as the blood–brain barrier (BBB) and transport of molecules across the BBB across is the key to understand how peripheral inflammation can produce prolonged neuroinflammation. Inflammatory cytokines and other protein-like substance were thought to be too large to enter the brain from blood. BBB active transport systems have been facilitating the delivery of TNF-α and IL –1β into the brain. Circumventricular organs (CVO) are particular areas of concentrated cytokine transport. Peripheral cytokines has direct influence on central nervous system and produce sickness behavior by vagal nerve stimulation and was recently reviewed as translation of peripheral inflammation to neuroinflammation (Fung et al 2012).
1.1.2.2 Glial cell activation

Inflammation is a common player in the brain’s response to injury and pathology. Loss of functional neurons on the integral role of reactive astrocytes and microglia in the initiation and exacerbation of CNS inflammation. Astrocytes, the most abundant cells in the CNS has many functions such as structure and preservation of blood brain barrier (BBB) and maintaining homeostasis of the external environment. Microglia, the resident macrophages of the CNS, identifies and engulfs cellular debris. However, in the presence of an inflammatory stimulus, astrocytes and microglia expression/release a large number of proteins. These secreted products include cytokine and reactive oxygen species. Glial activation plays a vital role of inflammation in neurodegeneration.

Microglia

Microglia is also called the fastest moving cell of the CNS and plays an integral part in the immune defense. These cells account for approximately 20% of the total glial population and around 5-20%of the total cells of the adult CNS (Aguzzi et al 2013). Microglia cells are the first line of defense in the CNS and are the key regulators of neuroinflammation (Smith et al 2012). While microglia activation is necessary and critical for host defense, over-activation of microglial is neurotoxic (McGeer et al 2005). Inflammation and microglial activation is a common component of the pathogenesis for multiple neurodegenerative diseases, including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease, Multiple sclerosis (MS), and Amyotrophic lateral sclerosis (ALS) (Nguyen et al 2002). In the event of an immunogenic stimuli or injury, the microglia is activated and functions similar to a macrophage, which might play a crucial role in the process of neuroinflammation.
Astrocytes

Astrocytes, also collectively known as astroglia, are characteristic star-shaped glial cells in the brain and spinal cord. They are the most abundant cell of the human brain and comprise the other family of glial cells that release pro-inflammatory signalling molecules such as TNF-α when stimulated in the cortex and midbrain (Kipp et al. 2008). It has been shown to serve many housekeeping functions, including maintenance of the extracellular environment and stabilization of cell-cell communications in the CNS.

In the diseased state such as in multiple sclerosis and AD, activated astrocytes are believed to facilitate leukocyte recruitment to the CNS by increasing leukocyte adhesion molecules and chemokine production (Moynagh 2005). Bacterial endotoxin LPS is able to stimulate astrocytes to produce prostaglandins and cytokines. These observations, taken together, indicate that astrocytes play an important role during immunological response.

Pathogen-associated molecular patterns (PAMPs)

The first line of defence against pathogens in mammals is the innate immune system. The innate immunity is non-antigen specific and its main responses are mediated by the glial cells such as microglia and astrocytes, which can identify the pathogens and become activated and lead to inflammation.

The innate immune cells identify the presence of pathogen through the specific molecule known as pathogen-associated molecular patterns from microorganisms such as bacteria, viruses and fungi. Pathogen-associated molecular patterns can be fragment bacterial cell (lipopolysaccharide and peptidoglycan) bacterial and viral DNA fragment (a protein derived from the
bacteria flagella). Toll-like receptor (TLR) is the recognition site for PAMP and its initial step of inflammatory reaction in the innate immune responses.

The main PAMPs derived from Gram negative bacteria will discussed below

**Lipopolysaccharide (LPS) activation**

Lipopolysaccharides are present in almost all gram-negative bacteria, amongst which several important pathogenic species (Escherichia coli, Salmonella ssp, Neisseria menigitidis, Haemophilus influenzae, Klebsiella pneumonias, Chlamydia trachomatis, Helicobacter pylori etc.,) (Caroff et al 2002). LPS, an endotoxin found in the outer membrane of gram-negative bacteria, is a strong inducer of innate immunity. The cell wall of gram negative bacteria is characterised by the presence of two lipid bilayers – outer and inner layer membrane separated by a space containing a network of peptidoglycan. LPS’s effects are mediated through with a member of the toll like receptor (TLR-4). TLRs are widely expressed in glial cells particularly by microglia and astrocytes and minor amount in neurons (Kielian 2006). The TLR family consists of 10 members (TLR1-TLR10). TLR are important signal transduction proteins in the innate immune system and induces systemic inflammatory response syndrome (SIRS) in animals, with human being particularly sensitive (Warren et al 2010). This pathway is commonly used to induce an inflammatory response in animal models (Hoshino et al 1999). The TLR-4 upon activation by LPS triggers a signaling cascade that ultimately induces the transcription of inflammatory cytokines such as TNF-α and IL-6 via NF-κB and oxidative stress activation (Qin et al 2004) as shown in the schematic diagram (Figure 1.1). Finally, LPS induced neuroinflammation contributes to neuronal death as shown in the schematic diagram (Figure 1.1). Inflammatory pathways involving the COX enzymes
and subsequent generation of prostaglandins are the potential target sites for treatments of neuroinflammatory conditions.

**Figure 1.1** Schematic diagram of LPS mediates activation of TLR4 and its signaling cascade in inducing the transcription of inflammatory cytokines

**1.2 OBJECTIVE**

Both COX-1 and COX-2 have been shown to play an important role in the inflammatory response. However, the exact role and preference of COX isoform in neuroinflammation is unclear. The main objective of the study is to explore the effect of selective / non selective COX inhibitors in
preventing the neuroinflammation induced by LPS. The specific objectives of the study are

- To evaluate the effect of selective and non selective COX inhibitors in LPS induced neuroinflammation in rats.
- To evaluate the mode of action of selective and non selective COX inhibitors in LPS induced neuroinflammation model in rats, and measure/ the neurochemical, biochemical and proinflammatory mediator alterations.
- To evaluate the effect of COX inhibitors in LPS induced neuronal damage histopathological analysis is performed.

1.3 RESEARCH OF LITERATURE

1.3.1 Inflammation

Inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen (Abbas & Lichtman et al 2009).

Table 1.1 Inflammation can be classified as either acute or chronic

<table>
<thead>
<tr>
<th>Factors</th>
<th>Acute</th>
<th>Chronic</th>
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<tbody>
<tr>
<td>Causative agent</td>
<td>Bacterial pathogen</td>
<td>Persistent acute inflammation</td>
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<tr>
<td>Major cells involved</td>
<td>Neutrophils and basophils</td>
<td>Mononuclear cells</td>
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<td>Primary mediators</td>
<td>Eicosanoids</td>
<td>IFN-γ and cytokines</td>
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<tr>
<td>Onset</td>
<td>Immediate</td>
<td>Delayed</td>
</tr>
<tr>
<td>Duration</td>
<td>Few days</td>
<td>Years</td>
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<td>Outcomes</td>
<td>Chronic inflammation</td>
<td>Fibrosis, necrosis</td>
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1.3.2 Inflammation in the CNS

Interest in Central Nervous System (CNS) inflammation has grown rapidly over the past decade driven by the increasing evidence for a role of neuroinflammation in the development of several important neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, stroke, traumatic brain injury, demyelinating disorders, as well as pathology associated with CNS infections.

In peripheral tissues inflammation generally has a protective role, limiting the survival and proliferation of invading pathogens, promoting tissue repair and recovery; it can be characterised by the cardinal signs described already by Celsus in the first century A.D., namely calor, rubor, tumor and dolor, i.e. increase in temperature, redness, swelling and pain which result from increased blood flow, increased vascular permeability, fluid accumulation and infiltration of blood-derived mediators of inflammation. In peripheral tissues it is directly associated with adherence and invasion of leukocytes (neutrophils, macrophages, lymphocytes) into injured or infected tissues. This innate, or immunologically non-specific, response normally resolves over a few weeks, with accompanying tissue repair aided by macrophages recruited to the site. If the stimulus is sufficiently great or persistent the inflammatory response may become chronic and is characterised by the presence of large numbers of macrophages and T-lymphocytes, and fibrosis. In the CNS a number of physiological and immunological processes appear to be differentially regulated.

1.3.2.1 Immunocompetent cells in the CNS

The CNS is constantly surveyed by a well developed network of innate immune cells that control all portals of entry for blood derived pathogens into the CNS parenchyma. Macrophages and dendritic cells have
been identified in the meninges and choroid plexus, in strategic locations to guard the ventricular/subarachnoid compartment. Perivascular macrophages, which surround small and medium size cerebral vessels, ensure protection of the CNS at the level of the blood-brain barrier displaying phagocytic and immune regulatory functions (Williams et al 2001). However, if the inflammatory stimulus (e.g. pathogen or its components) crosses the blood-brain barrier, the CNS parenchyma itself contains other cells that vigorously react to any immunological stimuli and to neuronal injury, and play an active role in the development of inflammation the glial cells.

Glial cells are generally classified into two groups: 1) macroglia, which include astrocytes and oligodendrocytes, of ectodermal origin, and 2) microglia, of mesodermal origin, that invade the CNS during embryonic development at the time of vascularization (Raine 1999).

Oligodendrocytes are restricted to the central nervous system; these cells produce a laminated, lipid-rich wrapping called myelin around some axons; myelin plays an important role in the conduction of action potential in neurons (increases the speed of potential propagation).

1.3.2.2 Microglia

The major resident inflammatory cells in the CNS are microglia. These are macrophage like cells, derived from bone marrow stem cells that populate the CNS early during the development and remain within the CNS as the resident macrophage population. Microglia comprise up to 20% of the total non-neuronal cell population (Davis et al 1994). These cells are especially important to guard the integrity and homeostasis of the brain. In normal conditions they are quiescent, but become activated by injury or infection and have been suggested to represent the first line of defence for the CNS, which normally lacks professional antigen presenting cells (APCs) and
intraparenchymal leukocytes, until they are recruited to the CNS by proinflammatory stimuli (Kreutzberg 1996).

Microglia has several morphological forms depending on their functional and developmental state (Figure 1.2). During embryonic development monocytes migrate to the CNS and convert into an intermediate form, amoeboid cells, with flat morphology and pseudopodia. It is a transient population present during the late prenatal to early postnatal period. Microglias present in adult brain are called ramified microglia. These cells have a small (5-10 µm) oval cell body with large nucleus and only a little amount of cytoplasm, as well as numerous long, branched processes. Ramified microglia have been characterised as down regulated (or inactive) macrophages, as they lack most of the characteristic markers and activities of this group (lack of phagocytic and endocytic activity, low expression of leukocyte common antigen (CD45), low levels of membrane ligands and receptors that are essential for mediating or inducing typical macrophage functions) (Davis et al 1994).

One of the most remarkable properties of microglia is to react to a stress signal from the inside (e.g. stressed or damaged cells, cytokines) as well as from outside (e.g. pathogens) and to direct their reaction for the purpose of tissue repair and for further induction of protective immune responses. Following the stimulus (e.g. neuronal injury) microglia migrate to the damaged sites of the CNS where they proliferate and become activated. During this process microglia undergo maturation, leading to the acquisition of macrophage differentiation markers and effector properties. They can assume two distinct forms activated and reactive microglia (Davis et al 1994). Activated microglia appears like swollen ramified cells and are characterised by a larger cell body with shorter processes. Reactive microglia is typically small, spherical cells and lack ramified processes. The different forms of brain microglia are schematically presented in Figure 1.2.
Figure 1.2 Cellular forms of brain microglia (adopted from Davis et al 1994)

The activation of microglia is an important host defence mechanism. Activated microglia release various pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) (Kreutzberg 1996; Aloisi 2001) as well as oxidative and nitrosative free radicals (superoxide, nitric oxide, peroxynitrite). Microglia can produce also anti-inflammatory mediators, such as TGF-β, PGE_2 and IL-1 receptor antagonist (IL-1ra) (Benveniste et al 2001). Due to their ability of phagocytosis microglia play also the role of scavengers for macromolecules and apoptotic or damaged brain cells as well as pathogens.

A constitutive and inducible expression of a variety of other surface receptors is indispensable for microglia to exhibit a proper response to a number of infectious and inflammatory stimuli. The receptors include: 1) pattern recognition receptors (Toll-like receptors, CD14) implicated in the recognition of pathogen associated molecules (such as Gram-positive and Gram-negative bacterial components, viral RNA and proteins, pathogen DNA, etc.); 2) complement receptors (called also opsonic receptors), which mediate or enhance phagocytosis; 3) cytokine and chemokine receptors (for
both pro and anti-inflammatory cytokines); these receptors regulate immune functions of microglia (Aloisi 2001).

Although microglia represents the first line of defence in the brain, the activation of these cells can also have negative effects. The inflammatory mediators released from activated microglia can contribute to CNS damage as neurotoxins (Aloisi 2001), and enhance the onset and progression of CNS diseases. Zhang & Fedoroff (1996) showed that in co-culture with neurons, microglial cells at a low degree of activation supported the neuronal survival, but when this microglia were pretreated with lipopolysaccharide (LPS), a known strong activator of immune cells, intensive neurotoxicity was found. The harmful effects of LPS activated microglia on neurons in vitro were also shown by other authors (Bal-Price & Brown 2001). Therefore, microglial activation can influence the extent of brain injury following an inflammatory stimulus and it is important to control the degree and duration of inflammation in the CNS. Excessive or chronic microglial activation has been implicated in a number of neurodegenerative diseases, such as Alzheimer’s disease (Blasko et al 2004), as well as trauma, ischemia, brain tumours and infectious diseases (Neumann 2003).

1.3.2.3 Astrocytes

Astrocytes make up a substantial proportion of the CNS and participate in a variety of physiological and pathological processes. In the adult, astrocytes constitute about 70% of the total population of brain cells. Their primary function is to provide structural, metabolic and trophic support to other cells (Raine 1999). Astrocytes act as a bridge to supply nutrients from blood capillaries to neurons and provide the major site of glycogen storage in the brain. Moreover, they are also able to synthesise and secrete a variety of neurotrophic and growth factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF) and
insulin-like growth factor-1 (IGF-1), which may be beneficial for neuronal survival (Moretto et al 1994).

Astrocytes are also responsible for maintaining a homeostatic environment in the brain by: 1) “buffering” or clearance of K+ released from electrically active neurons (Walz 2000); 2) detoxification of synaptically released glutamate by uptake and metabolic mechanisms (Bezzi et al 1999); 3) regulation of extracellular ionic gradients and pH (Lascola & Kraig 1997); 4) clearance and metabolism of arachidonic acid (Staub et al 1995).

Astrocytes not only support neuronal survival, they may also modulate neuronal signalling (Chvatal & Sykova 2000). Studies of Murphy et al (1993) showed that there is a direct communication between astrocytes and neurons in cultures of brain cells, probably through gap-junction channels (Froes et al 1999). Moreover, there is growing evidence that astrocytes play an active role in synaptic transmission, not directly forming synaptic contacts, but contributing to the physiological functioning of neurons by the integration of neuronal inputs, exhibition of calcium excitability and modulation of neighbouring neuronal responses (Araque et al 2001).

Apart from being involved in a variety of physiologic processes, astrocytes rapidly react to different neurological insults. A series of changes that occur in astrocytes upon activation has a common name of astrocytosis. The main feature of astrocytosis is the increase in the number and size of glial fibrillary acidic protein (GFAP) expressing cells. GFAP is an intermediate filament cytoskeletal protein expressed primarily by astrocytes and it is considered as the marker of astrocytes (Raine 1999). The precise function of GFAP molecule is still not clear, as well as it has not yet been established whether the increase in GFAP levels is a result of enhanced production of this protein by the cells or an increase in the number of astrocytes either due to proliferation or migration. Studies using double labelling with GFAP
antibodies and bromodeoxyuridine failed to show, at least in acute lesions, mitotic divisions of GFAP expressing cells (Norton et al 1992). Furthermore there is no convincing evidence that GFAP positive cells of adult brain are able to migrate. Therefore it is likely that the appearance of GFAP positive astrocytes in regions of acute neuronal injury is primarily due to a change in phenotype. Astroglial proliferation, however, cannot be excluded in chronic astrocytosis. Reactive astrocytes form a glial scar in areas of tissue necrosis, excluding the non-neuronal cells from parenchyma and filling in the space which results from neuronal loss (McGraw et al 2001). They also produce proteases and protease inhibitors (e.g. matrix metalloproteinases), which allow them to remodel the extracellular matrix at sites of neuronal injury and to clear the debris of degenerating cells (Wu et al 2004).

One of the important functions of activated astrocytes is the involvement in the immune functions in the CNS. Several studies performed both *in vivo* (where the expression of a particular molecule or its mRNA was co-localized to reactive astrocytes) as well as *in vitro* (using primary cultures of astrocytes), have demonstrated that activated astrocytes produce a large variety of molecules, which are involved in the initiation and regulation of the inflammatory response. These include several pro- and anti-inflammatory cytokines (IL-1, TNF-α, IL-6, TGF-α, TGF-β), chemokines (IL-8) and eicosanoids (leukotriens B4 and C4, prostaglandin E, thromboxanes A2 and B2) (Chao et al 1995; De Groot et al 1999; Dong & Benveniste 2001; De Groot & Woodroffe 2001; Xu et al 2003). Activation of astrocytes leads also to the expression of inducible nitric oxide synthase (iNOS) (Brown & Bal-Price 2003). Activated astrocytes express molecules involved in immune responses such as major histocompatibility complex antigens (MHC) and are able to present antigens to T lymphocytes (Cornet et al 2000). Moreover, they up-regulate the expression of several adhesion molecules (selectins, integrins, adherins), which play a role in the migration of leukocytes through the blood-brain barrier into the CNS parenchyma (Dietrich 2002).
Prominent reactive astrocytosis was found in AIDS dementia complex, a variety of viral infections, prion-associated spongiform encephalopaties, inflammatory demyelinating diseases, acute traumatic brain injury, ischemia and neurodegenerative diseases (e.g. Alzheimer’s disease).

Astrocytes are also essential for the morphological and physiological formation of a functional blood brain barrier in the CNS. The concept of the existence of a blood-brain barrier (BBB), which separates the brain from the rest of the body, was developed by Paul Ehlrich in 1906, when in his experiment he has shown that some dyes administered intravenously to rats stain all the organs except the brain. The BBB is a physical barrier between blood vessels in the CNS and the brain tissue that plays an important role in the protection of the CNS. It is formed by non-fenestrated endothelial cells that develop tight junctions among adjacent cells providing an increased resistance to passage of solutes between cells. In vitro studies have demonstrated that endothelial cells alone cannot provide a tight barrier without the presence of astrocytes. Several studies suggest that astrocytes secrete soluble factors essential for the development of specific BBB properties. The presence of a continuous sheet of astrocyte end feet around the capillaries results in a tight interaction between cerebral astrocytes and endothelial cells. This interaction results in a change in morphology and function of the cerebrovascular endothelium, and is especially important for the maturation and differentiation of tight junctions between endothelial cells (Abbott 2002).

The blood-brain barrier limits access of almost all molecules apart the smallest oxygen, carbon dioxide and sugars - which pass with no difficulty. Most drugs and hormones are too large to pass the barrier. In addition, the blood-brain barrier is an excellent way to protect the brain from common infections, as most microbes do not cross the BBB. Also the
components of the immune system (monocytes, macrophages, lymphocytes, antibodies) do not penetrate across the BBB under normal conditions.

The nature of the BBB can vary depending on the location; in certain areas of the brain, usually associated with ventricular organs and areas of endocrine regulation, commonly known as circumventricular system (consisting of area postrema, hypothalamus, pituitary and pineal gland, chorioid plexus), endothelial cells do not form tight junctions. Also some regions of the brain meninges have no functional barrier.

The integrity of the BBB can be also altered in some pathological conditions such as infection (bacterial, viral, parasitic), inflammation, brain injury, tumours and hypertensive encephalopathy, as well as by some drugs and hyperosmotic agents (e.g. mannitol). In these conditions, the increase in BBB permeability may allow access of leukocytes into the brain parenchyma where they can release neurotoxins, activate endogenous inflammatory processes or, in the case of macrophages, phagocytose cell debris.

Figure 1.3 Schematic representation of the blood-brain barrier
The cerebral capillaries lack fenestrations and have a dense basement membrane; endothelial cells form tight junctions between each other; several footplates of astrocytes are tightly opposed to the endothelium (adapted from Francis et al 2003)

1.3.2.4 Neurons

Dying neurons not only promote microgliosis and subsequent inflammation but almost surprisingly neurons themselves are also capable of producing inflammatory mediators such as the complement, TNF-α, IL-1, IL-6, M-CSF (Akiyama et al 2000). It is therefore possible that neurons themselves also promote the inflammatory reaction seen in AD brain, and thus contribute to their own damage and further activation of non neuronal cells. Alternatively release of proinflammatory mediators such as TNF-α may act as defence mechanisms against the glial mediated inflammation.

1.3.3 Evidence for Inflammation in CNS Pathology

The role of neuroinflammation as the potential pathogenic factor in a number of CNS diseases has been recognised only recently. The concept of “neuroinflammation” implies that specific innate immune responses in the brain are mediated mainly by activated microglia and astrocytes, which precedes and causes neuronal degeneration. Before “neuroinflammation” became a commonly used term, neuroscientists recognised “reactive gliosis” as the endogenous CNS tissue response to acute brain injury.

Acute pathological conditions such as cerebral ischemia and traumatic brain injury are characterised by rapid and usually severe insults to the brain, which lead to substantial loss of nerve cells and subsequent functional deficits. Several processes have been implicated in the neuronal damage including increased glutamate release (excitotoxicity), oxidative
stress and disturbances in ionic homeostasis (Chavarria & Alcocer-Varela 2004). There are also data suggesting the active role of inflammatory processes in these diseases - the activation of inflammatory cells (microglia, astrocytes) and increased expression of inflammatory mediators (cytokines, complement).

Although such specific responses in acute brain injury might be included in the term “neuroinflammation”, it is more commonly applied to chronic CNS diseases. Any chronic inflammatory process can damage healthy tissue and the brain may be particularly vulnerable, since neurons are post-mitotic cells and once lost cannot be replaced. There is vast evidence indicating that chronic inflammation in the brain may play an important role in the progressive neuronal cell death in many chronic CNS diseases such as Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis or multiple sclerosis.

1.3.3.1 Alzheimer’s disease

The greatest evidence of the role of inflammation in chronic CNS disorders comes from studies on Alzheimer’s disease (AD). Alzheimer’s disease is the most common neurodegenerative disease - approximately 10% of all people over the age of 65 and as many as 50% of those over the age of 85 are diagnosed with the condition. It is an irreversible, progressive disorder characterised by a loss of neurons predominantly in the cerebral cortex and hippocampus. Evidence for the importance of inflammation in AD comes from two directions. First, extensive immunohistochemical and molecular biology studies of brain tissues collected from patients who suffered from AD revealed the presence of many hallmarks of inflammation, including microglial activation, expression of cytokines, activation of the complement system, invasion of immune cells (McGeer & McGeer 2001). Secondly, there is an ongoing debate on the value of non-steroidal anti-inflammatory drugs
(NSAIDs, e.g. aspirin, ibuprofen) in AD treatment. Epidemiological studies suggest that patients on long term anti-inflammatory treatment (mainly because of arthritis) have a significantly reduced risk of developing AD.

As in the case of many neurodegenerative diseases, the aetiology of AD is not clear, however, evidences suggest that accumulation of β-amyloid in neuritic plaques and remnants of a cytoskeletal protein tau (neurofibrillary tangles) act as irritants, causing activation of complement system, the initiation of reactive changes in microglia, the release cytokines (IL-1β, IL-6, IL-8) (McGeer & McGeer 1997) and potentially neurotoxic products such as reactive oxygen species, nitric oxide, excessive extracellular glutamate (Klegeris et al 1997). All these toxic inflammatory products contribute to neuronal cell death, which further activates the immune reactions and leads to a chronic and progressive neurodegeneration.

1.3.3.2 Parkinson’s disease

Similar processes are observed in Parkinson’s disease (PD), a chronic, progressive neurodegenerative disorder characterised by degeneration of the nigrostriatal dopamine (DA) neurons in the substantia nigra. According to a common hypothesis, neurodegeneration in Parkinson's lays in abnormal accumulation of the protein α-synuclein in neurons, that similarly to β-amyloid in AD, aggregates and triggers activation of glial cells and the progression of inflammation. Indeed, activated microglia, and to a lesser extent reactive astrocytes, are found in the area associated with neuronal cell loss (Teismann & Schulz 2004), possibly contributing to the inflammatory process by the release of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 (Nagatsu & Sawada 2005), prostaglandins (PGE₂, PGD₂), reactive oxygen and nitrosative species (NO). The anti-inflammatory treatment with NSAID or dexamethasone has demonstrated to have a beneficial role also in PD, although it is much less evident than in the case of AD (Teismann et al 2003).
1.3.3.3 Multiple sclerosis

Multiple sclerosis (MS) is another example of a disease involving inflammation of the brain and associated neurological impairment. The key feature of MS is demyelination of axons in peripheral nerves and brain areas that leads to their subsequent degeneration and formation of plaques (Bitsch et al 2000). The aetiology of MS is still not fully understood. Considerable evidence suggests the main role of autoimmune reactions to an unknown antigen and the activation of T lymphocytes (or deactivation of suppressor T cells) that, together with macrophages, invade the CNS. There are suggestions that an initial infection – viral or bacterial – can trigger inflammation and activation of T cells cross-reacting with autoantigens present in the CNS. Many studies demonstrate that the cytokines TNF-α and IFN-γ mediate some aspects of the disease (Bettelli & Nicholson 2000). They are both toxic to oligodendrocytes and can stimulate local inflammatory cytokines production. Also clinical studies report the increased expression of cytokines in the brain parenchyma and CSF but the therapeutic strategies targeted to block TNF-α and IFN-γ have not yet been successful. The current treatments of MS use immunosuppressive drugs, anti-inflammatory approaches and the treatment with interferon-β. The mechanism of action of IFN-β is not fully known, but in in vitro studies it was shown to suppress many actions of IFN-γ, inhibit the expression and release of IL-1 and TNF-α, and induce the release of IL-1ra (Jungo et al 2001).

1.3.4 Innate Immunity

Host organisms detect the presence of infection by recognizing specific elements produced by microorganism. These elements also called as pathogen associated molecular patterns (PAMPs) are recognized by specific cells of the immune system as inducers of innate responses to bacterial infection. The reaction to endotoxin lipopolysaccharide (LPS), an important
component of the outer membranes of gram negative bacteria, is the best-characterized example of innate recognition that leads to a robust inflammatory response by phagocytic cells. Pathogen associated molecular patterns have the ability to activate the nuclear factor κB (NFκB) signalling pathways and the production of cytokines. The secretion of cytokines by circulating monocytes/neutrophils and tissue macrophages in response to PAMPs requires a cascade of signalling events, the details of which have been clarified in recent years. In particular, the involvement of toll-like receptors (TLRs) has received significant attention.

1.3.5 Lipopolysaccharide (LPS)

The first, most widely studied bacterial component that stimulates the innate immunity is the gram-negative bacterial lipopolysaccharide. More than a hundred years ago Richard Pfeiffer discovered that lysates of heat killed bacteria (Vibrio cholerae) caused toxic shock in guinea pigs; from this experiment he postulated that the toxic principle is localized inside the bacterial wall and named it endotoxin (from Greek endo = inside). Today we know that the cell wall of Gram-negative bacteria is characterised by the presence of two lipid bilayers – the outer and the inner (cytoplasmic) membrane, separated by the periplasmic space containing a network of peptidoglycan. The substance responsible for the biological effects is mainly lipopolysaccharide (LPS, also commonly known as endotoxin), a constituent of the outer membrane of the cell wall in Gram-negative bacteria.

1.3.5.1 Structure

Lipopolysaccharides are a class of heat stable amphiphilic glycolipid molecules composed of a hydrophilic poly or oligosaccharide core and a hydrophobic region known as lipid A (Caroff & Karibian 2003). The polysaccharide region of LPS is subdivided into the terminal O-specific chain.
and the core region most proximal to lipid A (Figure 1.4). The O-specific chain consists of 50 repeating oligosaccharide units formed of 2-8 monosaccharide moieties in a highly species and strain specific manner (Brandenburg & Wiese 2004). The core region can be divided into two parts – inner and outer core – differing in monosaccharide composition (Figure 1.4). The inner core shows the least variability within the polysaccharide region of LPS and in most bacteria is composed of 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo - a characteristic and essential component considered also as a diagnostic marker of LPS) and L-or D-glycero-D-manno-heptose. The outer core mainly consists of hexoses, such as D-glucose, D-galactose, D-glucosamine, N-acetylglucosamine or N-acetylgalactosamine (Rietschel et al 1994).

The conserved lipid A structure has been identified as the immunostimulatory principle of LPS (Brandenburg & Wiese 2004). The lipid A structure is quite homogenous within different Gram-negative bacterial species (Kusumoto et al 2003). The backbone consists of a central β-(1-6)-linked disaccharide units composed of D-glucosamine (D-GlcN) or D-2,3-diamino-2,3-dideoxyglucose (D-GlcN3N; DAG) (Caroff et al 2002).

![Figure 1.4 The general chemical structure of lipopolysaccharide (LPS)](image-url)
Comparative studies of lipids A from several bacterial species have shown that there is a considerable diversity in the specific acylation patterns (number, position and chemical nature of the acyl residues) and it is the acylation pattern that plays the major role in determining the immunostimulatory potential of LPS (Rietschel et al 1993).

### 1.3.5.2 Biological effects of LPS

LPS is released by bacteria during their growth or bacteriolysis. Recognition of LPS (or lipid A) by the professional phagocytes of the innate immune system - peripheral monocytes, macrophages and neutrophils - is a key event in host microbial defence reactions. Also microglia and astrocytes in the CNS have been shown to respond to LPS (Lee & Lee 2002).

In humans, especially mononuclear cells react with extreme sensitivity to LPS preparations - the threshold values for the activation of isolated human monocytes or monocytes in whole blood are about 1-10 pg/ml. The activation of mononuclear cells by LPS or free lipid A in vitro leads to the secretion of a wide spectrum of endogenous mediators among which: 1) the pro-inflammatory cytokines - TNF-α, IL-1β, IL-6, IL-8, IL-12, IL-15 and IL-18; 2) colony stimulating factors M-CSF, G-CSF and GM-CSF; 3) arachidonic acid derivatives such as PAF, PGE₂, thromboxane A₂, leukotriens; 4) reactive oxygen species (superoxide, hydroxyl radicals) and 5) nitric oxide. In addition, LPS may cause activation of the complement system - both via the classical and the alternative pathways.

By diverse mechanisms the autocrine and paracrine mediators released in the early phases of LPS-induced activation may initiate a complex network of secondary reactions, which include the stimulation of acute phase protein secretion by the liver, activation of blood cells from all lineages (thrombocytes, basophils, mast cells, eosinophils) (extensively reviewed by
Alexander & Rietschel 2001). The early activation of innate immune cells subsequently induces the recruitment of adaptive highly specific immune responses via the selection and clonal expansion of pathogen specific T and B lymphocytes (Ulmer et al 2000). According to current knowledge IL-1β and IL-6 are the primary mediators in the induction of fever by LPS (Dinarello 2004).

1.3.5.3 LPS in CNS diseases

The involvement of LPS in the pathogenesis of CNS diseases, and in particular meningitis, is well studied. In a rabbit model of meningitis, activities of pro-inflammatory TNF-α, IL-1, and IL-6 were rapidly detected in the CSF after injection of meningococcal LPS into the subarachnoidal space. Intracysternal administration of LPS in rats induced NO synthesis from the lateral and third ventricle choroid plexi and surface meninges. Several in vitro studies show that LPS activates glial cells – microglia and astrocytes – and induces production of inflammatory cytokines such as TNF-α, IL-1, IL-6, chemokines, nitric oxide, prostaglandins, adhesion molecules and matrix metalloproteinases (MMPs).

LPS can increase the permeability of the blood-brain barrier by the induction of cytokines and several adhesion molecules on the cerebral endothelium, which facilitate the infiltration of leukocytes in the CSF. Also, LPS induced NO, PGD₂ and MMPs were proposed to disrupt the BBB integrity in an experimental rat model of bacterial meningitis.

Finally, the LPS-induced neuroinflammation contributes to neuronal cell death. Recently it has been shown that inflammatory neurodegeneration is mediated by several factors released by LPS-stimulated microglia and astrocytes, such as cytokines TNF-α and IL-1β.
1.3.6 Toll-Like Receptors

The essential role in the activation of early immune responses to invading pathogens is played by a group of specialized receptors called Toll-like receptors (TLRs), which recognise specific pathogen associated molecular patterns (PAMPs). TLRs are transmembrane proteins with extracellular leucine rich repeat domains (LRR), and cytoplasmic signalling domains that are similar to the cytoplasmic domain of the interleukin-1 receptor (IL-1R), commonly termed as the Toll/IL-1 receptor homology domain (TIR). Both IL-1R and TLRs induce signal transduction pathways leading predominantly to activation of the transcription factor NF-κB, a key regulator of inflammatory responses.

1.3.6.1 Toll-like receptor 4

The first mammalian Toll-receptor discovered was TLR4. It has been identified as the specific receptor for Gram-negative bacterial lipopolysaccharide (LPS). The most important evidence for the essential role of TLR4 in LPS induced signalling was provided by the finding that the lps gene responsible for profound hypo responsiveness to LPS in the classical C3H/HeJ and C57BL/10ScCr strains of mice is identical to the gene of murine TLR4 (Vogel et al 1999).

According to current knowledge LPS released from bacteria is present in body fluids as free-floating aggregates, which can be monomerized by the LPS binding protein (LBP). LBP is a 58-kD serum glycoprotein that binds to the lipid A moiety of LPS and facilitates the extraction of single LPS molecules from LPS aggregates. In humans, LBP is present in plasma at 3-10 µg/ml but levels rise dramatically after acute phase response to bacteria (Prucha et al 2003). Immunodepletion of LBP from whole blood lowers the sensitivity of monocytes to LPS by at least two orders of magnitude. LBP
catalyses the transfer of monomerised LPS to CD14, a 55-kD glycoprotein present either in GPI-anchored form on the cell surface (mCD14), or in soluble form in the extracellular space (sCD14). The CD14 molecule is important for the activation of cells by LPS. It is constitutively expressed at high levels in monocytes ($10^5$ molecules/cell), tissue macrophages, neutrophils as well as microglia and astrocytes. CD14 is a co-factor for the LPS-induced cellular response in cultured cells, as addition of CD14 results in a 1000-fold increase in LPS-sensitivity. Antibodies to CD14 block the LBP-dependent activation of macrophages by LPS. The CD14 binds LPS with high affinity and is involved in mediating LPS responses. Since CD14 is a glycosylphosphatidylinositol (GPI) - anchored membrane protein devoid of a cytoplasmic domain, it does not elicit intracellular signaling events directly, but needs to bind to the TLR4 receptor (Triantafilou & Triantafilou 2002).

CD14 seems to have a role in amplifying the LPS responses, which was documented in numerous studies. Transfection of a CD14 negative cell line with CD14 expression vector induced strong sensitization to LPS. Monoclonal antibodies against CD14 had profound inhibitory effects on LPS-induced activation. Finally, transgenic mice lacking CD14 were hypo-responsive to LPS at lower concentrations and resistant to the lethal effects of LPS. However at higher concentrations of LPS, CD14 (-/-) mice responded normally to LPS.

LBP and CD14 have also been shown to mediate rapid cellular internalisation of LPS aggregates and even phagocytosis of intact gram-negative bacteria in vitro. This function is maintained in mononuclear cells from TLR4 deficient mice, showing that clearance of LPS aggregates and Gram-negative bacteria from the circulation or infected tissues does not depend on signal transduction via TLR4. TLR4 represents the central transmembrane signal transducer in the activation of mammalian cells by LPS.
Another characteristic and unique feature of TLR4 signaling is the presence of a MyD88-independent pathway, along with the MyD88-dependent pathway typical for all TLRs. Both pathways lead to the activation of MAP kinases and NF-κB. However, the MyD88-independent pathway seems to be responsible also for the activation of IFN regulatory factor 3 (IRF-3) and the subsequent induction of IFN-β and IFN-inducible genes, such as IP-10 (Toshchakov et al 2002).

1.3.7 COX-1 and -2 in the Central Nervous System

Cyclooxygenases (COX) exists in two isoforms COX-1 and COX-2 enzymes; it is encoded by different genes. COX enzymes plays an important role in the inflammatory cascade by converting arachidonic acid (AA) which is released from membrane phospholipids by a phospholipase A2 (PLA2) into bioactive prostanoids. Both COX isoforms catalyze the same reactions: dioxygenation of arachidonic acid (AA) to form prostaglandin G2 (PGG2), and a peroxidase reaction, which converts PGG2 to prostaglandin H2 (PGH2). PGH2 is then transformed into PGE2, PGF2a, PGD2, PGI2 and TXB2 by specific terminal synthases. Among these prostanoids synthases, PGE2 (microsomal PGE synthase 1 (m PGES-1), membrane PGES-2 (m PGES-2) and cytosolic PGES (cPGES)) (Thoren et al 2003) are potential targets in the treatment of inflammation have recently determined (Friesen et al 2008). Both mPGES-2 and cPGES are constitutively expressed enzymes, while mPGES-1 is an inducible enzyme by inflammatory stimuli such as LPS and primarily coupled with COX-2 (Murakami et al 2000).

COX isoenzymes known as COX-1 and COX-2 that have 65% homology in their amino acids sequence. COX enzymes are genetically independent proteins, the human gene for the COX enzymes are located on different chromosome and have different properties. The COX-1 gene which is 22 kB in size and located on chromosome 9 in humans and has few binding
sites for inducible transcriptional factors. The COX-2 gene is -8.3 kb in size and located on chromosome 1 the expression of which is tightly regulated. The COX-2 gene is responsive to many transcriptional regulatory factors such as growth factors and inflammatory mediators such as IL-1, TNF α and LPS. COX-1 enzyme is constitutive and present in most tissues and controls normal body function including stomach mucus secretion, kidney water excretion as well as platelet formation (Woodfork & Dyke 2004). COX-2 is not detectable in normal tissue but is detectable after induction by inflammatory stimuli. COX-2 enzyme induced by the action of macrophages, scavenger cells of the immune system and is producing prostaglandins for an inflammatory response (Chandrasekharan et al 2002). The important difference between the COX enzymes seems to be much large binding site in COX-2 enzyme. The COX-1 enzyme attachment site is smaller than that of COX-2 enzyme, so COX-1 can accepts a narrow range of substrates. In both COX genes a further difference can also be seen in the transcriptional elements. In COX-2 gene has several transcriptional regulatory elements such as NF-κB, Sp1, a TATA box, CAAT Enhancer Binding Protein Beta (C/EBP b), and cAMP response element-binding (CREB) sequences interacting with transacting factors produced by signalling pathways (Kang et al 2007). In contrast, COX-1 promoter lacks a TATA or CAAT box, has a high GC content, and contains several Sp1 elements.

1.3.7.1 **COX-1 and -2 in neuroinflammation**

Both COX-1 and COX-2 have been shown to play an important role in the inflammatory response. However, the exact role and preference of COX isoform in neuroinflammation is unclear. Both these isoforms have different roles both in normal physiology and pathological conditions. COX-1 expression leading to prostaglandin synthesis, seen in microglia activation of these cells could lead to an excessive release of prostaglandin (Hoozemans et
A recent study in an animal model suggests that COX-1 inhibition reduces neuroinflammation, neuropathology, and improves cognitive function (Choi et al 2013). COX-2 is an inducible enzyme that responds to proinflammatory stimuli and is mainly found in cortex, hippocampus and amygdala, with both neuronal and vascular association (Wang et al 2005) and in rat astrocytes and microglia (Hirst et al 1999). The physiological function for COX-2 enzyme such as long term potentiation, synaptic activity and long term depression. Even in physiological conditions, COX-1 or COX-2 derived prostanoids seem to have distinct functions, for instance, PGE\(_2\) results from COX-2, but not COX-1 activity. The major PGs in the CNS of most mammalian species including humans, monkeys, and rats are PGE\(_2\) and PGD\(_2\). Inflammatory processes associated with an increased COX-2 expression and elevated prostaglandins (PGE\(_2\)) levels have played a major role in neurodegeneration (Almer et al 2001). The COX-2 expression has been studied in various animal models of inflammation which can strongly be used as evidence for induction of COX-2 a pivotal role in inflammation. Increased COX-2 expression has been observed with head trauma, cerebral ischemia, spreading depression, and seizures, as well as in several progressive neurodegenerative conditions e.g., Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, Down’s syndrome, etc. (Dash et al 2000). Over expression of brain COX-2 in these disorders may reflect its role in chronic neuroinflammation and neural cell death. COX-2 deficient mice are protected against brain ischemia (Iadecola et al 2001) and COX-2 inhibition showed beneficial effects against ischemic damage and neuronal death (Candelario-Jali & Fiebich 2008).
Figure 1.5  COX-1 and COX-2 are regulated by the transcription factor NFκB

Membrane lipids are broken down by phospholipase A2 (PLA$_2$) into arachidonic acid (AA). Subsequently, the COX enzyme (either COX-1 or COX-2), first via the cyclooxygenase activity of the enzyme, converts AA to prostaglandin G2 (PGG$_2$) and then via the peroxidase step converts PGG$_2$ to Prostaglandin H$_2$ (PGH$_2$). The peroxidase step results in the production of free radicals. PGH$_2$ can then be converted by tissue-specific synthases to various prostaglandins not shown in this figure.

1.3.8  Effect of Inflammation on Sickness Behavior

Systemic administration of LPS is a component of the outer membrane of gram negative bacteria may cause inflammatory responses in the brain. LPS binding to immune cells initiates signalling cascade that activates the transcription factor NFκB and up regulate the expression of proinflammatory cytokines such as IL-1β, IL-6 and TNFα (Block et al 2007).
These inflammatory cytokine may mediate sickness behavior syndrome and affects the function of the brain (Konsman et al 2002). Many reports have demonstrated that LPS may lead to a collection of behavioral changes (Dunn & Swiergiel 2005). The mechanisms underlying sickness behavior have not been fully elucidated, but it has been suggested that cytokines and prostaglandins are involved (Teeling et al 2010). Interleukin-1β, interleukin-6 and tumor necrosis factor-α (TNF-α) may be secreted in response to infections and endotoxemia (Dantzer 2009). The inflammatory cytokines, IL-1β seems to be the most potent in inducing sickness behavior administered either by peripheral or central injection reproduces the set of non-specific symptoms of inflammation including decreased motor activity, social withdrawal, anorexia and fever.

### 1.3.9 Effect of Inflammation on Cognition

Learning and memory deficits occur when LPS induced neuroinflammation which affects hippocampus function (McGeer et al 2006). Elevation level of cytokines has been associated with cognitive deficits in neurodegenerative disease such as AD and mild cognitive impairment patients have been reported (Guerreiro et al 2007). Recent evidence suggests that LPS may also disrupt certain mnemonic processes: acute administration of LPS prior to training impairs contextual fear conditioning, a hippocampal dependent learning paradigm (Shaw et al 2005). Another hippocampal dependent learning task such as water maze in rats was impaired by IL-1challenged (Oitzl et al 1993). Long term potentiation (LTP) of synaptic transmission is a popular model of the biological processes that may underlie memory (Bliss & Collingridge et al 1993). LTP is readily induced in the hippocampus; blocking LTP causes impairment on the water maze task (Morris et al 1986). LPS inhibits LTP in the rat dentate gyrus in vitro (Cunningham et al 1996) and in the CA1 subiculum pathway in vivo (Commins et al 2001).
1.3.10 Effect of Inflammation on Oxidative Stress Generation

Oxidative stress has been considered a critical factor in the pathogenesis of many neurodegenerative diseases. LPS activates microglia and astrocytes in the brain and releases several cytokines and inflammatory mediators in response to several reactive oxygen species from cells such as macrophages, phagocytic cells and neutrophils and produce an oxidative stress. Oxidative stress may induce a rapid alteration in the antioxidant systems by inducing proteins that participate in these systems and/or depleting cellular stores of endogenous antioxidants such as glutathione. Reactive oxygen species serve as an intracellular second messenger to induce signal transduction and activate transcription factors such as NFκB (Macdonald et al. 2003). ROS is important for host defense and may influence sickness behavior via NFκB-dependent cytokine production. Recent reports strongly suggests that activated microglia releases inflammatory mediators such as IL-1β and TNF-α and also produces reactive oxygen and nitrogen-free radicals that contribute to the neurodegenerative processes (Tanaka et al. 2006). Antioxidant system plays an important role to regulate the inflammation (Carrillo et al. 2005) in neurodegenerative conditions.

1.3.11 Effect of Inflammation on Neurochemical Alterations

LPS treatment not only acts as an activator of the immune system by influencing cytokine secretion from macrophages but it also influences neuroendocrine function and central neurotransmitter activity (Dantzer et al. 2008). LPS induced activation of the immune system leads to the symptoms of sickness behavior such as decrease in locomotor activity, changes in body temperature, depression and anxiety. These behavior responses triggered by alterations in the neurotransmitter activity that are provoked by the effect of inflammatory mediators such as IL-1β, IL-6, TNF-α and COX expression levels in the brain (Dunn 2006). Treatment with IL-1β (20µg/kg, ip) produced
decreased hippocampal concentration of glutamate, glutamine and GABA 1 hr later (Bianchi et al 1995). Long-term potentiation (LTP) has been advanced as a leading candidate for the neurophysiological substrate of learning and memory (Brown et al 1988). Mechanisms of LTP appear to be dependent on activity of glutamatergic receptors (Bekkers & Stevens 1989). Also GABA seems to be involved in mechanisms of LTP, because administration of GABAergic antagonists results in facilitation of LTP induction (Mott & Lewis 1991). Thus, LTP may be related to both increased excitatory activity in glutamatergic systems and decreased inhibitory activity in GABAergic systems.

1.3.12 Effect of Inflammation on Proinflammatory Cytokines

LPS binds to the Toll like receptor (TLR 4)/ CD 14 complex which are present on the surface of mononuclear myeloid cells and activates the transcription factor NF-κB to up-regulate expression of pro-inflammatory cytokines such as IL-1b, IL-6, TNF-α, COX-2 and iNOS (Laflamme & Rivest 2001). Acute administration of LPS can induce expression of proinflammatory mediators. The two inducible enzymes of COX-2 and iNOS can be induced by LPS and play a vital role in the inflammation. Elevated COX-2 and iNOS expression level has been demonstrated to correlate with various inflammatory disorders (Choi et al 2003). These inflammatory cytokines may mediate sickness behavior and affect the normal brain functions. A principal player in the regulation of these molecules and brain cell death is the nuclear factor kappa B (NF-κB) family of transcription factors (Grilli & Memo 1999). NFκB can be activated by induced oxidative stress, bacterial endotoxin or cytokines and subsequently activate transcriptionally the genes encoding cytokines (Merrill & Benveniste 1996). It has been shown that upregulation of NFκB and other proinflammatory
cytokines plays a pivotal role in the cerebral inflammation and neuronal death in neurodegenerative conditions.

1.3.13 Neuroinflammation as a Neurodegenerative Disease Model

Neuroinflammation is a common feature in most neurodegenerative diseases. Recent report suggests that systemic infection and inflammation impacts on various neurological diseases correlate with an inflammatory component (Teeling & Perry 2009). Cunningham et al (2009) have shown that the onset and progression of neurodegenerative disease is exacerbated by systemic infection in both animals and humans with central cytokine production and neuronal damage.

Therefore, the main objective of our present study was to mimic this neuroinflammation in the rodent model. Intraperitoneal administration of LPS was used to induce inflammation in the CNS. LPS activates Toll-like receptor 4 (TLR-4) in the circumventricular organs and result in NFκB-dependent induction of proinflammatory mediators (Konsman et al 2002). The exact pathways by which systemic infection can alter brain function are not known. Epidemiological studies suggested that long term use of non-steroidal anti-inflammatory drugs (NSAIDs) has a protective effect in neuroinflammatory diseases such as AD. The most important mechanisms associated with the increased COX activity during neuroinflammation include production of PGE₂ in the potential target sites for treatments to neuroinflammatory conditions. Both COX-1 and COX-2 have been shown to play important role in the inflammatory response. However, the exact role and preference of COX isoform in neuroinflammation is unclear. Biological pathways, by which systemic inflammation influences the brain function in normal and diseased state, may lead to novel therapeutic strategies. With this background, the aim of present study is to explore the effect of selective-
non-selective COX inhibitors in preventing the neuroinflammation induced by LPS.

1.4 PLAN OF WORK

The study is conducted in three phases

Phase I

- Optimization of LPS dose in rats.
- Comparative study of pre-and post-treatment of aspirin with LPS to finalize the method of treatment.

Phase II

- Effect of non selective COX inhibitor (aspirin) and selective COX-1 and COX-2 inhibitors (resveratrol and celecoxib) in LPS induced behavioral alterations in rats.
- Comparison between selective- and non-selective COX inhibitors in LPS induced behavioral alterations in rats.
  - Locomotion – Actophotometer (Kozak et al 1994).
  - Anxiety – Open field test (de Paiva et al 2010).
  - Depression – Forced swim test (de Paiva et al 2010).
Phase III

- Effect of COX inhibitors in LPS-induced proinflammatory mediator modulations in rats.
  - Proinflammatory mediators: IL6, iNOS, TNF-α, COX-1 and COX-2 mRNA gene expression is determined by RT-PCR and NF κB protein expression is measured by Western blot.

- Effect of COX inhibitors in LPS-induced neurochemical alterations in rats.
  - GABA, glutamate and asparate neurotransmitters are estimated by HPTLC.

- Effect of COX inhibitors in LPS-induced biochemical alterations in rats.
  - Antioxidant evaluation catalase, SOD, GSH and TBARS are measured spectrophotometrically.

- Effect of COX inhibitors in LPS-induced neuronal damage in rats.
  - Histopathology-Neuronal degeneration using cresyl violet staining.
Figure 1.6 Schematic diagram represents the entire study protocol

RT-Rectal temperature, OFT-open field test, TL-transfer latency, PAR-Passive avoidance response, MWM-Morris water maze, LPS- Lipopolysaccharide. Body weight of the animals was recorded before the start of the drug treatment (before LPS administration) and on the last day of the study.