1. Introduction

1.1. The neuroglia

The glial cells are non-neuronal cells of the central nervous system (CNS). They exist in high density in the mammalian brain presenting almost 10 times as many cells as neurons (Streit et al., 1995). In contrast to the early beliefs that neuroglia (in old Greek, glia = glue) represent only connective and supportive tissue of the nervous system, nowadays, they are considered as participants in virtually every function of the brain. They may affect the proliferation, differentiation and degeneration of neurons (Kreutzberg et al., 1996; Asensio et al., 1999; Hesselgresser et al., 1999). They are also involved in the regulation of neuronal transmission, tissue homeostasis and immunological response (Aschner et al., 1999).

In the CNS, there are three main types of glial cells: astrocytes, oligodendrocytes, and microglia.

1.2. Resting vs. activated microglia; Role in CNS homeostasis and pathology

Microglia are macrophage equivalent, immunoeffector cells of the CNS. They are abundantly present in all regions of the CNS tissue including optic nerve and retina, representing approximately 10-20 % of the entire CNS glial population (Lawson et al., 1990). Microglia first appear as colonies in the embryonic brain and migrate throughout the CNS, becoming distributed homogeneously through the entire nervous parenchyma (for review Cuadros and Navascues, 1998). Precursor cells take on amoeboid shape during development and differentiate into quiescent, ramified forms which do not overlap with neighboring microglia`s processes (Nimmerjahn et al., 2005). In the adult CNS, microglia can assume at least three clearly identifiable states: (1) resting or ramified microglia, as are present under physiological conditions; (2) activated or reactive microglia that are found in pathological states but are non-phagocytic, and (3) phagocytic microglia, that represent full-fledged brain macrophages. Resting microglia are characterized by extended pseudopodia (Fig. 1a), “down-regulated” immunophenotype, and a low rate of cell proliferation (Lawson et al., 1992). Microglial activation is accompanied with morphological changes, namely assumption of an

amoeboid shape with reduction and shortening of ramifications and enlargement of the cell body (Streit et al., 1998; Gehrmann et al., 1995a; Fig. 1b). Activated microglia express immunologically relevant molecules, proliferate, migrate, release soluble factors (cytokines, chemokines, reactive oxygen species) (Streit et al. 1988; Gehrmann et al., 1995b; Kreutzberg, 1996; Town et al., 2005), show increased basal Ca^{2+} levels (Hoffmann et al., 2003) and changes in established electrophysiological properties (Farber and Kettenmann, 2005).

Microglia play a major role in adaptive immune reactions within the CNS, performing antigen-presenting cell (APC) functions by the expression of CD11a, CD40, CD54, CD58, CD80, CD86 and class II major histocompatibility (MHC II) molecules (Zielasek et al., 1993; Aloisi et al., 1998). On the other hand, they serve as cellular carriers of innate-defense mechanisms recognizing certain structures of non-self and acting as phagocytic cells (for review Aloisi, 2001).

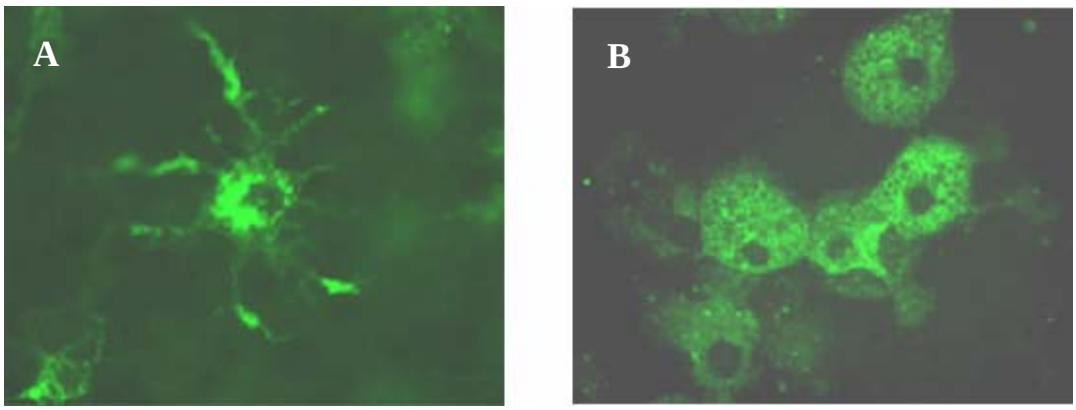

Figure 1. Morphology of "rested" and activated microglia in organotypic tissue culture.
Mouse retinal microglia in organotypic cultures stained with a fluorescent conjugate of the tomato lectin (A) before and (B) after a stimulation with LPS (10^{-7} g / ml. for 20 h).

While moderately activated microglia play the classical role as "scavengers" for the maintenance and restoration of the CNS, imbalanced activation or hyperactivation of microglia may cause significant neurodegeneration by producing excessive amounts of mediators, such as NO or inflammatory cytokines and chemokines, which are otherwise beneficial for killing bacteria and further activation of the immune system (Liu et al, 2001, Pocock and Liddle, 2001). Namely, microglia play a critical role in mediating

inflammatory processes in the CNS, which are associated with various neurodegenerative disorders. Presence of activated microglia has been demonstrated in pathological lesions in Alzheimer disease (AD), Parkinson disease (McGeer et al., 1993; Dehmer et al., 2000), multiple sclerosis (Boyle and McGeer, 1990), Huntington disease (Singhrao et al., 1999), and acquired immune deficiency syndrome dementia complex (Gelman, 1993). In addition to the progressive neurodegeneration in the CNS, microglial activation is proposed to be involved in the secondary damage following the primary damage after traumatic injury (Koshinaga et al., 2000).

1.3 Cytokines and chemokines as the key inflammatory mediators

Cytokines can be produced by virtually every nucleated cell type in the body, and they have pleiotropic regulatory effects on haematopoetic and other cell types that participate in host defense and repair processes (Hanisch, 2002). There are several characteristic features of cytokines (Thomson, 1994): (a) they are simple polypeptides or glycoproteins with a molecular weight usually $\leq$ 30 kD (some cytokines form higher molecular mass oligomers); (b) their constitutive production is usually low or absent; (c) their production is transient and the action radius is usually short (typical action is autocrine or paracrine) (d) they produce their actions by binding to specific high-affinity cell surface receptors (Kd in the range of 10^{-9}-10^{-12}M); (e) Most of their actions can be attributed to an altered pattern of gene expression in the target cells.

Microglial cyto/chemokine production is demonstrated both *in vitro* and *in vivo* (for review Hanisch, 2002) and may serve as a marker of the microglial activation. Among the long list of cytokines (including growth factors) and chemokines protein detection and mRNA-based techniques showed the ability of microglia to synthesise: interleukin-1 (IL-1) α, ß, and receptor antagonist -1ra, IL-3, -6, -8, -10, -12, -15, -18/interferon inducing factor (IGIF), tumour necrosis factor alpha (TNFα), transforming growth factor (TGFß), macrophage inflammatory protein-1alpha (MIP-1α) (CCL3), MIP-1ß (CCL4), -2ß (CXCL3), -3ß (CCL19), monocyte chemoattractant protein-1 (MCP-1) (CCL2), RANTES (regulated on activation, normal T cell expressed and secreted) (CCL5), macrophage derived chemokine (MDC) (CCL22), gamma interferon inducible protein-10 (IP-10) (CXCL10) and growth regulated oncogene alpha (GROα)/KC (CXCL1),

macrophage colony stimulating factor (M-CSF). Similarly, expression of related receptors has been demonstrated (Otero et al., 1994; Gebicke-Haerter et al., 2001).
In the CNS, an imbalanced cytokine production is mostly result of an excessive glial activation and is involved in neuropathologies like trauma, stroke, ischemia, infection or degenerative processes (Hopkins and Rothwell, 1995; Rothwell and Hopkins, 1995; Becher et al., 2000; Raivich et al., 1999a; Stoll et al., 2000; Hanisch et al., 2001).

1.4 Alzheimer's disease- potential contribution of inflammatory factors and processes

AD is a chronic, irreversible neurodegenerative disease that causes progressive impairment of memory and cognitive function. Neuropathological hallmarks of the disease include profound neuronal cell loss and brain atrophy, particularly in the hippocampus and cerebral cortex (for a review see Selkoe, 1999). There are three major pathogenic features of the disease: (a) the transformation of ß-amyloid precursor protein (APP) to form neurotoxic ß-amyloid (Aß) peptides and an aggregated insoluble polymer of Aß that forms senile plaques, (b) the formation of intraneuronal helical filaments of tau protein, so called "neurofibrilary tangles", and (c) the initiation and proliferation of a brain-specific inflammatory response (Akiyama et al., 2000). Two types of AD plaques have been identified: diffuse and neuritic. While diffuse plaques are composed of predominantly amorphous Aß but may contain a small population of the fibrils (Yamaguchi et al., 1989), neuritic senile plaques comprise clusters of Aß fibrils surrounded by dead and dying neurons as well as reactive astrocytes and microglia (Akiyama et al., 2000). The neurotic plaques are the foci of local inflammatory responses, cytokines, complement components and proteases (for reviews, see Eikelenboom et al., 1998, Cotter et al., 1999, Giulian 1999; McGeer and McGeer, 1999). Microglia associated with amyloid deposits, have a phenotype that is consistent with a state of activation, including immunoreactivity with antibodies to MHCII antigens, complement receptors and to inflammatory cytokines (IL-1ß, TNF-α etc.) (Dickson et al., 1993; Korotzer et al., 1995). Evidences from other studies in rodents indicate that microglia can be activated by neuronal degeneration (Ohno et al., 1992). These results suggest that microglial activation in AD may be secondary to neurodegeneration and

that, once activated, microglia may participate in a local inflammatory cascade that promotes tissue damage and contributes to amyloid formation (Dickson et al., 1993).

According to the amyloid hypothesis, accumulation of Aß in the brain is the primary influence driving AD pathogenesis. The rest of the disease process, including formation of neurofibrillary tangles containing tau protein, is proposed to result from an imbalance between Aß production and Aß clearance (Hardy and Higgins, 1992; Hardy and Selkoe, 2002). The amyloid cascade hypothesis suggests also that mismetabolism of the APP followed by subsequent formation of non-fibrillar and fibrillar Aß deposits leads to glial activation and subsequently to neurotoxicity (Bales et al., 1998).

The inflammatory hypothesis of AD suggests that neuronal injury results from the direct effects of inflammatory effectors-cytokines, activated complement and oxygen species- but also takes into account the indirect effects of cytokines or other inflammatory stimuli on increased production of neurotoxic Aß (Cotter et al., 1999; Giulian, 1999). Pronounced production of Aß1-40 and Aß1-42 was observed for instance, when primary astrocytes were stimulated with combination of INFγ+TNFα or INFγ+IL1ß (Blasko et al., 2000).

Several lines of evidence indicate that an inflammatory process contributes to the pathology of AD. First, inflammatory proteins have been identified as being associated with neuritic plaques and in glial cells surrounding these plaques (for review Giulian, 1999). Second, certain polymorphisms of acute-phase proteins and cytokines associated with AD plaques increase the risk or predispose for an earlier onset of developing AD (Ravaglia et al., 2005; Depboylu et al., 2006). Third, epidemiological studies indicate that anti-inflammatory drugs can retard the development of AD (Lim et al., 2000; Jantzen et al., 2002), and the mechanism is more likely to be through the suppression of microglial activity than by inhibiting the formation of senile plaques or neurofibrillary tangles (Mackenzie et al., 1998; Netland et al., 1998, Klegeris et al., 2002).

The cholinergic hypothesis of AD states that cholinergic neurons in the basal forebrain are severely affected and the resulting cerebral cholinergic deficit leads to memory loss and other cognitive and non-cognitive symptoms, which are characteristic for the illness (Bartus et al., 1982; Lawrence and Sahakian, 1998; Sivaprakasam, 2006).

Some alternative views point that it is the labile state of differentiation of a subset of neurons that - in the adult brain – retains a high degree of structural plasticity but at the same time renders these neurons particularly vulnerable and associates with the activation of mitogenic signalling and cell cycle activation (Arendt, 2002).

1.5 Aß, cytokines and other plaque-related proteins as microglia-activating agents

1.5.1. Amyloid beta peptide

ß-amyloid is the main protein component of the amyloid plaques found in the brains of patients with AD. It is a 39-43 amino acid fragment that is derived from the larger, membrane-spanning glycoprotein of approximately 700 amino acid residues called ß-amyloid precursor protein (APP) (Roher et al., 2000; Selkoe et al., 1994; Klein et al., 2001; Selkoe et al., 2001). Its gene is located on chromosome 21, which is also critically involved in Down syndrome, whose sufferers develop AD in their 30s (Beyreuther et al., 1993). APP is expressed in both neurons and glia (Schmechel et al., 1988). APP is cleaved to Aß through the proteolitic actions of ß- and γ-secretases (Busciglio et al., 1993; Shoji et al., 1992). The α-secretase activity cleaves APP within the Aß domain and thus precludes the generation of Aß. γ-secretase activity within transmembrane domains at the C-terminus determines releasing of either the 40- or 42-residue Aß peptides (Aß 1-40 and Aß 1-42) (Haass et al., 1992; Seubert et al., 1992; Shoji et al., 1992; Citron et al., 1996) (Fig. 2). The major Aß species is Aß 1-40, accounting for 70% of the Aß in brain, with about 15% counting for Aß 1-42. Mutations in the APP close to the γ-cleavage site have been shown to alter the cleavage specificity of the γ-secretase activity affecting the Aß 1-42/ Aß 1-40 ratio (Lichtenhalter et al., 1997; Evin et al., 2006). The prevalence of the hydrophobic Aß 1-42 peptide leads to extensive amyloid plaque formation. Aß peptides are capable of assembling into 60-100 Å diameter ß-sheet fibrils that exhibit the characteristic cross-ß X-ray fiber diffraction pattern. There is a nucleation-dependent polymerisation model, which explains the mechanisms of ß-amyloid fibril formation *in vitro* (Jarret et al., 1993; Harper and Lansbury, 1997; Lomakin et al., 1997). This model consists of two phases, i.e., nucleation and extension phases.

Once the nucleus has been formed, further addition of monomers becomes thermodynamically favourable, resulting in a rapid extension of amyloid fibrils.

Amyloid ß 1-40

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-OH

Amyloid ß 1-42

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile- Ala-OH

Figure 2. Primary structure of 1-40 and 1-42 amyloid ß protein. Aß 1-42 differs from Aß1-40 in two additional hydrophobic amino-acid residues: isoleucine and alanine, which could explain its higher potency to aggregate.

The fibril formation is required for Aß neurotoxicity (Lorenzo et al., 1994; Pike et al., 1995). Although it is well established that biological activity is dependent upon the aggregation state of the peptide (Pike et al., 1995; Howlett et al., 1995), the identity of the active species is unclear. Fibrillar synthetic peptide deposits, resembling those observed *in vivo* (Kirschner et al., 1987), are formed during *in vitro* aggregation or "aging" of Aß 1-40 in solution, and the presence of long straight fibrils appears to correlate with the neurotoxic properties of the peptide preparation (Howlett et al., 1995; Seilheimer et al., 1997). Other data suggest that smaller peptide oligomers may be the toxic form (Roher et al., 1996; Lambert et al., 1998).

1.5.2 Cytokines implicated in Alzheimer's disease

Virtually all the cytokines and chemokines that have been studied in AD, seem to be up-regulated in AD compared to non-AD control samples (Lanzrein et al., 1998; Grammas

and Ovase, 2001). However, Aß, the major component of the senile plaques in AD, has no direct stimulatory effect on microglial cytokine or reactive oxygen species production *in vitro* (Meda et al., 1995; Van Muiswinkel et al., 1996; Giulian, 1999) or induces only low levels of release activity (Lue et al., 2001). Furthermore, these studies showed that cytokine production and release of reactive oxygen species by Aß-stimulated microglia require co-stimulation with interferon-γ (INFγ), phorbol esters, or bacterial lipopolysaccharide (LPS) (Meda et al., 1995; Van Muiswinkel et al., 1996). However, since these compounds are not detectable/ present in AD brains, other co-stimulatory factors have been sought.

1.5.2.1 Interleukin-1

IL-1 is an immunostimulatory and proinflammatory cytokine that has a crucial role in innate defence and immune responses (Dinarello, 1997). In the CNS, it is known as an endogenous mediator of fever, sickness-behaviour, anorexia and slow-wave sleep, and a modulator of the hypothalamic pituitary adrenal axis (Rothwell and Hopkins, 1995; Rothwell, 1997). IL-1ß is massively secreted upon infection, ischemia, stroke, excitotoxicity or mechanical injury. It elicits a number of responses that lead to neuronal damage and death (Rothwell et al., 1997; Loddick et al., 1998; Rothwell and Luheshi, 2000), and is implicated in many neuropathological processes (Allan and Rothwell, 2001).

The IL-1 is overexpressed within affected cerebral cortical regions of the AD brain, as shown by quantitative assays of tissue IL-1 concentrations and by increased numbers of IL-1 immunoreactive microglia associated with AD plaques (Griffin et al., 1995; Griffin et al., 1998; Sheng et al., 1996). IL-1 overexpression seems to occur early in plaque evolution. It is already evident in diffuse, non-neuritic Aß (Griffin et al., 1995; Griffin et al., 1989). IL-1 promotes the synthesis (Goldgaber et al., 1989) and processing (Buxbaum et al., 1992) of APP and may therefore promote further amyloid production and deposition in plaques. A reciprocal relationship also seems to exist wherein the secreted form of APP (sAPP) activates microglia and induces their expression of IL-1 (Barger et al., 1997). IL-1 may also influence AD pathopharmacology through its ability to influence neuronal expression and activity of acetylcholineesterase (AchE) (Li et al.,

2000). IL-1 is a mediator of microglial pathological effects on tau phosphorylation in cortical neurons (Li et al., 2003).

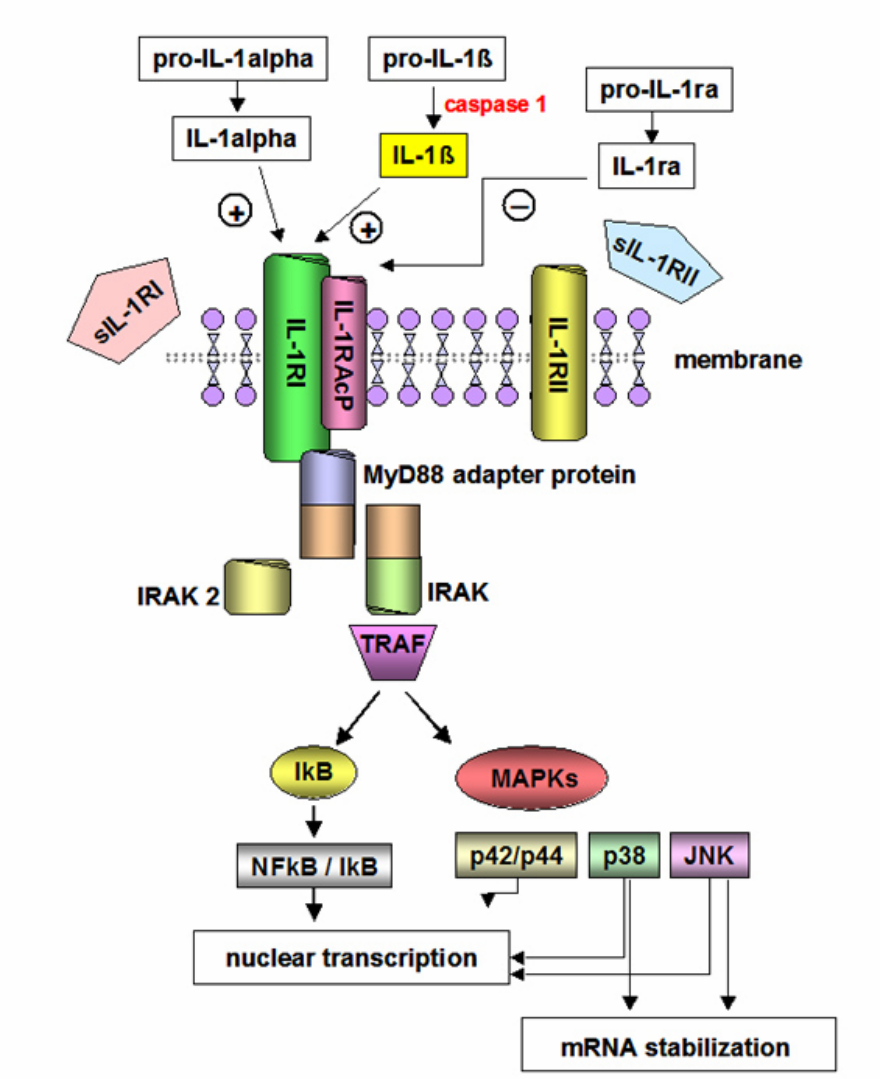

Figure 3. IL1: intracellular actions. Signalling cascade that follows binding of IL-1 ligand family members (IL-1α,ß) to IL-1 receptor

The IL-1 signalling pathway involves interleukin-1 receptor-associated kinases (IRAKs), serine/threonine kinases that act as signal transduction mediators for the Toll/IL-1

receptor (TIR) family members (Fig. 3). Their signalling pathways involve common intermediates including MyD88 (myeloid differentiation primary response gene 88), the members of the IRAK family (IRAK-1 and IRAK-2) and tumor necrosis factor receptor-associated factor 6 (TRAF6) (Cao et al., 1996; Li et al., 2002; Takatsuna et al. 2003). IL-1 intracellular signalling culminates in the activation of the NFκB and mitogen-activated protein kinases (MAPKs) p38, extracellular signal-regulated kinase p42/44 (ERK 1/2), and c-Jun N-terminal kinase (JNK) (reviewed in O`Neill and Greene, 1998). These MAPKs are activated by IL-1 also in glial cells (Zhang et al., 1998; Molina-Holgado et al., 2000) and drive the transcription of genes for other inflammatory mediators resulting in their release.

1.5.2.2 Interleukin-18

Based on sequence homology with IL-1 and IL-1ra, interleukin-18 (IL-18) (also called interferon-γ-inducing factor, IGIF or IL-1γ) was proposed to be a member of the IL-1 family. IL-18 and IL-1ß both depend on ICE/caspase-1 for maturation and release of functionally active forms (Akita et al., 1997). IL-18 signals through the IL-18 receptor complex (IL-18R), which although distinct from IL-1 receptors, also belong to the IL receptor family. The signalling cascade that follows IL-18 binding to IL-18 receptor complex and IL-1 binding to the IL-1RI complex involves in both cases IRAK association with TRAF6 and a following activation of downstream elements, such as protein kinases and NFκB (Cao et al., 1996; Kojima et al., 1998, Prinz et al., 1999). Some recent data from Lee et al. (2004) suggested that IL-18 may preferentially activate p38-MAPK and AP-1 and shows an inability of NF-κB activation.

Originally associated with the induction of Interferon-γ IL-18 is now regarded as a pleiotropic, proinflammatory cytokine (Dinarello et al., 1998). It has been shown in the brain that IL-18 is constitutively expressed in astrocytes and induced in microglia by LPS (Conti et al., 1999, Prinz et al., 1999). Only recently, its putative implication in AD was suggested. Malaguarnera and colleagues (2006) reported significantly elevated levels of IL-18 in plasma of AD patients compared to non-demented, age-matched subjects.

1.5.2.3 Interleukin-6

Interleukin 6 is a multifunctional cytokine, which regulates immune responses, and acute-phase reactions (Kopf et al., 1994). IL-6 exerts multiple effects on CNS cells, both beneficial and destructive. A variety of *in vitro* and *in vivo* studies provide evidence for IL-6 involvement in neuronal survival, protection, and differentiation (Hirota et al., 1996; März et al., 1997; Gadient and Otten, 1997; Loddick et al., 1998). It is involved in induction of sleep, fever, reduced food intake, and modulation of pain perception, functions that are also attributed to IL-1 actions in the brain. In glia, IL-6 promotes astrocyte proliferation, and is believed to be involved in astrogliosis, activation of microglia, and neurotoxicity (Campbell et al., 1993; Fattori et al., 1995). Together with IL-1, IL-6 was proposed as one of the first cytokines implicated in the pathology of Alzheimer's disease (Vandenabeele and Fiers, 1991). Activated microglia expressing IL-1ß and IL-6 immunoreactivity have been observed in close vicinity of the amyloid plaques in post-mortem tissue from AD patients (Thal et al., 1997). IL-6 mRNA induction was detected in the hippocampus and cortex of APPsw transgenic mice (Tehranian et al., 2001) as an early event in Aß-induced immune response cascade.

1.5.3 Alpha 2 macroglobulin

The acute phase proteins are a diverse set of molecules that arise early in inflammation as part of the acute phase response. Like many other inflammatory mediators, a wide range of acute phase reactants has been found in association with senile plaques and extracellular neurofibrillary tangles. These include the proteinase inhibitors α1-antichymotrypsin (Abraham et al., 1988), α1-antitrypsin (Gollin et al., 1992) and α_2-macroglobulin (Bauer et al., 1991).

Alpha 2-macroglobulin (α_2M) is a homotetrameric broad-spectrum protease inhibitor present at high concentration in plasma (Barrett et al., 1981). It is also distributed in various body fluids, including the cerebral spinal fluid (CSF) (Garton et al., 1991). In the brain, it is synthesized locally mainly by astrocytes (Businaro et al. 1992; Higuchi et al. 1994). The reaction with endoproteases or small nucleophile such as methylamine generates the activated α_2M by inducing a major conformational change in the inhibitor and exposing receptor recognition sites (Figler et al., 1991, Arakawa et al., 1994). α_2M

binding appears to involve two receptors: the low-density lipoprotein receptor-related protein (LRP) and the so-called “signalling receptor”. LRP participates in the uptake and degradation of several ligands implicated in the neuropathophysiology of AD, including apolipoprotein E (apoE), APP and α_2M. In the brain, LRP is present in pyramidal-type neurons of cortical and hippocampal regions and in activated astrocytes. The presence of LRP on microglial cells has been demonstrated by Marzolo et al. (2000). In macrophages, LRP is a scavenger receptor that binds to a variety of proteins, many of which trigger signal transduction. This pathway requires the activation of a pertussis toxin-sensitive G protein and is blocked by the receptor associated protein (RAP) or Ni^{2+} (Misra et al., 1993, 1994). The binding to the newly described α_2M signalling receptor appears to be coupled to a pertussis toxin (PTX)-insensitive G protein and triggers hydrolysis of membrane phosphoinositides, initiating Ca^{2+}-dependent cellular events and the activation of the $p21^{ras}$-dependent MAPK and phosphoinositide 3-kinase (PI 3-kinase) signalling cascades (Misra et al., 1993, 1994, 2002). Misra et al. (2002) have shown that the response of macrophages to activated α_2M requires the transcription factors NFκB and cAMP-responsive element-binding protein (CREB).

There is a whole line of evidence pointing to the implication of α_2M in the pathology of AD. Increased α_2M-immunoreactivity was found in AD brain. In particular, the large hippocampal neurons exhibited increased intracellular staining for α_2M (Bauer et al., 1991; Strauss et al., 1992). Later, α_2M was found to be localized specifically to neuritic but not to diffuse plaques (Van Gool et al., 1993). Increased α_2M levels, compared to non-AD controls, were found in AD temporal cortices by ELISA (Wood et al., 1993). α_2M shows the potency to bind Aß with high specificity, regardless of the activation state of the molecule (Du et al., 1997). Finally, the α_2M gene is listed among the factors, which show a genetic background for AD development. In addition, two independent studies have found that the α_2M receptor- LRP itself was associated with late onset familiar AD (Kang et al., 1997; Hollenbach et al., 1998).

The role of α_2M in the neurodegenerative process is still not clear, given that α_2M has been shown to exert both neuroprotective and neurotoxic effects on cultured neurons. Namely, it was suggested that α_2M might mediate Aß clearance in the LRP-independent

pathway when complexed with an unidentified serine protease (Qiu et al., 1999). Some studies reported that the high affinity binding of Aß to α_2M might prevent Aß fibril formation and neurotoxicity associated with aggregated Aß (Du et al., 1998; Hughes et al., 1998). Conversely, in human neuroblastoma cells, it was shown that activated α_2M increases Aß (25-35)-mediated toxicity (Fabrizi et al., 1999).

The interest in the potential effect on microglial cells is based on the fact that α_2M is considered an immunoregulatory protein. For instance, induction of cyclooxygenase-2 in macrophages has been demonstrated (Misra et al., 2001). α_2M as a TGFß carrier is also responsible for induction of nitric oxide (NO) and NO-dependent cytotoxicity (Lysiak et al., 1995; Webb et al., 1996). Data have shown that cytokines, like IL-2, bind directly to α_2M (James et al., 1994). While the native α_2M does not interfere with IL-2 biological activity, an interaction with activated forms of α_2M leads to a loss of IL-2 activity (Legres et al., 1995). In turn, cytokines like INF-γ and IL-6 have been shown to upregulate α_2M in human astrocytoma and neuroblastoma cells, respectively (Fabrizi et al., 1994; Ganter et al., 1991). An IL-1ß-induced synthesis and release of α_2M in human glia has been reported (Gao et al., 2002).

1.6 Lypopolysacharide (LPS) as a model stimulus for microglial activation

For the elucidation of microglial activation, many attempts have been reported using cell culture systems to specify a variety of factors in the rather complicated mechanism. In *in vitro* experiments using cultured microglia, LPS, glycolipid derived from the membrane surface of Gram-negative bacteria (endotoxin), has generally been used as a tool for activation. During CNS infections such as meningitis, microglia can be activated by encountering the bacterial cell wall. Thus, LPS is routinely used to stimulate microglia for full activation in *in vitro* experiments or to study the modulatory influences of other factors on activated cells. It can trigger a series of inflammatory reactions in microglia/ macrophages. With LPS stimulation, microglia are activated to change their cellular features drastically and to produce an array of cytokines, i.e. TNFα, IL-1ß, IL-6, IL-12p40, chemokines, i.e. MIP-1α, RANTES, neuronal growth factors, BDNF and NGF or prostaglandins (Prinz et al., 2001; van Rossum and Hanisch, 2004). In addition, LPS

stimulates inducible nitric oxide synthase (iNOS) expression and NO production (Colasanti et al., 1995). Activation of LPS-responsive cells occurs rapidly after LPS interacts with circulating LPS-binding protein (LPB) and CD14. CD14, a glycosylphosphatidylinositol (GPI)-anchored membrane protein that acts as a bacterial pattern recognition receptor (Da Silva Correia J. et al., 2001), has been identified as the main LPS receptor. However, CD14 binds LPS but is not capable of initiating a transmembrane activation signal since it does not contain a cytoplasmic domain. Indeed, CD14 serves as a member of the heteromeric LPS receptor complex that also contains Toll-Like receptor 4 (TLR-4) and MD2 (as shown in Fig. 4). Upon LPS binding, CD14 physically associates with TLR4, a member of Toll-Like receptor family that contains a conserved region called the Toll/IL-1 receptor (TIR) domain. Stimulation of TLRs by conserved structural motifs only expressed by microbial pathogens, so-called pathogen-associated microbial patterns (PAMPs), initiates a signalling cascade that involves a number of proteins, such as MyD88 and IRAK (Fig. 4). This signalling cascade leads to the activation of the transcription factor NF-kB, which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response. Interestingly, TLRs and members of the pro-inflammatory interleukin-1 receptor (IL-1R) family, share homologies in their cytoplasmic domains called Toll/IL-1R/plant R gene homology (TIR) domains. Intracellular signalling mechanisms mediated by TIRs are similar, with MyD88 (Wesche et al., 1997, Kawai et al., 1999) and TRAF6 (Cao et al., 1996b, Lomaga et al., 1999) having critical roles.

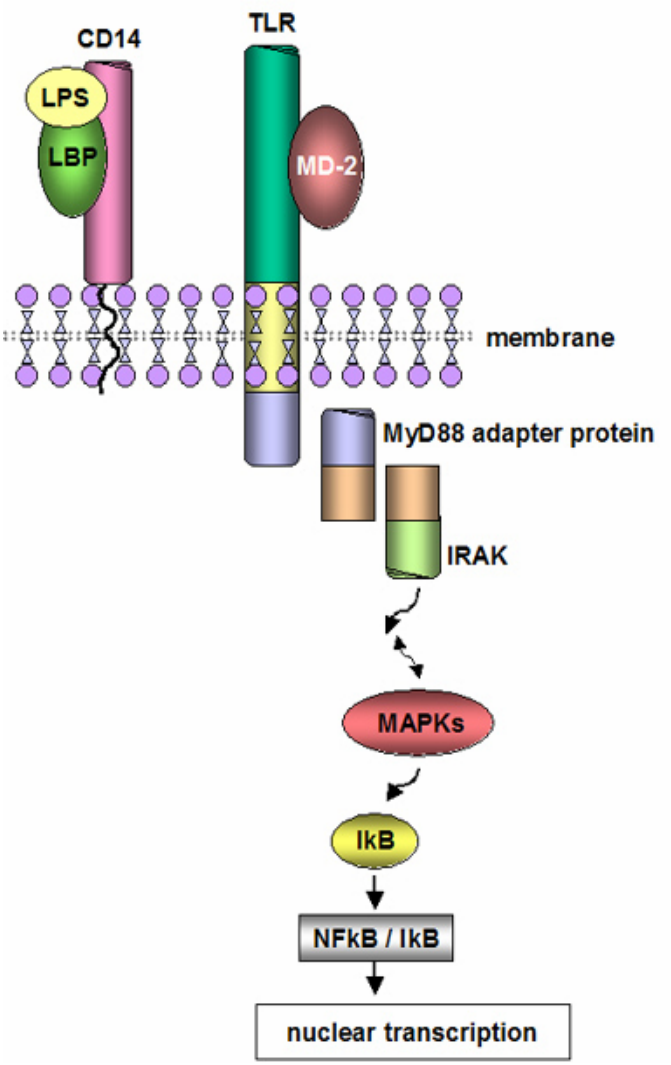

Figure 4. Intracelular signalling cascade upon LPS stimulation (modified from Aderem & Ulevitch, 2000); Involvment of MyD88 adapter protein, IRAK and TRAF point out an overlap with the IL-1 signalling pathway.