The Role of TNF Related Apoptosis-Inducing Ligand in Neurodegenerative Diseases

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A hallmark of all forms of neurodegenerative diseases is impairment of neuronal functions, and in many cases neuronal cell death. Although the etiology of neurodegenerative diseases may be distinct, different diseases display a similar pathogenesis, for example abnormal immunity within the central nervous system (CNS), activation of macrophage/microglia and the involvement of proinflammatory cytokines. Recent studies show that neurons in a neurodegenerative state undergo a highly regulated programmed cell death, also called apoptosis. TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF family, has been shown to be involved in apoptosis during many diseases. As one member of a death ligand family, TRAIL was originally thought to target only tumor cells and was not present in CNS. However, recent data showed that TRAIL was unregulated in HIV-1-infected and immune-activated macrophages, a major disease inducing cell during HIV-1-associated dementia (HAD). TRAIL is also induced on neuron by β-amyloid protein, an important pathogen for Alzheimer’s disease. In this review, we summarize the possible common aspects that TRAIL involved those neurodegenerative diseases, TRAIL induced apoptosis signaling in the CNS cells, and specific role of TRAIL in individual diseases.

Key Words: Alzheimer’s disease, HIV-1 associated dementia, multiple sclerosis, apoptosis, TRAIL, macrophage

Introduction

Neurodegeneration manifests in a variety of chronic diseases including HIV-1 associated dementia (HAD), Alzheimer’s disease (AD), and multiple sclerosis (MS). Emerging evidence suggests impairment of neuronal function or loss of neurons is prevailing to these diseases. Inflammation within the CNS; activation of macrophage/microglia; and involvement of proinflammatory cytokines among these diseases are very similar, although the molecular mechanisms of individual diseases are variable different (1, 2).

Many cytokines have been suggested to participate in neurodegeneration and neurotoxicity. Increase in factors such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) have been observed before neuronal death (3-7). However, the sum effect of proinflammatory cytokines such as IL-1β and TNF-α is controversial. Neither of these cytokines cause neuronal death in healthy brain tissue or normal neurons (8). But it is generally believed that in disease state those proinflammatory cytokines contribute to the uncontrolled inflammation in the CNS. Ultimately the chronic inflammation impairs neuronal function and leads to the neurodegeneration. TRAIL, a member of the TNF superfamily, was originally thought to target only tumor cells and not function in the CNS. However, recent data show TRAIL is expressed in macrophages and can be induced in neurons by Aβ (9). Induced TRAIL may then causes apoptosis of brain cells as well as virally infected cells.

In this review, we summarize the common aspects of TRAIL in neurodegenerative diseases, the intracellular mechanism of TRAIL induced CNS cells apoptosis, and the specific role of TRAIL in the pathogenesis of each individual disease.

TRAIL

TRAIL is a type II integral membrane protein. A member of the TNF superfamily, TRAIL is closely related to Fas ligand (10, 11). TRAIL interacts with five unique receptors found on
into a variety of tissues such as the liver (Kupffer cells), released into circulation as monocytes. These cells migrate

MP originate from a common bone marrow precursor and are released into circulation as monocytes. TRAIL forms a homotrimer (18), which triggers apoptosis through interactions with TRAIL-R1, -R2. While decoy receptors TRAIL-R3, -R4 can compete with TRAIL-R1, -R2 for ligand binding, which provides protection from the induction of apoptosis. TRAIL receptors form a homotrimer structure that is capable of signal transduction upon binding.

A significant level of TRAIL transcript has been detected in many human tissues and is expressed constitutively in some cell lines. Such widespread distribution of TRAIL transcripts differs from that of FasL, which suggests that TRAIL may not only be as cytotoxic molecule towards tissue in vivo. Originally thought to target only tumor cells (10, 11), TRAIL has recently been shown to induce apoptosis of normal liver (19) and brain cells (20, 21) as well as virally infected cells (22-24). TRAIL is up-regulated on monocytes in response to immune activators such as lipopolysaccharide (LPS) (25) and interferon (26) as well as infection by human immuno-deficiency virus (HIV) (21, 27). The up-regulation of TRAIL by these factors along with its ability to induce apoptosis in the brain (20) suggests that TRAIL may be immunologically involved in the pathogenesis of several neurodegenerative diseases including HAD (21, 28), AD (9) and MS (29).

Recently, new regulatory, pro-survival and proliferative effects are being attributed to TRAIL besides apoptosis (30). For example, TRAIL acts as a positive regulator of myeloid differentiation (31); also, in primary human endothelial cells TRAIL is able to promote either survival or proliferation as well as cell migration or cytoskeleton reorganization, without inducing NF-κB activation and inflammatory markers (32).

**Mononuclear phagocytes (MP) and neuronal injury**

MP originate from a common bone marrow precursor and are released into circulation as monocytes. These cells migrate into a variety of tissues such as the liver (Kupffer cells), spleen and lymph node (histiocytes), lung (alveolar macrophages) and brain (microglia) where they differentiate into macrophages. These cells are crucial components of both innate and adaptive immune system. As phagocytic cells, MP act as scavengers to engulf and clear pathogens and cell debris from the host system. In CNS, MP represent as perivascular macrophages, microglia and the number of MP increase in inflammatory disease such as HAD (33-35).

Somewhat paradoxically, MP play a pivotal role in the pathobiology of neurodegenerative diseases despite their importance to host defense. In those diseases, infiltration of MP is a characteristic finding confirmed by pathological observations. Moreover, MP in most case are in an active state, enhancing phagocytic capabilities, secreting cytokines, chemokines, contributing to the inflammatory state of the CNS. Conversely, MP are also the source of neurotrophic factors in the CNS, those factors have important beneficial effects on neuronal function (36, 37).

**TRAIL and general apoptosis signaling that lead to cell death**

TRAIL receptor mediated signaling events leading to apoptosis can be divided into two distinct pathways, involving either mitochondrial (intrinsic) or death receptors (extrinsic) (38, 39) (for reviews, see (40, 41)). The mitochondrial pathway is initiated through various stress signals that damage mitochondria. Bcl2 family proteins, including the anti-apoptotic members, i.e., Bcl-2 and Bcl-xL, and the pro-apoptotic members, i.e., Bax, Bak, play a critical role in this pathway (40, 42-44). The BH3-only Bcl-2 family proteins, such as Bid, Bad, Bim, and PUMA, serve as sentinels to these stress signals. They are activated through various means, including transcriptional activation, posttranslational modification, proteolytic cleavage, etc. (45). Once activated, these BH3-only proteins translocate to the outer membrane of mitochondria, where they trigger the oligomerization and activation of both Bax and Bak, which in turn cause the release of cytochrome c (cyto c) and other apoptogenic factors, including second mitochondrial-derived activator of caspase (SMAC), Htr2, apoptosis inducing factor (AIF), and endonuclease G (EndoG) (41). Cyto c then binds to the cytosolic adaptor protein, Apaf-1, which mediates the formation of the apoptosis complex “apoptosome”. Such complexes lead to the activation of caspase-9, which further processes and activates the effector caspases, pro-caspase-3, 6, or 7.

During this process, Bcl-2 and Bcl-xL appear to directly or indirectly preserve the integrity of the mitochondrial membrane, preventing cyto c release and mitochondria-mediated cell death initiation. The pro-apoptotic proteins Bax and Bak promote cyto c release from mitochondria. Bax has been implicated in apoptosis in many cell types under various conditions. Studies using Bax-deficient human colon cancer cells have provided direct evidence that Bax plays a key role in mediating apoptosis induced by certain anti-cancer agents. The Bax protein exerts at least part of its activity by triggering cyto c release from mitochondria. As mentioned, Bax is in a predominantly cytosolic latent state in healthy cells and translocates to mitochondria after death signal stimulation. Accumulating evidence suggests that Bax translocation is required for its pro-apoptotic function and that regulation of Bax’s association with the mitochondrial membrane represents a critical step in the transduction of apoptotic signaling (39).

In the death receptor (extrinsic) pathway, it has been suggested that death receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2) oligomerize upon TRAIL binding, the adapter protein Fas-associated death domain (FADD) is then recruited. The receptor-FADD complex then recruits pro-caspase-8, which forms the death-inducing signaling complex.
Figure 1. A schematic diagram illustrating the TRAIL and apoptosis signaling. TRAIL, upon binding to its cognate receptors, can recruit FADD and pro-caspase-8 to form DISC. DISC releases active caspase-8, two major pathways defined as mitochondria dependent (intrinsic) and mitochondria independent (extrinsic) pathway. Both pathways finally lead to the activation of effector caspases such as caspase-3, 6, or 7, cause chromatin condensation and the cell apoptosis.

(DISC) where pro-caspase-8 is activated (46, 47). Depending on the cell type, active caspase-8 can directly lead to the activation of downstream effector caspases, including pro-caspase-3, 6 and 7 (48).

While the death receptor (extrinsic) pathway and mitochondrial (intrinsic) pathway for apoptosis are capable of operating independently, recent accumulating evidence suggests that cross-talk between the two pathways exists in cells (41). The link between death receptor signaling and the mitochondrial pathway comes from the finding that a BH3-domain-only subfamily protein, Bid, is cleaved by active caspase-8 (Figure 1). The truncated Bid (tBid) translocates to mitochondria and triggers cyto c release. It has been suggested tBid regulates cyto c release by inducing the homo-oligomerization of pro-apoptotic family members Bak or Bax (49, 50). Cells lacking both Bak and Bax, but not cells lacking one of these components, are completely resistant to t-Bid-induced cyto c release and apoptosis. Thus, Bid appears to link the mitochondrial pathways to death receptor-mediated apoptosis, although the precise mitochondrial events required for this crosstalk remain unclear.

Caspases are regulated both at the level of activation and by endogenous inhibitors. Recently, Deng et al. (39) demonstrated that mitochondrial events are required for TRAIL mediated apoptosis using human colon cancer cells. It was discovered the reason for this requirement is the presence of negative regulation of the caspase cascade by XIAP, or X-linked IAP, a widely expressed inhibitor of apoptosis protein (IAP) member. IAPs contain baculoviral IAP repeat (BIR) domains, which can bind to and inhibit caspases. There are 8 members of IAPs so far. Although IAPs function as inhibitor of apoptosis, genetic ablation of individual IAPs doesn’t have a profound effect toward animals, indicating the overlapping function within the family.

In summary, while it is clear that TRAIL introduction can selectively cause neuronal cell death, the precise molecular mechanism and signaling pathways are just beginning to unfold.
TRAIL of different cell types in the CNS

TRAIL and CNS cell interaction may result in apoptosis. Recently, Nitsch and colleagues used a brain slice culture system to demonstrate TRAIL induced extensive and non-selective brain cell death. These results indicate that cells in the CNS may be particularly sensitive to TRAIL. TRAIL is absent in normal brain, but is induced under pathological conditions, the specific cellular location of TRAIL in these cases is now being explored. CNS neurons usually do not express TRAIL, however, neurons may be induced to express TRAIL in the presence of β-amyloid protein (9, 52) and ischemia (53) when using neuron derived neuroblastoma cells. In contrast to TRAIL, it is now clear that human brain cells express TRAIL receptors (20, 51). Further, in our previous published paper, we have demonstrated that TRAIL-R1, -R2, -R3 and -R4 are expressed on human neuronal cultures; TRAIL-R1, -R2 are expressed on neurons in HIV brain tissue (21). Multiple studies now indicate that neurons are susceptible to TRAIL induced apoptosis (21, 28, 54).

As the predominant population of cells in the CNS, astrocytes usually do not express TRAIL or express it at very low levels. Early studies showed that TRAIL was consistently expressed in low levels by astrocytomas, anaplastic astrocytomas, and glioblastomas (55-57). Although little published information is available on the relationship between primary astrocytes and TRAIL, Choi et al. has shown fetal brain astrocytes may be induced to express TRAIL by interferon-γ (58); in addition, murine astrocytes express TRAIL when stimulated by GDVII virus (59). Malignant glioma cells express one or a combination of TRAIL-R1, -R2, -R3 and -R4 at high levels. There is no direct evidence that TRAIL receptors are expressed on primary astrocytes, but presumably are, based upon functional studies. Astrocytes play a significant role in the inflammatory state of the CNS during neurodegenerative diseases. However, most studies indicate both astrocytes and reactive astrocytes are resistant to TRAIL induced apoptosis (60).

Brain MP such as macrophage and microglia both express TRAIL and expression is increased following immune activation by interferon-γ (IFN-γ) and lipopolysaccharide (LPS) (25, 61, 62). HIV-1 infection or tat protein also up-regulate TRAIL on macrophages (21, 27). Macrophages and microglia express one or a combination of TRAIL receptors according to different experiment culture system. Primary cultures of macrophages and microglia are quite resistant to TRAIL induced apoptosis (29), while viral infection or immune activation may reverse this.

Neural progenitor cells (NPC) represent a unique population of cells in the CNS. Interestingly NPC express death receptors TRAIL-R1, -R2. They challenged with cognitive ligand TRAIL and other death ligands were unable to conduct an apoptotic signal (63, 64).

So far there are no reports concerning TRAIL expression on oligodendrocytes, but members of the TNF receptor superfamily have been detected on oligodendrocytes including TRAIL receptors (29, 65).

TRAIL in neurodegenerative diseases

Proper balance of life and death at the cellular level is essential for development and homeostatic function within the CNS, especially in regard to neurons. Mature neurons last the life of the organism excluding accidental death, despite containing multiple components of death pathways. The normal function of neurons is usually highly regulated, however, in neurodegenerative disease, inflammation shifts the balance towards death. Consequently, selective cell loss manifests and apoptosis is predominant. A postulated mechanism of injury or apoptosis of cells in the CNS involves the family of death ligands.

Although TRAIL is not normally expressed in CNS, it is possible that in the neurodegenerative diseases TRAIL expressed on the macrophages which may infiltrate into the brain. Those macrophages may interact with different cell types in the CNS that possess TRAIL receptors causing cell injury or death. Alternatively, cells in the CNS are capable of produce TRAIL upon induction by immune activation such as IFN-γ or other pathogens, those cell include neurons (9), microglia (62), astrocytes (58).

Neurodegenerative disease may be indicative of impaired neurogenesis and repair. NPCs are resistant to TRAIL induced apoptosis and may have considerable implications in adult neurogenesis. In several pathological conditions, TRAIL has a deleterious effect on neurons, whereas NPCs migrate and survive in the site of lesion. Recent data suggest that NPC might potentially be a target cell of TRAIL (64). Certainly more functional studies of TRAIL need to be test on the NPCs before we can get a clear conclusion of the TRAIL-NPC interaction in neurodegenerative diseases.

TRAIL in HIV-1-associated dementia (HAD)

HAD manifests during the later stages of viral disease as a spectrum of neurological and psychiatric symptoms (66). Cognitive impairments predominate beginning with forgetfulness, loss of concentration, apathy, and may progress to include hallucinations, global cognitive dysfunction and coma. Motor deficits, although less common, may range from fine motor clumsiness to tremors or quadriaparesis (67-70). Highly active antiretroviral therapies (HAART) dramatically improved the survival of people living with AIDS and at least temporarily decreased the incidence of HAD. Once affecting 20-50% of adults and children respectively, now less than 10% of all HIV infected subjects develop neurological impairments (71, 72). Despite this decreased incidence, neurological dysfunction remains a significant complication in infected individuals. Increased resistance to antiretroviral therapy due to viral strain mutation, the inability of drugs to penetrate the blood-brain barrier or eliminate viral reservoirs, and the increased lifespan of infected individuals suggest that HAD will continue to be a significant complication of advanced HIV-1
Figure 2. Possible role of TRAIL in neurodegenerative diseases. The source of TRAIL basically comes from microglia and infiltrated macrophages in the CNS. But amyloid protein induced neuron and immune activated astrocytes may be another source of TRAIL. TRAIL in the CNS may interact with different cell types and cause apoptosis in specific population of cells. Neuron are the target of TRAIL in HAD and AD; HIV-infected macrophages and microglia are the possible target for TRAIL in HAD; Oligodendrocyte is the target of TRAIL in MS. While other cell types, including astrocytes and neural progenitor cells, might potentially be the target cells for TRAIL.
monocytes may preferentially enter the brain and cause disease (105-109). The neurotoxicity of these subsets may be enhanced not only by changes in functional properties but also by the up-regulation of specific cell surface factors such as TRAIL.

TRAIL is up-regulated on macrophages in response to lipopolysaccharide (LPS) (25) and IFN-α or γ (26). HIV infection also induces TRAIL (27). The regulation of TRAIL by these factors, along with its ability to induce neuronal apoptosis, suggests that TRAIL may be involved in the pathogenesis of HAD (21). TRAIL expressing HIV-1-infected macrophages could initiate neuronal injury through two possible means. First, TRAIL is capable of binding to at least four unique cell surface receptors; all four of these receptors, but not TRAIL itself, have been found in the normal human brain. TRAIL expressed on macrophages or low-level soluble form of TRAIL could interact directly with death receptors on neurons initiating neuronal apoptosis (21). Evidence from the in vitro study supports this conclusion, e.g., recombinant human TRAIL induces neuronal apoptosis on cultured human neurons through caspase-3 activation (21). Furthermore, a similar observation was recently demonstrated in a murine model using human peripheral blood mononuclear cell (PBMC)-transplanted nonobese diabetic (NOD)-severe combined immunodeficiency (SCID)-hu-PBMC-NOC-SCID-mice (54). In this work, LPS was administered to HIV-1-infected hu-PBMC-NOC-SCID-mice to induce infiltration of HIV-1-infected human cells into the perivascular region of the brain. Neuronal apoptosis was found in macrophage (M)-tropic but not T cell (T)-tropic HIV-1-infected brain. The apoptotic neurons frequently colocalized with the HIV-1 infected macrophages that expressed TRAIL. Administration of a neutralizing antibody against human TRAIL but not human TNF-α or Fas ligand blocked the neuronal apoptosis in the HIV-1-infected brain, suggesting a significant role for TRAIL in MP mediated neuronal apoptosis (54). Second, TRAIL could mediate macrophage-macrophage or macrophage-astrocyte interactions resulting in an inflammatory response and subsequent neuronal injury. In vitro studies show that recombinant human TRAIL can selectively induce HIV-1-infected macrophage to undergo apoptosis while leaving uninfected macrophage intact (110).

The novel role of TRAIL may reveal a therapeutic target for HAD. The up-regulation of infected brain MP may mediate neuronal death. Alternatively, viral infection could sensitize cells to apoptosis through increasing pro-apoptotic factor or decreasing anti-apoptotic factor. It is possible that, by preventing TRAIL upregulation, receptor binding or caspase activation, the devastating effects of HAD could be attenuated.

**TRAIL in Alzheimer’s disease (AD)**

AD is the most common chronic neurodegenerative disorder characterized by the presence of neuritic plaques in the cerebral cortex, neurofibrillary tangles, neurophil threads, and plaque deposits comprised of β-amyloid peptides (Aβ), cellular debris, and inflammatory proteins (111, 112). In AD, neurons of the hippocampus and cerebral cortex are selectively lost.

A number of factors have been thought to contribute redundantly to pathogenesis of AD, including unbalanced calcium homeostasis, cell-cycle protein dysregulation, excitatory amino acids, as well as DNA damage. Deposition of β-amyloid protein (βAP) in the brain parenchyma appears to be the crucial factor for onset of AD, although mechanisms underlying βAP effects are far from understood. Hypotheses have been proposed suggesting proinflammatory cytokines, such as interleukin-1 and tumor necrosis factor-α could partially promote neurodegnerative processes depending upon βAP.

Recently, Cantarella et al. reported neutralization of the TRAIL death pathway protects a human neuronal cell line from β-amyloid toxicity. This is the first paper reporting the possible involvement of the TRAIL pathway in neurodegenerative processes in AD. Additional evidence from the same group reports TRAIL is specifically expressed in the brain of AD patients and completely absent in the brain of non-demented patients. TRAIL-like immunoreactivity was localized in AD affected regions, such as cerebral cortex, often in the proximity of Congo-red-positive amyloid plaques (52).

**TRAIL in MS and experimental autoimmune encephalomyelitis (EAE)**

MS is a chronic neurological disease with pathological hallmarks of perivascular inflammation and demyelination. Disseminated white matter lesions of the CNS were first described by French neurologist Charcot in late 19th century. Histological sections were shown to contain perivascular inflammation and demyelination. Distribution of demyelinating plaques occur anywhere within the white matter of the CNS, but the most frequently affected sites are the optic nerves, the brainstem, the cerebellum and the spinal cord. The selective loss of oligodendrocytes and their myelin sheaths has been postulated to occur via a variety of different mechanisms. These may involve direct interaction with cellular immune mediators, demyelination antibodies as well as cytokines, including those of the TNF superfamily (113).

Elevated TRAIL expression in blood MP of MS patients had been reported (113). Oligodendrocytes, the myelin-forming cell, have recently been shown to be one of TRAIL’s targets. Using a primary culture of oligodendrocytes, M. Matsyiak et al. demonstrated that ligation of TRAIL-R1 induces oligodendrocytes death in the presence of protein synthesis inhibitor or pre-treatment with IFN-γ (29). The susceptibility to TRAIL-induced death is dependent on low expression of decoy TRAIL-R3. Another study by K. Wosik et al. suggests p53 mediated oligodendrocyte cell death is at least partially through the TRAIL receptor signaling pathway (65).

TRAIL and oligodendrocyte interaction may potentially be the cause of demyelination. However, study in EAE, has shown that rather than this direct interaction, TRAIL may act...
through indirect mechanism. EAE is a CD4$^+$ T cell-mediated, CNS demyelinating disease that serves as a model for MS. Comparing to the oligodendrocytes, those infiltrating immune cells especially CD4$^+$ T cells are more susceptible to TRAIL induced cell death. Thus the increase of TRAIL in the CNS has a protective role. Using a soluble TRAIL receptor to examine the consequences of TRAIL blockade in an animal model of multiple sclerosis, it was found that chronic TRAIL blockade in mice exacerbated EAE induced by myelin oligodendrocyte glycoprotein. The exacerbation was evidenced primarily by increases in disease score and degree of inflammation in the CNS. Interestingly, the degree of apoptosis of inflammatory cells in the CNS was not affected by TRAIL blockade, suggesting that TRAIL may not regulate apoptosis of inflammatory cells in experimental autoimmune encephalomyelitis. By contrast, myelin oligodendrocyte glycoprotein-specific Th1 and Th2 cell responses were significantly enhanced in animals treated with the soluble TRAIL receptor. These suggest that TRAIL inhibits autoimmune encephalomyelitis and prevents activation of autoreactive T cells (29).

**Conclusion**

Since discovered in 1995, TRAIL has been extensively studied in the cancer area because of its selective apoptosis inducing capability in numerous tumor cell lines. The past few years have witnessed the novel TRAIL-cell interactions in the CNS, supported by different approaches and experimental systems. The role of TRAIL in the pathogenesis of different neurodegenerative diseases may foresee a therapeutic target for those diseases. Nevertheless, given the non-apoptotic functions of TRAIL, the complex system of neurodegenerative diseases are even more intriguing.

**Acknowledgements**

This work was supported in part by research grants by the National Institutes of Health: R01 NS 41858, P20 RR15635 and P01 NS043985 (JZ). We kindly acknowledge Drs. Howard E. Gendelman, Yuri Persidsky, Anuja Ghorpade, Lisa Ryan Xu Luo and Robin Cotter for valuable suggestions and criticisms of this work. Shelley Herek, David Erichsen, Alicia Lopez, Jianxing Zhao and Li Wu provided technical support for this work. Julie Ditter, Robin Taylor, Myanh Che, Nell Ingraham, Theresa Grutel, Lesley Gendelman and Connie Curro provided outstanding administrative support.

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