Chapter 1: Neuroinflammation and the role of microglia - an overview

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Neuroinflammation and the role of microglia - an overview

1.1 Neuroinflammation

Inflammation is a cardinal host defense response to injury, tissue ischemia, autoimmune responses or infectious agents. Classical signs of swelling, redness, heat and pain are witnessed in all tissues except that of brain as manifestations of inflammation. These symptoms are caused as a result of increased amount of blood flow to the site of injury to get more nutrients and immune cells to an area in need (Benacerraf and MCcluskey, 1963). Invasion of circulating immune cells (lymphocytes and macrophages), induction or activation of inflammatory mediators such as cytokines, kinins, reducing and oxidizing species aid the repair and removal of injured or infected cells (Eming et al., 2007).

The central nervous system differs from the other systems and its response to pathogenic challenges is significantly different. In contrast to the historic view that the central nervous system (CNS) is an immune-privileged organ, lacking a lymphatic system and shielded from the circulatory system by the blood-brain barrier recent studies and evidences have revised the idea significantly. The CNS has been found to have its own system of combat through inflammatory response and an adapted system of immunosurveillance with coordination with the systemic immune system is also evident (Hao et al., 2002; Badie et al., 2000). Nevertheless the inflammatory response of the other tissues and the brain are varied. This is most evident in leukocyte recruitment, which is rapid in many systemic organs, but modest and delayed in the brain. While leukocyte invasion may be delayed in response to acute insults, activation of brain’s own immune cells and release of inflammatory mediators are rapid, occurring within minutes or hours (Lucas et al., 2006).

Inflammation in the brain is characterized by activation of glial cells (mainly microglia and astrocytes) and expression of key inflammatory mediators as well as neurotoxic free radicals. In the central nervous system, microglia is the resident phagocytes of the innate immune system and astrocytes provide nutrients to the nervous tissue and help maintain the extracellular ion balance (Stoll and Jander, 1999).
1.1.1 Neuroinflammation as physiological response

Neuroinflammation is a process in which the brain responds to infections, diseases and injuries through release of proinflammatory molecules (Nencini et al., 2003; Schmidt et al., 2005). These responses are mediated by two types of immune cells: lymphocytes, monocytes and macrophages of the hematopoietic system and glial cells of the CNS (astrocytes and microglia-the supporting cells of the CNS) (Stoll and Jander, 1999; Sreit et al., 1999).

In response to a brain insult, glial cells are the first to be activated. The astrocytes upon activation, increases expression of glial fibrillary acidic protein, and produces cytokines; also contributing to the formation of the glial scar, which isolates the damaged area. These reactive astrocytes produce neurotrophic factors such as nerve growth factor and brain-derived growth factor which help in blood brain barrier repair and remyelination (Faulkner et al., 2004). On the other hand, within any scenario of immune-mediated brain injury, microglia qualifies as the main intrinsic immune effector cells of the brain. They are potentially phagocytic cells, have a pronounced cytotoxic potential (reviewed by Banati et al., 1993) may express several immunomolecules on their surface, may effectively present antigen to T-lymphocytes (Frej et al., 1987; Matsumoto et al., 1992) and are capable of releasing a plethora of mediator substances such as inflammatory cytokines.

Most inflammatory mediators have relatively few actions in healthy CNS tissue and are expressed at very low or undetectable levels. Nevertheless some cytokines also modulate neuronal activities in the mature CNS and participate in neuro-immune–endocrine communication. However, they are induced rapidly in response to tissue injury or infection; certain cytokines appear in the affected brain region and the cerebrospinal fluid (CSF) when the CNS homeostasis is disturbed as a result of trauma, stroke, ischemia, infection, or degenerative processes. This increased cytokine levels in the CNS may result from blood–brain barrier (BBB) disruption or synthesis by invading immune cells, both of which originate from extraneuronal sources. These disruption of the blood-brain barrier (BBB), allows cells from the hematopoietic immune cells to leave the blood stream and come in contact to the injury site (Lossinsky and Shivers, 2004) The immune cells respond to injuries by eliminating debris and, synthesizing and releasing a host of powerful regulatory substances, like complements, cytokines, chemokines, glutamate, interleukins,
nitric oxide, reactive oxygen species and transforming growth factors (Ghirnikar et al., 1998; Jander et al., 2002; Stoll et al., 2004; Hensley et al., 2006; Bonifati and Kishore, 2002) which in turn start the cycle all over.

Neuroinflammation can be further explained as two distinct responses—acute and chronic neuroinflammation.

1.1.1.1 Acute neuroinflammation

Acute neuroinflammation is more of a physiological response either to injury or insult to the CNS. Before "neuroinflammation" became a commonly used term, endogenous CNS tissue responses to injury were referred to as ‘reactive gliosis’. Reactive gliosis entails accumulation of enlarged glial cells, notably microglia and astrocytes, appearing immediately after CNS injury has occurred (Wolfgang et al., 1999). Glial reactivity is majorly a passive response to injury whereas glial activation implies a more aggressive role in responding to activating stimuli. Activated glial cells release factors that act on and engender responses in target cells equivalent to the responses of activated immune cells in the periphery; however, peripheral immune cells activation leads to leukocyte infiltration of tissues, which is notably absent in the brain unless there has been destruction or compromise of the blood brain barrier (Sroga et al., 2003; Sreit et al., 1998). In the presence of such destruction or compromise, peripheral leukocytes do enter the brain producing a scenario similar to that seen in inflammatory responses in the periphery.

In limited, acute reactions to injury, in the absence of blood-brain barrier breakdown, there is the subtler response of the brain's own immune system (Illustration 1A), composed largely of rapid activation of glial cells. These responses represent the other end of the spectrum of CNS injury, where limited neuronal insults trigger glial cell activation without breakdown of the blood brain barrier and without concomitant leukocytic infiltration. This form of "pure" glial response occurs in neuronal injury caused by either loss of afferents (Kreutzberg, 1996) or loss of efferents (Ito et al., 1997).

The term "neuroinflammation" as generally used and understood applies to more chronic, sustained cycles of injury and response, in which the cumulative ill effects of
immunological microglial and astrocytic activation contribute to and expand the initial neurodestructive effects, thus maintaining and worsening the disease process through their actions.

1.1.1.2 Chronic neuroinflammation

Chronic inflammation is often associated in the understanding of CNS disease as opposed to acute inflammation which is linked with CNS injury. It is proposed that chronic inflammation is a causative factor to the pathogenesis of neurological diseases and disorders (Eikelenboom et al., 2006; Whitton et al., 2007). The immune cells and pro-inflammatory chemicals involved in neuroinflammation would underlie the mechanisms of diseases and neurodegeneration. The activation, or over activation, of immune cells involved in neuroinflammation and release of pro-inflammatory substances would result in reduced neuroprotection and neuronal repair, and increased neurodegeneration, leading to neurodegenerative diseases (Zilka et al., 2006; Donnelly et al., 2007). To elaborate, during disease states (for example, Parkinson's (PD), Multiple sclerosis (MS), Alzheimer's disease (AD)) the inflammatory responses damage the BBB, increase oxidative stress and release pro-inflammatory and pro-apoptotic cytokines and other neurotoxic factors that affect neuronal damage or dropout. The damage and stress signals enhance microglial activation, resulting in positive feedback in the release of chemokines and cytotoxic cytokines that cause further ingress of immune cells into the brain and expand inflammatory responses (Illustration 1B).
Illustration 1: Brain perivascular macrophages and microglia in nervous system health and disease. (A, top panel) Under steady state conditions, microglia secretes neurotrophic factors and engages other glial elements to promote tissue homeostasis. (B, bottom panel) In neurodegenerative disease states, stress signals activate microglia and a vicious cycle of inflammatory mediator synthesis is initiated.

1.1.2 Neuroinflammation - association with neurodegeneration and diseases

Chronic inflammation could influence the pathogenesis of neurodegenerative diseases. It is clearly evident that physiological or pathological responses like neuroinflammation, if goes awry, is tightly linked to the neurodegenerative disorders and diseases. A few examples neuroinflammatory response being the key drivers in disease and infection progression would bring out a better understanding of this concept.

Neurodegenerative diseases particularly Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and Huntington's disease (HT) lack the prominent infiltrates of blood-derived mononuclear cells that characterize autoimmune diseases. On the other hand, there is abundant evidence that many substances involved in the promotion of neuroinflammatory processes are present in the CNS of patients with such neurodegenerative diseases.

Alzheimer’s disease (AD) is characterized by the progressive inability to form new memories and access existing ones, mainly because of neuronal cell death in the hippocampus and frontal cortex of the brain (Selkoe et al., 2002). Besides such atrophy in the brain, microscopically there are a number of changes in the AD patient’s brain too. The two major findings in the Alzheimer's brain are amyloid plaques and neurofibrillary tangles. β amyloid (Aβ) is a peptide that forms insoluble and pathological extracellular aggregates forming plaques, which seem to attract microglial cells, as suggested by the clustering of microglia at sites of Aβ deposition (reviewed by Streit, 2004). This proinflammatory response of microglial recruitment initially poses as a neuroprotective effect, but when prolonged or continuous turns into a neurotoxic effect. This suggests that CNS inflammation at least participates in amplification of the disease state.

Parkinson’s disease (PD) whose clinical symptoms include tremor, postural instability and slowness of movement (Gao et al., 2003) is attributed to the loss of dopaminergic neurons in the substantia nigra pars compacta and the subsequent loss of projecting nerve fibres in the striatum (Block et al., 2004; Qin et al., 2004). PD is sporadic, and various environmental agents including pesticides and infections, may contribute to the disease. A role of inflammation has been strongly implicated (reviewed by Gao et al.,
2003), and activated microglia are found close to degenerating substantia nigral neurones of patients with PD.

Multiple sclerosis (MS) is a chronic disorder in which inflammation plays a clear role. Invasion of the CNS by T cells and macrophages leads to damage to the myelin sheaths surrounding axons, loss of neuronal function and death. Since the injured areas of the CNS vary widely, the clinical symptoms are heterogeneous, and can include fatigue, muscle weakness, areas of numbness and paralysis. Microarray analysis has revealed that many genes related to inflammatory processes are upregulated in the marginal zones of active demyelinating lesions (Mycko et al., 2003).

Autism is a neurodevelopmental disorder characterized by impaired communication and social interaction and may be accompanied by mental retardation and epilepsy. Its cause remains unknown, despite evidence that genetic, environmental, and immunological factors may play a role in its pathogenesis. Recent studies show that in autistic cases, microglial and astroglial activation was present in the absence of lymphocyte infiltration or immunoglobulin deposition in the CNS (Vargas et al., 2005).

Inflammation undoubtedly contributes to other chronic CNS disorders, such as amyotrophic lateral sclerosis (ALS) and Creutzfeldt–Jakob disease (CJD). ALS, a rapidly progressing motor neuron disease, is associated with mutation of superoxide dismutase (SOD1) gene, and mice that overexpress mutant SOD1 show upregulation of proinflammatory cytokines (Yoshihara et al., 2002), suggesting activation of microglia. Similarly, cytokine levels are elevated in CSF of CJD patients, and activated microglia are detected in mice infected with CJD (Van et al., 2002).

Infections are another group of diseases that are classically recognized as inflammatory in nature, with meningeal, perivascular, or even parenchymal infiltrates of peripheral leukocytes. However there are exceptions, microglial cells have found to be the potential mediator of neurodegeneration in some infections. Rabies is a disease in which the peripheral immune response is slow and inadequate, and in which classic inflammatory changes are less striking than those found in other viral encephalidites. Babes (1892) described microglial activation in rabies infection, although he did not recognize the nodules he found as clusters of activated microglia. Similar small collections of activated
Microglia are subsequently found to occur in a wide variety of viral brain infections. Presently, the most important example of a chronic brain infection is human immunodeficiency virus (HIV). The virus targets and disables precisely those cells that are key players in neuroinflammation; microglia in the brain and T lymphocytes in the periphery. HIV enters and persists in the CNS via myelomonocytic cells: monocytes, perivascular cells, and microglia (Garden, 2002). Chronic HIV encephalitis is characterized by the same nodules of activated microglia that was described in rabies. Overall the activated microglial cells contribute significantly to the HIV associated neuropathogenic processes.

Prion diseases represent another chronic infectious CNS disease that is not accompanied by leukocytic infiltrates. Microglial activation, again, appears to be the most prominent inflammatory component of prion diseases (Eikelenboom et al., 2002; Perry et al., 2002), although there are a few reports describing T cell infiltration as well (Betmouni, 1996; Lewicki et al., 2003).

Thus it clearly evident that chronic microglial activation is an important component of neurodegenerative diseases, and this chronic neuroinflammatory component likely contributes to neuronal dysfunction, injury, and loss (and hence to disease progression) in these diseases. The recognition of microglia as the brain's intrinsic immune system, and the understanding that chronic activation of this system leads to pathologic sequel, has widened the modern concept of neuroinflammation. The idea of microglia-driven neuroinflammatory responses, with neuropathological consequences, has comprehensively replaced the older vision of passive glial responses inherent in the reactive gliosis hypothesis.

1.2 Microglia in the Central nervous system and its role in neuroinflammation

1.2.1 Microglia in the brain

Microglia — from micro (small) and glia (glue) — are the resident immune cells of the brain and constantly patrol the cerebral microenvironment to respond to pathogens and damage. These cells constitute about 5-12% of the total glial population (Ling and
Leblond, 1973). They are present throughout the central nervous system, including the spinal cord, although some regions are more populated than others, with the white matter generally containing fewer microglia than the grey matter.

Nissl first recognized microglia (1899) in the brain and subsequently they were classified as a distinct cell type apart from oligodendrocytes and astrocytes (del Rio-Hortega, 1932). It is widely accepted that microglia originate and are derived from mesodermal hematopoietic cells which in mammals originate from the yolk sac. It has been demonstrated that circulating monocytes or precursor cells in the monocyte-macrophage lineage invade the developing brain during embryonic, fetal or postnatal stages (Kaur et al., 2001) and transform into microglial cells which express several proteins which are able to recognize cells of the monocyte/macrophage lineage. These findings together with the phagocytic activity of microglia suggest that microglia are related to monocytes and belong to the mononuclear phagocytic system.

According to the morphology microglial cells have been classified into three types: amoeboid, ramified and reactive. In the prenatal brain, the amoeboid phagocytic microglia are the predominant form, with a large spherical cell body and short processes (Hess et al., 2004). During postnatal maturation, amoeboid microglia transform into ramified resting microglia, and these cells remain a semi-permanent population with relatively slow turnover rates when compared to peripheral macrophages (Dick et al., 1995; Becher et al., 1996). As resting ramified microglia, they monitor their microenvironment and adapt their morphology and express cell surface markers accordingly (Lawson et al., 1990; 1992). The resident microglial cells in the healthy brain can be seen in all regions of the CNS including optic nerve and retina (Kaur et al., 2006). They remain quiescent until stimuli from injury, infection or neurodegenerative process activate their transformation into amoeboid phagocytic cells (Schroeter et al., 1997; Zhang et al., 2001).
1.2.2. Activation of microglia in neuroinflammation:

As microglia have been recognized as the prime components of an intrinsic brain immune system they have become a main focus in cellular neuroimmunology and therefore in neuroinflammation. There are various stimuli that activate microglial cells and cause neuroinflammation. The in vivo studies reveal the stimuli associated as neural infections, ischemia, neurodegeneration and prion diseases. Lipopolysaccharide (LPS), β-amyloid (βA), interferon (IFN-γ), thrombin and some proinflammatory cytokines are some of the proven stimuli in in vitro neuropathological conditions (Banati, 2002;2003; Wierzba-Bobrowicz et al., 2004)

The magnitude of microglial activation depends on extrinsic and intrinsic conditions: for example, the type of insult, potency of the stimulus, distance from the stimulus, immediate microenvironment, and the “primed” (sensitized) state of microglia that have been exposed to prior and existing stimuli.

It is believed that microglia activated by these stimuli exert cytotoxic effects through two very different and yet complementary processes (Banati et al., 1993). They either act as phagocytes which involve direct cell-to-cell contact or release large variety of potentially noxious substances (Kaur et al., 2006).

Phagocytosis by activated microglia:

Microglia are able to phagocytose particles via different described phagocytic receptors and digest the taken-up material by a professional lysosomal machinery. Depending on the type of the phagocytic receptor microglia responds differently in their downstream cytokine signaling, either pro- or anti-inflammatory. In the presence of apoptotic cells, microglia phagocytose without inflammation and by production of anti-inflammatory cytokines such as transforming growth factor β (TGFβ) (Moller 2000; Wu et al.,2002). Also adenosine triphosphate (ATP) released from injured/necrotic neurons can activate microglia through binding to purinergic receptors and convert them to the neurotoxic phenotype (Beal et al., 2007; Koutsilieri, et al.,2002). Normal microglia phagocytoses injured neurites and myelin debris in a slow process (Gao et al., 2003),
whereas activation of microglia increases this ability to phagocytose axonal and myelin debris (Block et al., 2004).

**Release of noxious factors by activated microglia**

Activated microglia expresses Toll like receptor (TLR) and initiates innate responses with the production of cytokines, chemokines and nitric oxide (NO). Specifically, cytokines released by activated microglia include interleukins 1 and 6 (IL-1, IL-6) and tumor necrosis factor alpha (TNFα), as well as monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1), RANTES (regulated on activation Normal T Cell expressed and secreted) and chemokines for lymphocyte recruitment (reviewed by Yang et al., 2010). These results show that microglia are involved in first line innate immunity of the CNS. Once the microenvironment of the CNS becomes activated, local cells also produce proinflammatory cytokines, chemokines and upregulate immunomodulatory surface markers. These changes in turn decrease the stringency of the blood-brain barrier, allowing entry of soluble factors and peripheral immune cells, including macrophages, natural killer cells and lymphocytes. The specific sequence of events demonstrating that microglia activation precedes peripheral cell infiltration has been demonstrated in bone marrow chimeric mice in an well-designed study by Schilling et al. (2003). For self protection against oxidative stress, microglial cells are equipped with efficient antioxidative defense mechanisms. Microglial cells contain glutathione, substantial activities of the antioxidative enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase as well as nicotinamide adenine dinucleotide phosphate (NADPH) -regenerating enzymes. Their good antioxidative potential protects microglial cells against oxidative damage (Dringen, 2005).

Activated microglia cells trigger and maintain an inflammatory response, deluging neurons with a whole host of inflammatory mediators that may ultimately lead to neuronal cell death. Thus microglial activation and chronic inflammation thereafter is the starting point for elevated levels of a wide array of potentially neurotoxic molecules which are believed to contribute to neurodegenerative processes (Glass et al., 2010; Ransohoff et al., 2009; Cagnin et al., 2001). Several methods have become available for identifying activated microglia, and their presence has been demonstrated in a variety of
neuroinflammatory/neurodegenerative diseases such as AD, PD, ALS and MS (Chen et al., 2004; Turner et al., 2004). This participation of activated microglia and the release of neurotoxic products in the demise of neurons have now been postulated in most, if not all, neurodegenerative diseases.

1.2.3 Signaling pathways mediating microglial activation

Kinase and phosphate cascades mediate microglial response to extracellular stimuli. p38 mitogen-activated protein kinases are a class of mitogen-activated protein kinases (MAPK) that are responsive to stress stimuli. Several reports have demonstrated that p38 and p44/42 families of mitogen activated protein kinase pathways play a significant role in activation of microglial cells which in turn leads to release of neurotoxic molecules and neuroinflammation (Lee et al., 2000; Li et al., 2001; Waetzig et al., 2005). In vivo evidence also implicates that p38 and p44/42 MAPKs play an important role in microglial activation in acute brain injury such as stroke and in chronic neurodegenerative diseases such as Alzheimer’s disease. A MAPK pathway generally consists of 4 sub-pathways: 1) extracellular-signal-regulated kinases (ERK1/2, also known as p44/42 MAPK); 2) c-jun N-terminal kinases/stress-activated protein kinases (JNKs./SAPKs); 3) ERK5/big MAPK 1 (BMK1), and 4) p38 MAPK. Microglia activation could effect through any these if not all of the MAPK pathways.

The other proinflammatory pathway that could respond to microglia activation is the NFκB pathway. Any of the microglia mediated pro-inflammatory stimuli can activate NFκB expression (Sparacio et al., 1992), which can further induce specific genes that regulate the expression of inflammation and acute phase genes leading to the continued elevation of inflammatory proteins.
Illustration 2. The role of microglia in normal homeostasis and its link to neurodegenerative diseases under excessive activation signals. (Adapted from Dheen et al., 2007)
1.2.4 Targeted inhibition of microglial activation as therapeutic strategy

Microglia, since it plays so much of a key and critical role in neuroinflammation and thereby neurodisease progression has been the reason why they have been put in focus as intervention targets in disease treatment. A corollary of neuroinflammation proposes that suppression of microglial production of neurotoxic mediators will result in neuroprotection. Although several drugs alleviate symptoms of neurodegenerative diseases, chronic use of these drugs is often associated with debilitating side effects, and none seems to dampen the progression of these diseases. So far, the development of effective neuroprotective therapies is impeded by our limited knowledge of the pathogenesis of neurodegenerative diseases. Since several studies have demonstrated that the inhibition of microglial activation control the pathogenesis, thus the activation of counter regulatory mechanisms is essential to avoid the escalation of CNS inflammatory processes (McCarty, 2006). This may be possible through the identification of agents that target over activated microglial cells and the determination of their anti-inflammatory mechanisms. This may help development of better therapeutic strategies for neurodegenerative diseases.

Some of the well studied inhibitors of inflammation are glucocorticoids, minocyclines, vitamin E, D, endocannbinoids, transforming growth factor beta1 and several synthetic drugs (Dheen et al, 2007). Though several anti-inflammatory drugs have been shown to diminish neuroinflammation, a very few have direct functional effects on microglial activity (Lleo et al., 2007).

Non steroidal anti-inflammatory drugs (NSAIDS) which include ibuprofen, naproxen and many other generic drugs, have been identified with a tendency to irritate the stomach, with the possibility of more serious complications such as an ulcer, stomach bleeding, colon or small bowel irritation over long term usage(Silverstein et al., 2000). Though synthetic cycloxygenase 2(COX-2) selective NSAIDs were brought into the market as alternatives highlighting its association with less gastrointestinal toxicity than nonselective NSAIDs, recent studies have shown that they cause more serious adverse effects like cardiovascular events and other life threatening side effects (Mukherjee et al., 2001). Therefore, there is an urgent need to develop drugs that have wide spectrum anti-
inflammatory effects and which are able to slow down or curtail the progression of the degenerative process without causing any debilitating side effects.

In the recent decade, there has been a widespread surging interest in naturally occurring plants and plant derived compounds which prove to be highly efficacious drugs with less adverse side effects. With the great strides in the technology of extraction, isolation and activity detection, natural product studies are being propelled as alternatives to synthetic drugs. 63% of the low molecular drugs developed from 1981 to 2006 are natural products or natural product-derived compounds (Newman and Cragg, 2007). In this encouraging scenario, any naturally occurring medicinal compound which can efficaciously inhibit microglial activation could open avenues for better ameliorating microglia-associated neurodegenerative and neuroinflammatory diseases. A whole spectrum of natural compounds has been studied for its ability to curtail microglia caused neurodegeneration and neuroinflammation associated diseases (Table 1). But there has been a few setbacks in pursuing some of them as therapeutic alternatives; some plant compounds are not taken up for further large scale trial studies because of its limited availability, cost demanding extraction methods, low bioavailability and side effects. Therefore finding natural compounds that overrule the above mentioned impediments and pursuing those that have been established as having promising therapeutic potential, with absolutely nil to significantly less physiological adverse side effects, could be effective ways of identifying drug alternatives for treating neurodegeneration. A compound that has already been sufficiently established for its various therapeutic potentials is costunolide, but its effect on microglial activation has never been studied so far. Hence investigating its ability to inhibit microglial activation could open avenues for this broad spectrum pharmacological compound for further exploration as a drug in treating neuroinflammation.
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1.3 Hypothesis and Research objective of this study

Costunolide, an active compound isolated from *Costus speciosus*, has long been used in traditional practices of oriental medicine. In the recent years its curative properties has been studied and scientific evidence confirming its mode of action has been mounting. Though there were many reports on the anti-inflammatory effect of the compound in numerous peripheral macrophages (discussed in Chapter 2), surprisingly no data was available on the usage and properties of this compound in the treatment of neuroinflammation diseases.

In many aspects, the neuropathology of neuroinflammation associated diseases is closely associated with microglial activation. So, the inhibition of the neuroglial activation may provide an effective therapeutic intervention that alleviates the progression of the neurodegenerative diseases. Continual investigation of the mechanisms underlying neuroglial activation, regulation of neuroinflammation, modulatory role of herbal medicines like costunolide in these processes would not only lead to the discovery of novel neuroprotective agents based on medicinal herbs, but also help to understand complex pathophysiology of neurodegenerative diseases.

The **hypothesis of this study** is that

- Costunolide (Cos) has an anti-inflammatory effect in neuropathological context and is a potent neuroprotector against brain pathologies
- Costunolide exerts its anti-neuroinflammation effect by interfering or modulating microglial activation.

**Research Objective**

Through the exploitation of *in vitro, in vivo* and *in silico* systems the following questions will be addressed:

- Does costunolide exert a reversal effect on microglia, activated by an inflammatory agent?
- What exact events/inflammatory molecules does Cos modulate in activated microglia?
- Which receptors could costunolide interact with, to bring about its pronounced anti-inflammatory effect?