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TNF- α /NF- κ B signaling in the CNS: possible connection to EPHB2

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Abstract

Tumor necrosis factor- α , TNF- α , is a cytokine that is a well-known factor in multiple disease conditions and is recognized for its major role in central nervous system signaling. TNF- α signaling is most commonly associated with neurotoxicity, but in some conditions it has been found to be neuroprotective. TNF- α has long been known to induce nuclear factor- κ B, NF- κ B, signaling by, in most cases, translocating the p65 (RelA) DNA binding factor to the nucleus. p65 is a key member of NF- κ B, which is well established as a family of transcription factors that regulates many signaling events, including growth and process development, in neuronal cell populations. NF- κ B has been shown to affect both the receiving aspect of neuronal signaling events in dendritic development as well as the sending of neuronal signals in axonal development. In both cases, NF- κ B functions as a promoter and/or inhibitor of growth, depending on the environmental conditions and signaling cascade. In addition, NF- κ B is involved in memory formation or neurogenesis, depending on the region of the brain in which the signaling occurs. The ephrin (Eph) receptor family represents a subfamily of receptor tyrosine kinases, RTKs, which received much attention due to its potential involvement in neuronal cell health and function. There are two subsets of ephrin receptors, Eph A and Eph B, each with distinct functions in cardiovascular and skeletal development and axon guidance and synaptic plasticity. The presence of multiple binding sites for NF- κ B within the regulatory region of EphB2 gene and its potential regulation by NF- κ B pathway suggests that TNF- α may modulate EphB2 via NF- κ B and that this may contribute to the neuroprotective activity of TNF- α .

1. Introduction

TNF- α is a proinflammatory cytokine that has many important physiological and pathological roles, including cell necrosis apoptosis. TNF- α plays an important role in a broad range of biological events including the regulation of embryo development, the sleep-wake cycle, lymph node follicle and germinal center formation, host defense against bacterial and viral infections, and serves as an endogenous pyrogen that causes fever (reviewed by Chu, 2013). TNF- α induces the production of other proinflammatory cytokines and chemokines and by an autocrine pathway, increases its own production. TNF- α plays a central role in autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis, systemic lupus erythematosus and systemic sclerosis, and inflammatory bowel diseases including Crohn's disease and ulcerative colitis. Moreover, TNF- α has emerged as an

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CONFLICT OF INTERESTS

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important risk factor for tumorigenesis, tumor progression, invasion, and metastasis (Locksley et al. 2001).

TNF- α triggers activation of the I κ B kinase (IKK)/NF- κ B and mitogen-activated protein kinase (MAPK)/AP-1 pathways, which are essential for the expression of proinflammatory cytokines and induction of many biological events occurring downstream of TNF- α , including apoptosis and necrosis (Baud and Karin, 2001). There are two receptors for TNF- α , TNFR1 and TNFR2, which can either be membrane bound or present in the cytoplasm as soluble proteins. Each TNFR1 and TNFR2 can interact with both membrane-bound TNF- α (mTNF- α) as well as soluble TNF- α (sTNF- α). The difference between mTNF- α and sTNF- α rests on the extent of the signaling events, as TNFR1 signaling is strongly activated by both mTNF- α and sTNF- α , while TNFR2 signaling can only be efficiently activated by mTNF- α (Wajan et al. 2003). Another key difference is that TNFR1 is ubiquitously expressed while TNFR2 is mainly expressed on lymphocytes and endoepithelial cells (Chu, 2013). Signaling events downstream of TNF- α are shown in Fig. 1.

This review offers a possible linkage between TNF- α and Ephrin B2 via activation of the NF- κ B pathway. In general, Ephrin receptors (Eph) and ephrin ligands (ephrin) are expressed in nearly all tissues of a developing embryo, and they are involved in a variety of developmental processes. Eph receptors form a large family of receptor tyrosine kinases (RTKs). With respect to its role in the brain, EphB2 directly impacts neuronal development and has a major role in synaptic plasticity.

2. TNF- α signaling in the brain

Both TNF- α receptors in the brain are expressed by neurons and glial cells. Yet this distribution is dictated by apoptotic signaling or inflammatory cascades (Kinouchi et al. 1991; Tchélégrian et al. 1995; Botchkina et al. 1997; Dopp et al. 1997; Sairanen et al. 2001; Figiel et al. 2007). Because of their differences in signaling, TNFR1 and TNFR2 may have opposite effects on neurons, with TNFR1 having a damaging impact on neuronal cells whereas TNFR2 appears to have a neuroprotective effect (Fintaine et al. 2002). In an experiment with primary cortical neurons from TNFR1- or TNFR2-deficient mice, glutamate treatment studies revealed that TNFR1-induced persistent NF- κ B activity is not sufficient for neuronal survival, whereas transient NF- κ B activation induced by TNFR2 is able to provide neuroprotection (Marchetti et al. 2004).

Another mode of neuroprotection associated with TNF- α is mediated through manganese superoxide dismutase (Mn-SOD), a scavenger of reactive oxygen species (ROS). Pretreatment of neurons with TNF- α increased Mn-SOD activity and significantly attenuated 3-nitropropionic acid (3-NP)-induced superoxide accumulation and loss of mitochondrial transmembrane potential (Bruce-Keller et al. 1999). 3-NP is a mitochondrial toxin that inhibits succinate dehydrogenase, which causes a reduction in energy metabolism that can lead to oxidative stress resulting in the formation of reactive oxygen and nitrogen species (Garcia et al. 2002). TNF- α also exhibits the ability to attenuate β -amyloid-induced elevation of calcium and ROS. It is likely that this activity involves NF- κ B since it is well established that the Mn-SOD promoter contains an NF- κ B consensus sequence and Mn-SOD expression is increased by TNF- α (Wong and Goeddel, 1988; Barger et al. 1995). TNF- α appears to have a role in signaling long term potentiation (LTP) (Albensi and Mattson, 2000). TNF- α treatment stimulated NF- κ B signaling that regulated synaptic plasticity in the hippocampus as LTP was not induced at low frequency stimulation in TNF- α receptor knockout mice in the hippocampus (Albensi and Mattson, 2000) implying that TNF- α may impact α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

receptors. Accordingly, TNF- α increases cell-surface expression of AMPA receptors in cultured hippocampal neurons and affects synaptic efficacy in both primary cultures and hippocampal slices. Treatment with the soluble form of the TNFR1, a TNF- α antagonist, had a negative effect on AMPA receptor expression and decreased synaptic strength (Beattie et al. 2002).

In addition to neurons, astrocytes and oligodendrocytes, the two main supporters of neuronal cells, can also be regulated by TNF- α signaling. Similar to the study performed in neurons, pretreatment of astrocytes with TNF- α elevated Mn-SOD activity and significantly reduced superoxide accumulation and loss of mitochondrial transmembrane potential (Bruce-Keller et al. 1999). The levels of two important growth factors are also affected by the TNF- α signaling pathway as up-regulation of glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) occurred in TNF- α -treated primary astrocytes (Kuno et al. 2006; Saha et al. 2006). NF- κ B activation is one possible avenue for the BDNF up-regulation by TNF- α in astrocytes, along with the C/EBP β transcription factor connected with the ERK/MAP kinase pathway (Saha et al. 2006). Both exogenous TNF- α and TNF- α released by astrocytes after stimulation with LPS induce NGF and GDNF production by astrocytes, further demonstrating, albeit indirectly, a neuroprotective role for TNF- α (Kuno et al. 2006).

On the other hand, some studies have revealed a neurotoxic effect of TNF- α . For example, TNF- α has been shown to directly prevent glutamate uptake, which leads to glutamate neurotoxicity, and this effect can be reversed by inhibiting NF- κ B (Zou and Crews, 2005). The effects of TNF- α on brain damage are evident through acutely inhibiting endogenous TNF- α by treatment with soluble TNF- α receptors, antisense blockers, or neutralizing antibodies that resulted in a reduction of traumatic and ischemic brain damage in rodents. (Zou and Crews, 2005).

Aside from cell death, TNF- α has a negative impact on the formation of neurites. Treatment of neuronal cells from mice cultured on astrocytes with recombinant TNF- α or when the glial cells were stimulated to secrete TNF- α , neurite outgrowth and branching was drastically reduced (Neumann et al. 2002). Evidently, this event required a functional TNF- α pathway as this reduction in neuronal process development was not observed in TNF- α receptor null cells (Neumann et al. 2002).

Thus, it is possible to speculate that the diverse effects of TNF- α on neuronal cells, either direct or indirect, reflect a necessary balance to ensure the survival of healthy and functional cells in the CNS. In some cases, TNF- α signaling is neurotoxic and results in damage or death in the neuronal population. The receptor activation is a crucial aspect of TNF- α signaling, as the outcome of the signaling seems to be dependent on which receptor is activated. This would allow dysfunctional or damaged neurons to be targeted by TNF- α for apoptosis while sparing healthy neurons. This dichotomy allows for more dynamic signaling, in that it prevents inflammation from resulting in complete neuronal death but rather ensuring strong survival even under alternative conditions. If the surrounding environment is unfitting to survival from overall damage, it would be ideal that the dysfunctional neuron be eliminated. In the case that healthy neurons are targeted for apoptosis, the environment may not be suitable for survival. If the neuron is capable of persisting through the disease conditions, ideally it would be targeted for neuroprotection to promote survival.

In Alzheimer's Disease (AD), recent studies demonstrate that TNF- α is located in compromised brain regions in postmortem brain tissue from patients with AD (Zhao et al. 2003). TNF- α levels were significantly elevated in cortical and hippocampal regions in

patients with mild cognitive impairment (MCI) and with AD compared with age-matched controls (Tarkowski et al. 1999, 2003; Alvarez et al. 2007). Studies using AD mouse models revealed a role for TNF- α in AD-associated pathogenesis and cognitive deficits, as deficits in learning and memory directly correlated with elevated hippocampal TNF- α mRNA levels (Mediros et al. 2007) in mice receiving A β 1–40 by intracerebroventricular injections. In other AD mouse models, including Tg2576 (Mehlhorn et al. 2000), APPswe/PS1dE9 (Ruan et al. 2009) and 3 $\times$ Tg-AD (Janelins et al. 2005), TNF- α is upregulated, co-localized with amyloid plaques, and is neurotoxic. It has been shown in AD studies that short-term inhibition of TNF- α improves cognitive deficits, but complete elimination of TNF- α signaling did not show any cognitive improvement (Gabbita et al, 2012). Although the inflammation has been linked to the progression of AD, no direct pathogenic role has been established for TNF- α in amyloid plaque formation.

In addition, TNF- α also plays a role in the immune response to HIV. TNF- α has been shown to increase the permeability of the blood-brain-barrier to allow infected macrophages to infiltrate and increase the progression of HIV-encephalitis (Fiala et al, 1997). Also, a positive-feedback loop involving NF- κ B and TNF- α regulates the transcriptional activity of the HIV long terminal repeat (LTR). In the inactivated form, NF- κ B is unable to bind DNA because it is associated with I κ B proteins, which keep NF- κ B in the cytoplasm (Ganchi et al. 1992; Henkel et al. 1992; Baldwin, 1996). When activated by TNF- α , NF- κ B is translocated into the nucleus, which leads to the transcriptional activation of the HIV LTR (Duh et al. 1989). TNF- α has also been shown to impact microglia-induced neuronal cell death through HIV Tat-induced apoptotic cell death (Zou and Crews, 2005). TNF- α and TNFRs levels are increased and correlate with a neurological decline in HIV-associated dementia patients, and TNF- α has been shown to cooperate with Tat and gp120 to induce oxidative stress and ultimately neuronal death (Saha and Pahan, 2003). On the other hand, TNF- α has been found to reduce HIV-1 replication, in a dose-dependent manner, in HIV-infected peripheral blood monocytes and alveolar macrophages (Lane et al, 1999). These varying effects may be dependent on other factors such as the state of the disease and health of the patient.

3. NF- κ B in the brain

Also known as the Rel family of proteins, NF- κ B proteins consist of subunits of p50, p52, p65 (RelA), c-Rel and RelB, all of which are ubiquitously expressed in the mammalian nervous system and are highly conserved across species. The active molecule of NF- κ B is a homo- or hetero-dimer of these subunits, and activation of NF- κ B is a result of signaling cascades of specific receptors that are activated by growth factors, cytokines, chemokines, oxidants and pathogens associated with oxidative stress, which further categorizes NF- κ B as a redox-sensitive transcription factor (Srivastava and Ramana, 2009). Two main signaling pathways lead to activation of NF- κ B. In the canonical, or classical, pathway, the ligand binds to its associated receptor and activates the I κ B kinase (IKK) complex that contains the catalytic kinase subunits (IKK α , IKK β) and the regulatory non-enzymatic scaffold protein called NEMO (NF- κ B essential modulator or IKK γ), which leads to the phosphorylation of I κ B (Srivastava and Ramana, 2009). The non-canonical (or alternative) pathway differs in the route of activation as ligand binding to the coupled receptor leads to activation of the IKK complex via the activation of NF- κ B-inducing kinase (NIK). This results in phosphorylation of the inactive NF- κ B p100/RelB dimer, which is further processed to form the active p52/RelB dimer. In both pathways, the phosphorylation event leads to translocation of the NF- κ B subunit to the nucleus leading to changes in target gene transcription (Srivastava and Ramana, 2009). Thus, activation of NF- κ B is required for the differentiation, survival, proliferation and apoptosis in neuronal cell types, among others (Hoffman and Baltimore, 2006; Kaltschmidt and Kaltschmidt, 2009; Srivastava and

Ramana, 2009). Inactivation of NF- κ B is achieved by I κ B proteins in the canonical pathway. Three forms of I κ B (I κ B α , I κ B β , and I κ B ϵ) are classified as traditional I κ B proteins in that they inhibit transcription by isolating NF- κ B dimers in the cytoplasm away from κ B elements of the target genes. Each of the I κ B proteins has its own functions, and it has been shown that canonical activation of NF- κ B requires all three typical I κ B proteins (Srivastava and Ramana, 2009; Hayden and Ghosh, 2008).

As suggested above, TNF- α signaling through NF- κ B has various roles in the brain. Constitutive NF- κ B activity was first found in glutamatergic neurons of the CNS, in the hippocampus and cerebral cortex. Transgenic mouse models verified the initial findings and also reported constitutive NF- κ B activity in several other brain regions such as the amygdala, olfactory lobes, cerebellum, and hypothalamus (Kaltschmidt and Kaltschmidt, 2009). Neuroprotection during neuronal development has been well established by multiple studies, but has been shown to be time point- and cell-limited as in some cases it promotes apoptosis and in some neuronal survival. NF- κ B signaling has also been well documented to be involved in dendritic arborization and axonal growth. Interestingly, after glutamatergic stimulation, p65 has been shown to travel in a retrograde fashion from active synapses back to the nucleus, where it has a central role in regulating synaptic gene expression (Kaltschmidt and Kaltschmidt, 2009; Memet, 2006).

Various neurotransmitters and neurotrophic factors and cytokines allow constitutively active NF- κ B persist. In some CNS neurons, excitatory amino acids produce stimuli that also contribute to the constitutive activation of NF- κ B. This NF- κ B activity can be suppressed by glutamate antagonists and L-type Ca²⁺ channel blockers, which suggests that constitutive NF- κ B results from physiological basal synaptic transmission (Pizzi and Spano, 2006). Activation of NF- κ B can protect neurons against amyloid β peptide toxicity and excitotoxic or oxidative stress. In vivo experiments in models of brain preconditioning with kainate, ischemia or linolenic acid showed NF- κ B dependent protection against neuronal death. Transgenic inhibition of NF- κ B by neuronal over-expression of the I κ B α super-repressor decreased neuroprotection after kainic acid or Fe⁺⁺ application (Pizzi and Spano, 2006).

NF- κ B signaling is involved in the proliferation and migration of neural progenitor cells. NF- κ B p65 regulates proliferation of adult neural stem cells isolated from the subventricular zone (SVZ) through the NF- κ B target genes c-myc and cyclin D1. NF- κ B p65 and p50 expression continues into adulthood in SVZ astrocyte-like cells and in migrating neuronal precursors (Kaltschmidt and Kaltschmidt, 2009). Many studies have demonstrated that multiple stimuli initiate NF- κ B activation in astrocytes and microglia, which triggers production of proinflammatory mediators that may be either neuroprotective or apoptotic. NF- κ B can also induce differentiation of neuronal stem cells into astrocytes depending on the signaling cascade (Kaltschmidt and Kaltschmidt, 2009).

NF- κ B mediates myelination in Schwann cells, so many consider it likely that NF- κ B exerts the same function in oligodendrocytes, but this assumption needs to be tested. It may also be the case that NF- κ B regulates remyelination by oligodendrocytes since TNF- α is required for both remyelination and proliferation of oligodendrocyte progenitors cells (OPCs). NF- κ B has an antagonistic role in oligodendrocytes as the signaling is prosurvival role and promotes maturation of OPCs, through a platelet-derived growth factor- α (PDGF- α) receptor signaling pathway triggered by binding of a soluble factor produced by non-activated microglia, as well as survival of oligodendrocytes after TNF- α exposure (Kaltschmidt and Kaltschmidt, 2009; Memet, 2006). NF- κ B is also involved in dendritogenesis in the developing cerebellum as analysis of the Purkinje cell degeneration mutant mouse, psdSid, shows reduced expression and nuclear localization of p65 in the Purkinje cells both in vivo and in vitro and also reduced dendritic development (Gutierrez and Davies, 2011).

A strong decrease in nuclear NF- κ B p65 immunoreactivity was demonstrated by the analysis of plaque stages in AD patients. In the cells surrounding plaques, this decrease was shown from early to late stages of the disease in comparison to healthy controls (Teraï et al. 1996). A β stimulates NOS-II expression and NO production via a NF- κ B -dependent mechanism in rat cortical astrocytes, supporting the importance of oxidative damage and astrocyte NF- κ B signaling in AD (Lee et al. 2011). Up-regulation of many NF- κ B target genes, including IL-8, is seen in gene expression profiling of post-mortem human cortical microglia treated with A β (Memet, 2006). A β neurotoxicity can be reduced by inhibition of NF- κ B activity in microglia by expression of the dominant-negative super-repressor, I κ B α -SR, in which two critical N-terminal serine residues are replaced with alanine residues preventing its phosphorylation and activation (Chen et al. 2005; Memet, 2006). Thus, NF- κ B plays a significant role in AD, influencing survival in neurons and mediating A β toxicity in glial cells.

NF- κ B also has multiple roles in HIV-1 pathogenesis. It is well demonstrated by in vitro studies and genome-wide analysis that HIV-1 transcription can be initiated by prototypical NF- κ B p50/p65 heterodimers and components of the canonical NF- κ B pathway. The enhancer region of the HIV-1 LTR has two identical and highly conserved NF- κ B binding sites and when these are mutated, HIV-1 can infect cells but fails to produce de novo transcripts. The NF- κ B p50/p65 heterodimer mediates LTR transcriptional initiation and elongation, and NF- κ B p50/p65 initiates HIV-1 transcription by associating with the acetyltransferases p300, leading to acetylation of LTR-associated chromatin, promoting improved access for RNA polymerase II (RNAPII). Recruitment of p300 also leads to the acetylation of NF- κ B itself, which can increase its activation of transcription (Quivy and Van Lint 2004; Calao et al. 2008). NF- κ B p50/p65 stimulates the early rounds of RNAPII elongation by recruiting the P-TEFb complex to phosphorylate serine-2 heptad repeats within the carboxyl domain (CTD) of RNAPII, which converts this low activity enzyme into a more active form. This NF- κ B-mediated RNAPII conversion is transient as interference can occur by the action of an okadaic acid (OA)-sensitive phosphatase that dephosphorylates the CTD of RNAPII. Production of short transcripts containing HIV-1 trans-activation response (TAR) RNA element predominates, but the transcriptional lag is overcome when sufficient Tat, which is OA-phosphatase insensitive, is produced. High-level, sustained phosphorylation and transcriptional elongation by RNAPII results as Tat powerfully amplifies HIV-1 expression by binding to TAR and directly recruiting PTEFb from latent complexes. HIV-1 mediates NF- κ B action in a cell type- and context-dependent manner to promote viral replication and immune evasion. In all of the Jurkat-based models of HIV-1 latency, NF- κ B agonists consistently induce proviral reactivation. However, in recent primary CD4⁺ T-cell latency models, the role of NF- κ B as an effective antagonist of HIV-1 latency is debated (Leghmari et al. 2008).

4. Ephrin B2 in the brain

Eph receptors (Ephs) and ephrin receptor ligands (ephrins) are expressed in nearly all tissues of the developing embryo, and they are involved in a variety of developmental processes such as cardiovascular and skeletal development, axon guidance, and tissue patterning. In many processes, the function of Eph/ephrin signaling involves cell adhesion including growth cone retraction in axon guidance, cell sorting in embryo patterning, cell migration and fusion in craniofacial development, and platelet aggregation, etc. Although Eph/ephrins have been studied mostly in a developmental context, their physiological functions in the adult are now a major focus. They have recently been shown to be involved in learning and memory, in bone homeostasis, and in insulin secretion. Alterations of Eph/ephrin signaling in humans have been implicated in congenital diseases and cancer (Arvantis and Davy, 2008).

Ephrin receptors form the largest subfamily of receptor tyrosine kinases (RTKs). They interact with cell surface-bound ligands that are also part of a family of related proteins. Two classes of ephrins are distinguished by structural differences: Ephrins-A (A1–A6) are tethered to the plasma membrane via a glycosyl phosphatidyl inositol moiety, and ephrins-B (B1–B3) span the plasma membrane and possess a short cytoplasmic tail. The Eph receptors are categorized based on their affinity for their ligands, EphA (A1-8 and A10) interact with ephrin A (A1-5) and EphB (B1-4 and B6) interact with ephrin B (B1-3). A unique feature of Eph/ephrin signaling is the ability of both receptors and ligands to transduce a signaling cascade upon interaction. Eph-activated signaling is considered forward, and ephrin-activated signaling is considered reverse. Another level of complexity stems from the fact that interactions between Eph receptors and ephrins can happen in trans (between two opposing cells) or in cis (within the same cell). It is commonly assumed that trans interactions are activating while cis interactions are inhibiting (Arvantis and Davy, 2008). EphB-ephrinB, particularly B2, interactions have recently emerged as major role players in synaptic plasticity and neuronal process development. A general scheme of ephrin signaling is shown in Fig. 2.

Evidence shows that EphB receptor signaling pathways are required for spine morphogenesis as dendritic extensions remain filopodial in the presence of a dominant negative form of EphB2. In addition, the rapid formation of dendritic extensions with enlarged heads in immature hippocampal and cortical neurons is induced by the activation of EphB receptors by exogenous ligand (immunoglobulin Fc fusion proteins of ephrin-B1 or ephrin-B2). Deficits in NMDA-dependent synaptic plasticity in the hippocampus, such as LTP and long-term depression (LTD), and possibly minor defects in spatial memory, are demonstrated in EphB2 knockout mice. Synaptic efficiency in dorsal horn neurons receiving nociceptor afferents in the spinal cord is regulated by EphB–ephrin-B signaling. The Eph–ephrin system might operate in various types of synaptic plasticity, not only in the hippocampus, as similarities exist between LTP and central sensitization of nociceptive neurons in the dorsal horn (Grunwald et al. 2001; Henkemeyer, 2003; Penzes et al. 2003; Murai and Pasquale, 2004).

EphB2 has recently been implicated in AD pathology in the reduction in functional synapses. A decrease in EphB2 levels are correlated with amyloid- β -induced neuronal dysfunction as overexpression of EphB2 in human amyloid precursor protein transgenic mice recovered NMDA-receptor-dependent LTP and memory deficits (Cissé et al. 2011). In vivo, gammasecretase inhibitors block Notch signaling, resulting in the formation of dense capillary networks similar to those in the brains of patients and mice. Notch inhibition reduces EphB2 receptor expression, which could also contribute to synapse dysfunction (Ethel, 2010). EphB receptors play a major role in the maintenance of dendritic spine morphology, so a loss or reduction in EphB results in immature spines that lack synaptic activity. This reduction in EphB may directly contribute to AD pathology (Lacor et al. 2007).

In addition to Alzheimer's disease, EphB2 levels are altered in HIV. A decrease in ephrin-B2 in the caudate and EPHB2 in the anterior cingulate in postmortem brain of HIV-infected subjects was found when compared to non-infected subjects. When the HIV-infected subjects were analyzed in groups of non-cognitively impaired and cognitively impaired, an increase in EPHB2 was found in the cognitively impaired. Decreases in caudate ephrin-B2 were associated with worsening immune status, whereas increases in cingulate EPHB2 were associated with worse cognitive status (Yuferov et al. 2013). This seems to present a contradicting role of EPHB2, but not when considering the effects of EPHB2. A decrease in some areas of the brain may be due to the state of the affected area. If the diseased area of the brain is beyond repair and is degenerative, a decrease in EPHB2 would be expected as it

would correlate with a decrease in neuronal processes since EPHB2 is a membrane bound receptor. In the case of an increase in EPHB2, perhaps that region of the brain is undergoing repair signaling, which would upregulate EPHB2 in an attempt to restructure neuronal processes. The timing of the changes of EPHB2 expression must be examined in parallel with the state of the disease in order to fully understand the dynamics of EPHB2 signaling in HIV and HAND.

In recent studies, variations in Eph and ephrin gene expression have been found in various types of human tumors such as neuroblastoma, carcinomas of the breast, lung, prostate, ovarian, and melanoma (Tang et al. 2001; Surawska et al. 2004). The EphB2/R-Ras signaling pathway may influence the malignant behavior of glioblastoma, promoting the invasion of glioma cells into normal brain tissue as decreased adhesion has been proposed to promote invasion of tumor cells (Nakada et al. 2005). EphB2 signaling has been shown to lead to phosphorylation of R-Ras, resulting in a reduction in adhesion of cells to the ECM that was reversed by R-Ras knockdown (Nakada et al. 2010). Thus the EphB2 receptor has a role in the regulation of invasion of glioma cells.

5. Conclusion and Future Directions

As described above, the TNF- α signaling axis and NF- κ B translocation may affect cells in different ways, depending on the location and duration of the signaling events involved. Both can be pro-survival or pro-apoptotic depending on their interactions, and they can be either neuroprotective or neurotoxic. As mentioned, the outcome of the interactions occurring downstream of TNF- α directly depends on the initiation of signaling and which TNF- α receptor is activated. Upon activation by TNF- α , NF- κ B can initiate transcription of many target genes and the presence of NF- κ B motifs and EPHB2 may be one of these. EphB2 has a multifaceted involvement in many physiological processes, including development of neurons and synaptic plasticity. Upon promoter analysis using PROMO, a software program for the prediction of transcription factor binding site (Farré et al, 2003), there were found multiple NF- κ B binding sites present near the transcription start site of the EphB2 gene. This presents the possibility of a connection of three signaling modules; TNF- α , NF- κ B and EphB2 – creating a very complex signaling cascade. In the event of TNF- α being neuroprotective, EphB2 could be the missing link. As TNF- α signaling increases, this results in NF- κ B being activated, which could in turn result in the transcription of the predicted NF- κ B target gene, EphB2. Activation of EphB2 could, then, result in neuroprotection and survival of neuronal cells. In the case of injury or disease, where all three are upregulated, the ultimate activation of EphB2 could promote cellular repair in damaged regions. However, if the surrounding environment is unfavorable for regrowth or survival, apoptosis could be the outcome. A study in post-mortem brain tissue from diseased patients could provide insight into the relationship between the three. If, for example, TNFR1 or TNFR2 levels correlated with EPHB2 levels, this would show whether TNF- α levels have an impact on EPHB2 levels and would provide strength to the argument that TNF- α initiates EPHB2 signaling. This would also provide evidence of whether or not EPHB2 has a neuroprotective role since the type of TNF- α receptor would be distinguished. Promoter studies and binding assays could also provide insight into the predicted effect of TNF- α signaling on the interaction of NF- κ B with the EPHB2 gene. If this signaling cascade occurs as speculated, this could provide a new drug target, EPHB2, for neurodegenerative diseases. It was described that TNF- α inhibitors improve cognitive status in neurodegenerative diseases, but completely abolishing TNF- α signaling showed no effect. This clearly demonstrates that either more sensitive modulation of TNF- α needs to be developed or another route must be taken. NF- κ B has also been described to have an apoptotic role, so EPHB2 would be an ideal candidate since it has a major role in neuronal development. Up-regulating EPHB2 may be able to increase development from surviving

neurons to rebuild a degenerating network of neurons. EPHB2 would not result in an inflammatory response by the immune system, unlike TNF- α , so it seems to be a more ideal target for therapy. Future studies are needed to address these unknown aspects of the EPHB2 signaling cascade and could dramatically affect our attempt at improving the quality of life for patients with neurodegenerative disease.

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REFERENCES

- Albensi BC, Mattson MP. Evidence for the involvement of TNF and NF-kappaB in hippocampal synaptic plasticity. *Synapse*. 2000; 35:151–159. [PubMed: 10611641]
- Álvarez A, Cacabelos R, Sanpedro C, García-Fantini M, Aleixandre M. Serum TNF-alpha levels are increased and correlate negatively with free IGF-1 in Alzheimer disease. *Neurobiol Aging*. 2007; 28:533–536. [PubMed: 16569464]
- Arvanitis D, Davy A. Eph/ephrin signaling: networks. *Genes Dev*. 2008; 22:416–429. [PubMed: 18281458]
- Baldwin AS. The NF-kappaB AND IkappaB proteins: new discoveries and insights. *Ann Rev Immunol*. 1996; 14:649–681. [PubMed: 8717528]
- Barger SW, Horster D, Furukawa K, Goodman Y, Kriegstein J, Mattson MP. Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa b-binding factor and attenuation of peroxide and Ca2+ Accumulation. *Proc Natl Acad Sci USA*. 2005; 92:9328–9332. [PubMed: 7568127]
- Baud V, Karin M. Signal transduction by Tumor Necrosis Factor and its relatives. *Trends In Cell Biology*. 2001; 11:372–377. [PubMed: 11514191]
- Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC. Control of synaptic strength by glial TNF-alpha. *Science*. 2002; 295:5563:2282–2285. [PubMed: 11910117]
- Botchkina GI, Meistrell ME, Botchkina IL, Tracey KJ. Expression of TNF and TNF Receptors (p55 and P75) in the rat brain after focal cerebral ischemia. *Mol Med*. 1997; 3:765–781. [PubMed: 9407552]
- Bruce-Keller AJ, Geddes JW, Knapp PE, McFall RW, Keller JN, Holtsberg FW, Parthasarathy S, Steiner SM, Mattson MP. Anti-death properties of TNF against metabolic poisoning: mitochondrial stabilization by MnSOD. *J Neuroimmunol*. 1999; 93:53–71. [PubMed: 10378869]
- Calao M, Burny A, Quivy V, Dekoninck A, Van Lint C. A pervasive role of histone acetyltransferases and deacetylases in an NF-kappaB-signaling code. *Trends Biochem Sci*. 2008; 33:339–349. [PubMed: 18585916]
- Chen J, Zhou Y, Mueller-Steiner S, Chen LF, Kwon H, Yi S, Mucke L, Gan L. SIRT1 protects against microglia-dependent amyloid- β toxicity through inhibiting NF- κ B signaling. *J Bio Chem*. 2005; 280:40364–40374. [PubMed: 16183991]
- Chu W. Tumor Necrosis Factor. *Cancer Lett*. 2013; 328:222–225. [PubMed: 23085193]
- Cissé M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, Orr A, Lotz G, Kim DH, Hamto P, Ho K, Yu GQ, Mucke L. Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature*. 2011; 469:47–52. [PubMed: 21113149]
- Dopp JM, Mackenzie-Graham A, Otero GC, Merrill JE. Differential expression, cytokine modulation, and specific functions of type-1 and type-2 tumor necrosis factor receptors in rat glia. *J Neuroimmunol*. 1997; 75:104–112. [PubMed: 9143243]
- Duh EJ, Maury WJ, Folks TM, Fauci AS, Rabson AB. Tumor necrosis factor alpha activates Human Immunodeficiency Virus Type 1 through induction of nuclear factor binding to the NF-kappa B sites in the long terminal repeat. *Proc Natl Acad Sci USA*. 1989; 86:5974–5978. [PubMed: 2762307]

- Ethell DW. An amyloid-notch hypothesis for Alzheimer's disease. *Neuroscientist*. 2010; 16:614–617. [PubMed: 20592273]
- Farré D, Roset R, Huerta M, Adsua JE, Roselló L, Albà MM, Messeguer X. Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. *Nucleic Acids Res*. 2003; 31:3651–3653. [PubMed: 12824386]
- Figiel I, Dzwonek K. TNF α and TNF receptor 1 expression in the mixed neuronal–glial cultures of hippocampal dentate gyrus exposed to glutamate or trimethyltin. *Brain Res*. 2007; 1131:17–28. [PubMed: 17161388]
- Fontaine V, Mohand-Said S, Hanoteau N, Fuchs C, Pfizenmaier K, Eisel U. Neurodegenerative and neuroprotective effects of tumor necrosis factor (TNF) in retinal ischemia: opposite roles of TNF Receptor 1 and TNF Receptor 2. *J Neurosci*. 2002; 2:RC216. [PubMed: 11917000]
- Fiala M, Looney DJ, Stins M, Way DD, Zhang L, Gan X, Chiappelli F, Schweitzer ES, Shapshak P, Weinand M, Graves MC, Witte M, Kim KS. TNF-alpha opens a paracellular route for HIV-1 invasion across the blood-brain barrier. *Molecular Medicine*. 1997; 3:553–564. [PubMed: 9307983]
- Gabbita SP, Srivastava MK, Eslami P, Johnson MF, Kobritz NK, Tweedie D, Greig NH, Zemlan FP, Sharma SP, Harris-White ME. early intervention with a small molecule inhibitor for tumor necrosis factor- α prevents cognitive deficits in a triple transgenic mouse model of Alzheimer's disease. *J Neuroinflammation*. 2012; 9:99. [PubMed: 22632257]
- Ganchi PA, Sun SC, Greene WC, Ballard DW. IkappaB/MAD-3 masks the nuclear localization signal of NF-kappaB P65 and requires the transactivation domain to Inhibit NF-kappaB p65 DNA binding. *Mol Biol Cell*. 1992; 3:1339–1352. [PubMed: 1493333]
- Garcia M, Vanhoutte P, Pages C, Besson M, Brouillet E, Caboche J. The mitochondrial toxin 3-nitropropionic acid induces striatal neurodegeneration via a C-Jun N-terminal kinase/c-Jun module. *J Neurosci*. 2002; 22:2174–2184. [PubMed: 11896157]
- Grunwald IC, Korte M, Wolfer D, Wilkinson GA, Unsicker K, Lipp H, Bonhoeffer T, Klein R. Kinase-independent requirement of EphB2 receptors in hippocampal synaptic plasticity. *Neuron*. 2001; 32:1027–1040. [PubMed: 11754835]
- Gutierrez H, Davies AM. Regulation of neural process growth, elaboration and structural plasticity by NF- κ B. *Trends Neurosci*. 2011; 34:316–325. [PubMed: 21459462]
- Hayden M, Ghosh S. Shared principles in NF- κ B signaling. *Cell*. 2008; 132:344–362. [PubMed: 18267068]
- Henkel T, Zabel U, Van Zee K, Muller JM, Fanning E, Baeuerle PA. Intramolecular masking of the nuclear location signal and dimerization domain in the precursor for the p50 NF- κ B subunit. *Cell*. 1992; 68:1121–1133. [PubMed: 1547506]
- Henkemeyer M. Multiple EphB receptor tyrosine kinases shape dendritic spines in the hippocampus. *J Cell Biol*. 2003; 163:1313–1326. [PubMed: 14691139]
- Hoffmann A, Baltimore D. circuitry of nuclear factor kappaB signaling. *Immunol Rev*. 2006; 210:171–186. [PubMed: 16623771]
- Janelins MC, Mastangelo MA, Oddo S, LaFerla FM, Federoff HJ, Bowers WJ. Early correlation of microglial activation with enhanced tumor necrosis factor-alpha and monocyte chemoattractant protein-1 expression specifically within the entorhinal cortex of triple transgenic Alzheimer's disease mice. *J Neuroinflammation*. 2005; 2:23. [PubMed: 16232318]
- Kaltschmidt B, Kaltschmidt C. NF- κ B in the nervous system. *Cold Spring Harbor Perspect Biol*. 2009; 1:A001271.
- Kinouchi K, Brown G, Donner DB. Identification and characterization of receptors for tumor necrosis factor-alpha in the brain. *Biochem Biophys Res Commun*. 1991; 181:1532–1538. [PubMed: 1662500]
- Kuno R, Yoshida Y, Nitta A, Nabeshima T, Wang J, Sonobe Y, Kawanokuchi J, Takeuchi H, Mizuno T, Suzumura A. The role of TNF-alpha and its receptors in the production of NGF and GDNF by astrocytes. *Brain Res*. 2006; 1116:12–18. [PubMed: 16956589]
- Lacor PN, Buniel MC, Furlow PW, Sanz Clemente A, Velasco PT, Wood M, Viola KL, Klein WL. A β oligomer-induced aberrations in synapse composition, shape, and density provide a molecular

- basis for loss of connectivity in Alzheimer's disease. *J Neurosci.* 2007; 27:796–807. [PubMed: 17251419]
- Lane BR, Markovitz DM, Woodford NL, Rochford R, Strieter RM, Coffey MJ. TNF- α inhibits HIV-1 replication in peripheral blood monocytes and alveolar macrophages by inducing the production of RANTES and decreasing C-C Chemokine Receptor 5 (CCR5) expression. *J Immunol.* 1999; 163:3653–3661. [PubMed: 10490959]
- Lee YJ, Choi DY, Choi IS, Han JY, Jeong HS, Han SB, Oh KW, Hong JT. Inhibitory effect of a tyrosine-fructose Maillard reaction product, 2,4-bis(*p*-hydroxyphenyl)-2-butenal on amyloid- β generation and inflammatory reactions via inhibition of NF- κ B and STAT3 activation in cultured astrocytes and microglial BV-2 cells. *J Neuroinflammation.* 2011; 8:132. [PubMed: 21982455]
- Leghmari K, Bennasser Y, Bahraoui E. HIV-1 Tat protein induces IL-10 production in monocytes by classical and alternative NF- κ B pathways. *Eur J Cell Biol.* 2008; 87:947–962. [PubMed: 18760861]
- Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell.* 2001; 104:487–501. [PubMed: 11239407]
- Marchetti L, Klein M, Schlett K, Pfizenmaier K, Eisel UL. Tumor Necrosis Factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-Methyl-D-aspartate receptor activation: essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF- κ B pathway. *J Biol Chem.* 2004; 279:32869–32881. [PubMed: 15155767]
- Medeiros R, Prediger RD, Passos GF, Pandolfo P, Duarte FS, Franco JL, Dafre AL, Di Giunta G, Figueiredo CP, Takahashi RN, Campos MM, Calixto JB. Connecting TNF- α signaling pathways to iNOS expression in a mouse model of Alzheimer's disease: relevance for the behavioral and synaptic deficits induced by amyloid β protein. *J Neurosci.* 2007; 27:5394–5404. [PubMed: 17507561]
- Mehlhorn G, Hollborn M, Schliebs R. Induction of cytokines in glial cells surrounding cortical beta-amyloid plaques in transgenic Tg2576 mice with Alzheimer pathology. *Int J Dev Neurosci.* 2000; 18:423–431. [PubMed: 10817926]
- Memet S. NF- κ B functions in the nervous system: from development to disease. *Biochem Pharmacol.* 2006; 72:1180–1195. [PubMed: 16997282]
- Murai KK, Pasquale EB. Eph receptors, ephrins, and synaptic function. *Neuroscientist.* 2004; 10:304–314. [PubMed: 15271258]
- Nakada M, Anderson EM, Demuth T, Nakada S, Reavie LB, Drake KL, Hoelzinger DB, Berens ME. The phosphorylation of Ephrin-B2 ligand promotes glioma cell migration and invasion. *Int J Cancer.* 2010; 126:1155–1165. [PubMed: 19728339]
- Nakada M, Niska JA, Tran NL, McDonough WS, Berens ME. EphB2/R-Ras signaling regulates glioma cell adhesion, growth, and invasion. *Am J Pathol.* 2005; 167:565–576. [PubMed: 16049340]
- Neumann H, Scheigreiter R, Yamashita T, Rosenkranz K, Wekerle H, Barde Y. Tumor necrosis factor inhibits neurite outgrowth and branching of hippocampal neurons by a rho-dependent mechanism. *J Neurosci.* 2002; 22:854–862. [PubMed: 11826115]
- Penzes P, Beeser A, Chernoff J, Schiller MR, Eipper BA, Mains RE, Huganir RL. Rapid induction of dendritic spine morphogenesis by trans-synaptic EphrinB-EphB receptor activation of the Rho-GEF kalirin. *Neuron.* 2003; 37:263–274. [PubMed: 12546821]
- Pizzi M, Spano P. Distinct roles of diverse nuclear factor- κ B complexes in neuropathological mechanisms. *Eur J Pharmacol.* 2006; 545:22–28. [PubMed: 16854410]
- Quivy V, Van Lint C. Regulation at multiple levels of NF-kappaB-mediated transactivation by protein acetylation. *Biochem Pharmacol.* 2004; 68:1221–1229. [PubMed: 15313420]
- Ruan L, Kang Z, Le Y. amyloid deposition and inflammation in APP^{swe}/PS1^{dE9} mouse model of Alzheimer's disease. *Curr Alzheimer Res.* 2009; 6:531–540. [PubMed: 19747158]
- Saha RN, Liu X, Pahan K. Up-regulation of BDNF in astrocytes by TNF- α : A case for the neuroprotective role of cytokine. *J Neuroimmune Pharmacol.* 2006; 1:212–222. [PubMed: 18040799]
- Saha RN, Pahan K. Tumor necrosis factor- α at the crossroads of neuronal life and death during HIV-associated dementia. *J Neurochemistry.* 2003; 86:1057–1071.

- Sairanen TR, Lindsberg PJ, Brenner M, Carpén O, Sirén AL. Differential cellular expression of tumor necrosis factor- α and type I tumor necrosis factor receptor after transient global forebrain ischemia. *J Neurol Sci.* 2001; 186:87–99. [PubMed: 11412877]
- Srivastava SK, Ramana KV. Focus on molecules: Nuclear factor-kappaB. *Exp Eye Res.* 2009; 88:2–3. [PubMed: 18472097]
- Surawska H, Ma P, Salgia R. The role of Ephrins and Eph receptors in cancer. *Cytokine & Growth Factor Rev.* 2004; 15:419–433. [PubMed: 15561600]
- Tang XX, Evans AE, Zhao H, Cnaan A, Brodeur GM, Ikegaki N. Association among EPHB2, TrkA, and MYCN Expression in Low-stage Neuroblastomas. *Med Pediatr Oncol.* 2001; 36:80–82.
- Tarkowski E, Blennow K, Wallin A, Tarkowski A. Intracerebral production of tumor necrosis factor- α , a local neuroprotective agent, in Alzheimer disease and vascular dementia. *J Clin Immunol.* 1999; 19:223–230. [PubMed: 10471976]
- Tarkowski E, Andreassen N, Tarkowski A, Blennow K. Intrathecal inflammation precedes development of Alzheimer's disease. *J Neurology Neurosurg Psych.* 2003; 74:1200–1205.
- Tchélingérian J, Monge M, Le Saux F, Zalc B, Jacque C. Differential oligodendroglial expression of the tumor necrosis factor receptors in vivo and in vitro. *J Neurochem.* 1995; 65:2377–2380. [PubMed: 7595529]
- Terai K, Matsuo A, McGeer PL. Enhancement of immunoreactivity for NF-kappa B in the hippocampal formation and cerebral cortex of Alzheimer's disease. *Brain Res.* 1996; 735:159–168. [PubMed: 8905182]
- Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death and Differentiation.* 2003; 10:45–65. [PubMed: 12655295]
- Wong G, Goeddel D. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. *Science.* 1988; 242:941–944. [PubMed: 3263703]
- Yuferov V, Ho A, Morgello S, Yang Y, Ott J, Kreek MJ. Expression of ephrin receptors and ligands in postmortem brains of HIV-infected subjects with and without cognitive impairment. *J Neuroimmune Pharmacol.* 2013; 8:333–344. [PubMed: 23314923]
- Zhao M, Cribbs DH, Anderson AJ, Cummings BJ, Su JH, Wasserman AJ, Cotman CW. The induction of the TNF α death domain signaling pathway in Alzheimer's disease brain. *Neurochem Res.* 2003; 28:307–318. [PubMed: 12608703]
- Zou JY, Crews FT. TNF α potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: Neuroprotection by NF κ B inhibition. *Brain Res.* 2005; 1034:11–24. [PubMed: 15713255]

Figure 1. The interaction of TNF- α , NF- κ B, and EphB2

Once TNF- α binds to TNFR1, trimerization occurs and results in recruitment of adapter molecules. This leads to the release of NF- κ B from I κ B and leads to the transcriptional activation of EphB2, which causes insertion of EphB2 into plasma membrane. The binding of EphrinB2 with EphB2 ultimately leads to the modulation of NMDAR signaling, which leads to various downstream events, including dendritic arborization, spine formation, and LTP. Synapse diagram modified from [Motifolio.com](https://www.motifolio.com).

Figure 2. Ephrin signaling

The general characteristics of ephrins and Eph receptors are shown. The top plasma membrane represents a cell expressing ephrinA and ephrinB ligands and the bottom membrane represents a cell expressing an Eph receptor. Structures of ephrins and Ephs are indicated as well as the direction of forward and reverse signaling. Abbreviations: Cys-rich domain – Cysteine-rich domain; EphR-BD – Eph-receptor binding domain; Ephrin-BD – Ephrin binding domain; GPI, Glycosylphosphatidylinositol; PDZ-BD – PDZ-binding domain – PDZ is an acronym combining the first letters of three proteins: post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1); SAM, sterile α -motif. Cell signaling diagram modified from Motifolio.com.

Table 1
Variations in ephrin B2 receptor and ligand levels in AD, brain cancer, and HIV-1 affected brains

EphB2 and ephrin B2 levels are reduced in disease that correlates to cognitive decline, but elevated in cancer.

	Disease	Levels	Reference
EphB2	AD	Down	Lacor et al., 2007
	Glioblastoma	Up	Nakada et al., 2005
	Neuroblastoma	Up	Tang et al., 2001
	HIV-1	Down	Yuferov et al., 2013
Ephrin B2	Glioblastoma	Up	Nakada et al., 2010
	HIV-1	Down	Yuferov et al., 2013