CHAPTER 8

Summary and general discussion
The hypothesis of an immune privileged central nervous system (CNS) dates back to the beginning of the 20th century when several studies reported potential immune privileged regions in the brain (Shirai, 1921; Billingham and Boswell, 1953; Medawar, 1948). Immune privilege is defined as an evolutionary adaptation to ensure protection of vital body regions from the inflammatory immune response. Nowadays, CNS immune privilege is considered relative and rather an active than a passive process (Galea et al., 2007). An example of the influence of the immune system on the regulation of brain neuronal homeostasis is represented by cytokine activity. Cytokines, such as TNF-α are involved in a myriad of cellular responses including inflammation, growth, and cellular differentiation in immune and non-immune cells. Under several pathological conditions, such as neurodegeneration, cytokine expression was found to be up-regulated (Fillit et al., 1991).

Neurodegeneration is a detrimental process that results in progressive loss of neuronal function and ultimately in neuronal death. Thus, the investigation of the role of neuroprotective molecular mechanisms in preventing and treating neurodegenerative diseases is of great importance. In neurodegenerative diseases glutamate is released in high quantities and represents the major cause of neuronal death (Olney et al., 1972).

One model to study in vitro the molecular mechanisms underlying neurodegeneration is the glutamate-induced excitotoxicity paradigm, where neurons are treated with glutamate to induce neuronal death and several substances are tested for their potency to prevent this neuronal death. Hitherto, a number of studies showed that the cytokine TNF-α is able to promote neuroprotection against glutamate-induced excitotoxicity in cortical neurons (Barger et al., 1995; Cheng et al., 1994; Fontaine et al., 2002; Marchetti et al., 2004). Until now, the molecular mechanisms underlying these beneficial effects of TNF-α are largely unknown. An initial study showed that TNF-α increases neuronal survival by activating the TNF-R2 pathways (Marchetti et al., 2004). TNF-R2-associated neuroprotective effects are mediated by the activation of PKB/Akt (Fontaine et al., 2002) and subsequent sustained NF-κB activation (Marchetti et al., 2004).

In this thesis the underlying molecular mechanism of the neuroprotective TNF-α effect was investigated in more detail. Furthermore, the kinetics of the protective TNF-α signaling, upstream molecules and downstream targets involved in this beneficial TNF-α effect were studied.

8.1 Long term TNF-α treatment changes the neuronal state from a vulnerable to a resistant state against excitotoxic insults

8.1.1 PKB/Akt and NF-κB play a major role in TNF-α mediated neuroprotection signaling.
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Chapters 2 and 4 of this thesis investigate the neuroprotective mechanisms by which TNF-α enhances neuronal resistance against glutamate-induced excitotoxicity. First, the kinetics of the neuroprotective effect of TNF-α in primary cortical neurons was studied. Short-term exposure of neurons for 2-4 h to TNF-α did not rescue neurons from the glutamate-induced cellular death. However, after 6-8 h or longer of TNF-α treatment neuronal survival was significantly increased.

Several studies showed the influence of TNF-α treatment on synaptic transmission (Beattie et al., 2002; Stellwagen and Malenka, 2006; Stellwagen et al., 2005). Interestingly, TNF-α acting on neuronal TNF-R1 receptors, but not on TNF-R2 receptors increases surface AMPA receptors expression through a PI3-Kinase-dependent pathway after 20 to 40 min (Stellwagen et al., 2005). This fast increase of excitatory synaptic transmission (AMPARs) is concomitant with endocytosis of GABA receptors that weakens the inhibitory synaptic strength. This TNF-α effect could explain why the first hours of TNF-α treatment were not able to induce neuroprotection against glutamate excitotoxicity.

It was previously shown that long term (24 h) TNF-α treatment causes an increase of PKB/Akt phosphorylation (Marchetti et al., 2004). PKB/Akt phosphorylation in cortical neurons is associated with increased cellular survival (Lawlor and Alessi, 2001). Chapter 2 describes the PKB/Akt kinetics upon TNF-α treatment. Since PTEN is the major negative regulator of PKB/Akt activation (Bellacosa et al., 2004) the PTEN expression upon TNF-α treatment was investigated. In previous studies, an increase of PTEN expression in cortical neurons was shown to induce neuronal death (Gary and Mattson, 2002). Short term TNF-α treatment (2-4 h) induced an increase of PTEN expression and a decrease of PKB/Akt phosphorylation. However, long term TNF-α treatment (6-8 to 24 h) resulted in a decrease of PTEN expression and augmented PKB/Akt phosphorylation in a time dependent manner. The fact that high levels of PTEN coincided with low levels of PKB/Akt phosphorylation and vice versa is in accordance with the inverse relationship between PTEN and PKB/Akt activation.

To assess the contribution of both TNF-receptors, TNF-R1 and/or TNF-R2 to the neuroprotective TNF-α mediated pathway, PKB/Akt and PTEN expression in TNF-R1−/− and TNF-R2−/− neurons was investigated. It appeared that in TNF-R1−/− neurons, PKB/Akt was phosphorylated after long term TNF-α treatment with visible effects after 6-8 h, while in TNF-R2−/− PKB/Akt phosphorylation was down regulated in a TNF-α treatment dependent manner (Chapter 2). From these data we conclude that in wild-type neurons the increase of PTEN expression and the decrease of PKB/Akt phosphorylation observed in the first hours of TNF-α treatment could be attributed to TNF-R1 signaling, while the later decrease of PTEN levels and increase of PKB/Akt phosphorylation are due to TNF-R2 signaling (Fig. 8.1).

The increase of PKB/Akt activation can be explained by the low levels of PTEN expression but the remaining question is how is PTEN expression down regulated after several hours of TNF-α treatment. This may occur through the TNF-α-mediated NF-
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Figure 8.1: The inverse relationship between PTEN and PKB/Akt activation in neuronal survival mechanisms. A. PTEN expression and B. PKB/Akt phosphorylation in wild-type, TNF-R1\(^{-/-}\) and TNF-R2\(^{-/-}\) neurons treated with TNF-\(\alpha\) for 24 h.

\(\kappa B\) activation signaling (Kim et al., 2004).

In neurons, activation of NF-\(\kappa B\) is associated with increased neuronal survival (Mattson and Camandola, 2001). Evidence for the hypothesis that NF-\(\kappa B\) might be involved in PTEN down regulation comes from the fact that in cells that are deficient in NF-\(\kappa B\) activation, TNF did not repress PTEN (Vasudevan et al., 2004). Indeed, it was demonstrated that a decrease of PTEN levels by NIK/NF-\(\kappa B\) results in activation of the PI3K/Akt pathway and an increase of TNF-\(\alpha\)-induced PI3K/Akt activation. NF-\(\kappa B\)-inducing kinase (NIK) participates in the regulation of NF-\(\kappa B\) pathway by activating the I(\(\kappaappa\)B) kinase (I\(\kappa kappa\)B) both in vivo and in vitro. I\(\kappa kappa\)B is a temporal regulatory switch to turn off NF-\(\kappa B\) activity. However it is not known how the activity of NIK is regulated (Xiao and Sun, 2000).

TNF-\(\alpha\) mediated activation of NF-\(\kappa B\) via PKB/Akt was shown to be dependent on the inhibitors of IKK complex (I\(\kappa kappa\)B-\(\alpha\) or I\(\kappa kappa\)B-\(\beta\)). Cells with higher I\(\kappa kappa\)B-\(\alpha\) tend to be more responsive to PKB/Akt-induced NF-\(\kappa B\) activation, whereas cells with higher I\(\kappa kappa\)B-\(\beta\) tend to be less responsive to TNF/PKB/Akt-induced NF-\(\kappa B\) activation (Gustin et al., 2004). These results are in good agreement with our findings, since PTEN levels were down regulated after long term TNF-\(\alpha\) treatment (Chapter 2), a time frame that coincided well with an increased and persistent NF-\(\kappa B\) activation (Marchetti et al., 2004).

Besides PTEN, PKB/Akt signaling is modulated by several other molecules, \textit{inter alia} by carboxyl-terminal modulator protein (CTMP) through a mechanism that involves direct protein-protein interaction (Maira et al., 2001) or by a recently discovered PH domain leucine-rich repeat phosphatase (PHLPP) (Gao et al., 2005).

Since PKB/Akt pathway could be regulated by cyclic adenosine monophosphate (cAMP)-dependent signaling as well, Chapter 3 investigated the effect of two main-stream pathways initiated by cAMP, cAMP-dependent protein kinase (PKA) and
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Exchange proteins directly activated by cAMP (Epac1 and Epac2) on PKB/Akt phosphorylation in primary cortical neurons. PKA activation led to a decrease of PKB/Akt phosphorylation, whereas activation of Epac increased PKB/Akt phosphorylation. PKA, PKB/Akt and Epacs were all shown to be complexed with the neuronal A-kinase anchoring protein 150 (AKAP150). Particularly, activation of Epac2 increased phosphorylation of PKB/Akt complexed to AKAP150, whereas silencing of cellular Epac2 diminished PKB/Akt phosphorylation. From experiments using PKA binding deficient AKAP150 and PKA disrupting anchoring to AKAP150 peptides, AKAP150 was found to act as a key regulator in the two cAMP pathways that control PKB/Akt phosphorylation (Fig. 8.2).

Several reports showed that PKB/Akt activation is essential for cellular survival and for TNF-α mediated neuroprotective pathway (Marchetti et al. (2004); Lawlor and Alessi (2001), Chapter 2 of this thesis). The CNS is endowed with three PKB/Akt isoforms: PKBα/Akt1, PKBβ/Akt2 and PKBγ/Akt3, therefore we were interested in the question which PKB/Akt isoforms are important for TNF-α mediated neuroprotection. To answer this question we transfected primary cortical neurons with siRNA probes for individual PKB/Akt isoforms. NR2B/TNF neurons were chosen since they express constitutively elevated levels of activated PKB/Akt compared with wild-type neurons. Furthermore, NR2B/TNF neurons express regionally high levels of TNF-α and are totally resistant to excitotoxic effects induced by glutamate treatment (Marchetti et al., 2004). In NR2B/TNF mice TNF-α is expressed under the control of the murine NMDAR subunit NR2B promoter. Using this system, PKB/Akt1 and PKB/Akt3 isoforms were found important for TNF-α mediated neuroprotection, while PKB/Akt2 had minimal effects on PKB/Akt phosphorylation (Chapter 2).

Another essential player in TNF-α induced neuroprotective signaling is NF-κB (Marchetti et al., 2004). One possible downstream molecule of NF-κB activation is represented by small conductance Ca\(^{2+}\)-activated potassium (SK) channel. Recently two NF-κB binding sites were shown to regulate the murine SK2 promoter (Kye et al., 2007). Therefore, in Chapter 4 it was investigated whether SK channels are possible downstream targets of TNF-α signaling (Chapter 4).

SK channels modulate synaptic excitability and neuronal activity e.g. by contributing to afterhyperpolarization processes. The hypothesis of the neuroprotective properties of SK channels in cortical neurons originates from the SK channels’ capability to lower the firing frequency of action potentials and to dampen NMDA receptor activity. Therefore, SK channels may enhance the resistant state of neurons against glutamate excitotoxicity (Stocker, 2004). The connection between potassium channels and TNF-α mediated neuroprotection was suggested by Houzen and colleagues (1997) who showed that the TNF-α induced neuroprotective effect is dependent on the increase of outward potassium current (A-current) density in cortical neurons. The A-current regulates the firing activity of neurons and it reduces the neuronal excitability. Interestingly, a specific blocker of A-current inhibits the TNF-α neuroprotective
effect (Houzen et al., 1997).

In the present study, SK2 channel expression is augmented upon TNF-α treatment. This increase of SK2 channel expression corresponded with the time point of increased PKB/Akt phosphorylation and increased cellular survival against glutamate toxicity (Chapter 2). The neuroprotective function of SK channels was demonstrated by the finding that the SK channel activators, NS309 and CyPPA, promoted neuroprotection against glutamate-induced excitotoxicity in primary cortical neurons. In addition, apamin, a specific antagonist of SK2 channels, partially blocks the protection mediated by TNF-α indicating that SK channel activity may underlie the TNF-α-induced neuroprotective mechanisms. The increase of SK2 channel expression after TNF-α treatment was shown to be NF-κB-dependent since the NF-κB inhibitor BAY11-7082 blocked the TNF-α-induced increase in SK2 channel expression.

Although TNF-R2, PKB/Akt and NF-κB were suggested to be important in TNF-α induced neuroprotective mechanisms (Fontaine et al. (2002); Marchetti et al. (2004), this thesis), data on potential up-stream activators of these signaling pathways were not available yet. Interestingly, a commercially available drug, lovastatin commonly used in coronary artery disease was recently discovered to specifically enhance TNF-R2 expression in HUVEC cells (Nübel et al., 2005b). In addition, lovastatin was proposed as neuroprotective agent for several neurodegenerative disorders. Although both lovastatin and TNF-α were shown to be neuroprotective, so far their signaling pathways had been studied independently. The study described in Chapter 5 shows that lovastatin can induce TNF-R2 expression in primary cortical neurons. It was demonstrated that NF-κB is responsible for the lovastatin-dependent increase of TNF-R2 expression (Chapter 5). Since in TNF-R1−/− neurons inhibition of PKB/Akt activity reverted the protective effect induced by lovastatin (Figure 8.2) it was concluded that lovastatin-induced TNF-R2 expression contributes to neuronal survival through PKB/Akt activation.

The mechanisms through which lovastatin activates the neuroprotective TNF-R2/PKB/Akt pathway remain elusive (Chapter 5). One possibility is that lovastatin influences TNF-R2 signaling by reducing the cellular cholesterol levels. In endothelial cells statins induce the recruitment of PKB/Akt to the plasma membrane, which results in the activation of PKB/Akt. This process could be attributed to the disruption of cholesterol-rich microdomains of the plasma membrane called lipid rafts (Skaletz-Rorowski et al., 2003). Statins deplete the lipid rafts and promote receptor clustering and initiation of TNF-R signaling. Related to this hypothesis, it is still under debate whether statins are able to reduce cholesterol levels in the brain (Wolozin, 2004). In cultured cortical neurons it was shown that statins reduce the total pool of cholesterol (Zacco et al., 2003). However, in the brain, due to tight cholesterol regulation, even high doses and long-exposure time (3 months) to statins had a minimal effect on brain cholesterol levels (Johnson-Amna et al., 2005).
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Another possibility to explain the lovastatin-induced neuroprotective mechanisms via TNF-R2/PKB/Akt signaling is the recently discovered relationship between Rho-associated kinase (ROCK) signaling and PTEN. ROCK activates PTEN in HEK cells and leukocytes through Rho-associated kinase (RhoA). By blocking the isoprenylation of RhoA and ROCK, statins could inhibit the activation of PTEN, which in turn would induce PKB/Akt phosphorylation. Interestingly, TNF-α activates RhoA through TNF-R1 signaling. Depletion of cholesterol from lipid rafts caused a redistribution of TNFR1 to non-lipid raft area of the plasma membrane and prevented ligand-induced RhoA activation (Lotocki et al., 2004). These findings are in agreement with the observation that TNF-R1 increases PTEN expression in cortical neurons (Chapter 2). In addition, lovastatin treatment could abolish PTEN activation (Pedrini et al., 2005), repress TNF-R1-mediated RhoA activation (Lotocki et al., 2004) and increase PKB/Akt phosphorylation via TNF-R2 (Chapter 5). Overall, these findings may explain how lovastatin mediates neuroprotection via TNF-R2/PKB/Akt pathways (Figure 8.2).

Chapter 6 consolidates the role of PKB/Akt as a downstream molecule in lovastatin-mediated neuroprotective signaling. Using an in vivo model, in which NMDA was infused in the nucleus basalis of Meynert (MNB), located in the basal forebrain cholinergic complex, the effect of lovastatin on cortical cholinergic fibers was investigated. Since the basal forebrain provides the major input in cholinergic projections to the cerebral cortex and hippocampus (Mesulam and Geula, 1988; Gaykema et al., 1990) the cortical cholinergic target area was quantified to determine the magnitude of cholinergic fiber loss produced by NMDA lesions in MNB. Although brain cholinergic neurons have different sensitivity to pathogenic insults, the basal forebrain cholinergic neurons are particularly susceptible to neuronal death when exposed to toxic agents, such as glutamate or nitric oxide (Fass et al., 2000; Schliebs and Arendt, 2006). Lovastatin treatment significantly protected cholinergic neurons and their cortical projections against NMDA induced cell death in MNB. Furthermore, lovastatin-mediated neuroprotection was shown to be dependent on protein kinase B (PKB)/Akt signaling since inhibition of PKB/Akt with LY294002 blocked the lovastatin-induced neuroprotective effect (Chapter 6).

Several studies already provided evidence of a decrease of presynaptic cholinergic markers in the cerebral cortex of patients with early-onset of AD (Davies and Moloney, 1976; Bowen and Davison, 1980). The decline of cortical cholinergic markers is highly linked with the memory deficiency in AD patients. A correlational study of the cognitive performance and the cholinergic neurons of the basal forebrain in both aged human patients and mild cognitive impaired human patients shows that the cognitive deficits are detectable when at least 30% of the total cholinergic basal forebrain neurons are degenerated (Arendt, 1999). Since lovastatin treatment was shown to prevent cortical cholinergic fiber loss produced by NMDA infusion in MNB (Chapter 6) the memory performance was tested in a passive avoidance behavior paradigm.
Figure 8.2: Proposed TNF-α mediated neuroprotective mechanisms in primary cortical neurons

and a spontaneous alternation test. Lovastatin treatment improved memory deficits in NMDA-lesioned mice. Several studies showed as well that statins improve the spatial learning and memory deficits after hypoxic-ischemic or traumatic brain injuries in rats (Balduini et al., 2003; Lu et al., 2007); and learning and attention in a mouse model of neurofibromatosis type I (Li et al., 2005). Overall, the study presented in chapter 6 substantiates the lovastatin-induced increase in learning and memory performance effect.

The presence of distinct NMDA-Rs subunits in the CNS and the fact that these NMDA-Rs subunits may play different roles in the brain suggests a relationship be-
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tween receptor subunit composition and receptor function (Cull-Candy and Leszkiewicz, 2004). The structure and function of NMDA-Rs subunits is altered in several neurodegenerative disorders (Wang et al., 2000). For example in AD NMDA receptor subunits are selectively and differentially reduced in the cortex and hippocampus, brain regions known to be involved in learning and memory (Maragos et al., 1987). Particularly, the NR2B receptor subunit is significantly diminished in the hippocampus (40%) and the entorhinal cortex (31%) when compared with the levels in age-matched control human patients (Sze et al., 2001; Hynd et al., 2004).

The functional properties of NMDA-Rs are influenced mainly by the type of NR2 subunit (Cull-Candy et al., 2001). Several studies showed that NMDA-R featuring NR2B subunits compared with NMDA-R bearing NR2C subunits, provide special properties, e.g. a higher Ca$^{2+}$ conductance, higher Mg$^{2+}$ sensitivity, higher excitatory postsynaptic potentials (EPSPs) (Nakanishi and Masu, 1994). Generation of NR2C-2B mutant mice, in which NR2B cDNA insertion into the gene locus of the NR2C gene, replaced NR2C by NR2B throughout the whole brain allowed to further investigate the consequences of this subunit exchange. Alterations in cerebellar morphology together with motor deficits were found in NR2C-2B mutant mice. In addition, the amplitude of NMDA currents was found increased in cerebellar slices of NR2C-2B mutant mice compared with wild-type neurons (Schlett et al., 2004).

Since the overexpression of the NR2B subunit in the forebrain improves object recognition memory (Tang et al., 1999) and increased cortical ACh levels are associated with memory performance (Abe and Iwasaki, 2001), NR2C-2B mutant mice were used to examine whether a subunit exchange in cholinergic neurons would affect acetylcholine (ACh) content in several brain structures (Chapter 7). Interestingly, this NR2C-2B receptor subunit exchange induced an increase in the content of ACh in cholinergic target regions from MNB, such as the frontal cortex and amygdala. Although brain ACh modulates neuroplasticity and novelty-induced arousal, the behavioral analysis in adult mice (novel object recognition) did not indicate any gross behavioral alteration in the adult mutant mice compared with the adult wild-type mice (Chapter 7). A follow up study in juvenile mice investigated dopamine, serotonin content in the frontal cortex and in regions related to the defense system, such as periaqueductal grey matter (De Souza Silva et al., 2007). Furthermore, juvenile NR2C-2B mice showed increased open arm avoidance in the elevated plus-maze and increased fear-induced immobility. The increase of serotonin content in the frontal cortex and the decrease of ACh levels in periaqueductal grey matter was associated with enhanced anxiety- and fear-related behaviors possibly due to the replacement of subunit NR2C by NR2B in juvenile mice (De Souza Silva et al., 2007).

Increase of cholinergic transmission in human patients with mild cognitive impairment has been shown to improve hippocampal function (Grön et al., 2006). Therefore, it would be interesting to test in future experiments whether the increased level of ACh in the frontal cortex of NR2C-2B transgenic mice could compensate the memory deficit induced by NMDA lesions in MNB.
8.2 Degenerative and protective effects of TNF-α in CNS

Several studies investigating the effect of TNF-α on cellular survival/death showed that TNF-α could trigger both neuroprotective and neurodegenerative signaling pathways.

Excess TNF-α causes cell death of human cortical neurons and oligodendrocytes (Chao and Hu, 1994). Another degenerative TNF-α effect was attributed to the so-called neurotoxic synergy between TNF-α and glutamate. For example, in a spinal cord trauma model, TNF-α was shown to exacerbate cellular injury processes through excitotoxic interactions with glutamate and increased c-Fos expression (Hermann et al., 2001). In organotypic hippocampal brain slices, TNF-α potentiates cellular death initiated by glutamate through an NMDA receptor-dependent pathway and by NF-κB activation (Zou and Crews, 2005).

On the other hand, accumulating evidence indicates that TNF-α contributes to neuroprotection against glutamate-induced excitotoxicity in cortical neurons (Cheng et al. (1994); Marchetti et al. (2004), this thesis).

An explanation for this dual effect of TNF-α may lie within different experimental protocols (e.g. duration, concentration), cellular types (e.g. neurons, T cell, lymphocytes) cellular models or source of TNF-α (murine, human). To make things even more complicated, within CNS some neuronal populations vary in their expression levels of the endogenous TNF-α and TNF-Rs levels in diseased and non-diseased brain (Fontaine et al., 2002). Several studies faced the difficulty of differentiating the dual effect of TNF-α on cellular viability. Most of the protocols used soluble TNF-α (sTNF-α) to investigate TNF-α signaling (Shohami et al., 1999). Since sTNF-α binds with a higher affinity to TNF-R1 it was for a long time rather impossible to recognize subtle TNF-R2-mediated effects. The finding that inhibitors of TNF-α converting enzyme (responsible for cleaving sTNF-α from the membrane-bound TNF-α) protected neurons from focal brain ischemia (Wang et al., 2004) indicated a possible involvement of TNF-R2 in the neuroprotective pathways. By using mice lacking TNF-R1, TNF-R2 or both receptors, it has become much easier to study the contribution of TNF-Rs to neuroprotective/neurodegenerative pathways.

8.2.1 “Est consensus in rebus” (There is a link between all things).

First of all, “Est modus in rebus” (There is a proper measure in all things) is a quote that describes well the effect of TNF-α doses on neuronal survival. An important example related to the effect of different concentrations of TNF-α on neuronal viability is represented by the effect of TNF-α on dopaminergic neurons. Loss of dopaminergic neuronal function leads to typical symptoms of Parkinson’s disease (Rinne et al., 1987). In experimental animal models for Parkinson’s disease 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or MPTP is widely used to induce Parkinson’s-like symptoms (Jenner and Marsden, 1986). However, the loss of dopaminergic neurons in
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the substantia nigra and subsequently the Parkinson’s like symptoms induced by MPTP treatment are reduced in the absence of TNF-α (Ferger et al., 2004). Interesting, though, is the fact that low endogenous levels of TNF-α promote neuroprotection against 6-hydroxy-dopamine (6-OHDA)-induced lesions in dopaminergic cells (Pitossi et al., 2003). Overall, these experiments showed that elevated TNF-α levels in dopaminergic neurons of the substantia nigra promote neuronal death, whereas low levels of TNF-α exert neuroprotective functions.

Secondly, duration of TNF-α treatment or “Est tempus in rebus” (There is a time to all things) is crucial in promoting neuroprotective signaling. In primary cortical neurons short-exposure time (2-4 h) to TNF-α failed to induce neuroprotection (Chapter 2), whereas longer exposure time (6-8 h) induced increased cellular survival against glutamate toxicity.

The inter-connectivity between up-stream molecules and downstream targets of TNF-α plays an important role in TNF-α protective mechanisms (“Est consensus in rebus” (Everything is inter-connected)). For example, lovastatin (described as one of the up-stream molecules of TNF-α signaling), PKB/Akt and NF-κB activation (as down-stream targets) are associated to TNF-α-mediated cellular survival mechanisms (Chapters 2, 4, 5). Understanding and controlling these neuroprotective pathways could lead to the development of new treatments for preventing neuronal death that occurs in several neurodegenerative disorders.

8.2.2 “Est evolvere in rebus” (All things evolve and adapt).

Necrosis is a cellular death process characterized by an increase in cellular entropy due to the cell’s inability to balance internal-external flux of ions and to perform normal functions. In order to prevent the spread of cellular death to other cells, organisms have evolved over millions of years a form of adaptation, a programmed, ordered, non-energy dependent mechanism called apoptosis. This “programmed cell-death” mechanism has several switch-off points. For example, TNF-α signaling in neurons could interfere in the apoptotic pathways and change the balance towards programmed-cell survival.

Microarray study of approximately 22,600 genes attempted to clarify the down-stream TNF-α signaling pathways (Quintana et al., 2007). An important group of genes involved in the development and cellular survival (heat shock proteins, NF-κB, c-fos) were found down regulated in the cortex of TNF-R2⁻/⁻ mice. Furthermore, TNF-R2 depletion decreased the expression level of several genes involved in the modulation of synaptic plasticity (synaptotagmin (syt1), dynamin-1 like (dpl1), SH3-domain GRB2-like 2 (Sh3gl2), and potassium voltage-gated channel, shaker-related subfamily, beta member 1 (kcnab1)) (Quintana et al., 2007). Analysis of the gene expression profile of the cortex of TNF-R1⁻/⁻ mice and of wild-type mice showed lower expression of the genes involved in pro-inflammatory response in TNF-R1⁻/⁻ mice compared with wild-type mice (e.g. signal transducer, and activator of transcription-3 (stat-3)). This is in agreement with the overall decreased inflammatory response of TNF-R1⁻/⁻ mice.
(Quintana et al., 2005). In addition, lovastatin treatment, which activates TNF-R2 neuroprotective signaling in neurons (Chapter 4) and in endothelial cells (Nübel et al., 2005b), was shown to enhance the expression of several anti-apoptotic genes (Bcl-2, c-fos, H1.2, and c-myc) in the mouse cortex (Johnson-Amuna et al., 2005).

Taken together, these results suggest that TNF-R2 signaling could elicit an anti-inflammatory and neuroprotective effect under various brain injury conditions (Suvannavejli et al., 2000; Quintana et al., 2007).

8.3 Molecular basis for new therapeutics

Cytokine expression is drastically altered in various neurodegenerative conditions, including AD and Parkinson’s disease (Fillit et al., 1991). TNF-R1, TRADD and TNF-α are increased 3- to 10-fold in AD patients as compared to the levels in age-matched control brains. In contrast, TNF-R2 and Fas-associated death domain-like interleukin-1-beta-converting enzyme-inhibitory protein (FLIP) (Taoufik et al., 2007), which mediate neuroprotective mechanisms, are decreased in late pathological conditions of AD, at the same time as progressive neuronal injury occurs (Zhao et al., 2003). The increase of TNF-R1-death receptor expression is first seen in the entorhinal cortex, then in hippocampus and prefrontal cortex, in agreement with the predicted progression of AD pathology.

Together these observations suggest a strong connection between TNF-α and the initiation and progression of AD disease. Since extensive clinical reports suggest that statins may protect against AD pathology (Wolozin et al., 2006) and that lovastatin can increase the expression of TNF-R2 levels, as we demonstrated in this thesis, one approach to restore the balance in TNF-R1/TNF-R2 expression in AD patients could be a treatment with statins. Another approach would be anti-TNF-α therapy, by which the expression of TNF-α and TNF-R would be down regulated. However, anti-TNF-α therapy in neuroinflammatory and neurodegenerative disorders has so far resulted in conflicting outcomes. In clinical trials of rheumatoid arthritis or inflammatory bowel disease, blockade of TNF-α produced impressive beneficial outcomes for the majority of patients. In some cases, however, it led to lupus and manifestations of neuroinflammatory disease (Feldmann et al., 1996; Sandborn and Hanauer, 1999). In multiple sclerosis patients, anti-TNF-α therapy induced disease exacerbation (Sicotte and Voskuhl, 2001).

Interestingly, a recent study showed that treatment for 6 months with Etanercept, (an TNF-α scavenger drug) improved learning and memory in AD patients (Tobinick et al., 2006). In their most recent study, Tobinick and colleagues reported in a one-case study a tremendous memory improvement within 30-45 minutes after Etanercept administration (Tobinick and Gross, 2008). Noteworthy is the fact that this patient received a combination of Etanercept and statins, which could mean that Etanercept supressed soluble TNF-α whereas statins induced TNF-R2 expression and therefore changed the balance of TNF-R1/TNF-R2 towards neuroprotective TNF-R2 signaling.
In conclusion, our studies suggest potential for therapeutic manipulation of TNF-α signaling in the treatment of neurodegenerative disorders.

References


References


