

## The role of TRAIL/TRAIL receptors in central nervous system pathology

Orhan Aktas, Ulf Schulze-Topphoff, Frauke Zipp

*Institute of Neuroimmunology, Clinical and Experimental Neuroimmunology, Neuroscience Research Center NWFZ 2680, Charité, Universitätsmedizin Berlin, Charité Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany*

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Expression of TRAIL and its receptors on immune cells and in the CNS
  - 3.1. Immune cells
  - 3.2. CNS
4. TRAIL for treatment of primary brain tumors
5. Dual role of TRAIL in multiple sclerosis
  - 5.1. TRAIL as an inflammatory effector molecule responsible for collateral damage
  - 5.2. TRAIL as a critical regulator of autoimmune T cell activation
6. TRAIL in other neurological diseases
7. Conclusion
8. Acknowledgement
9. References

### 1. ABSTRACT

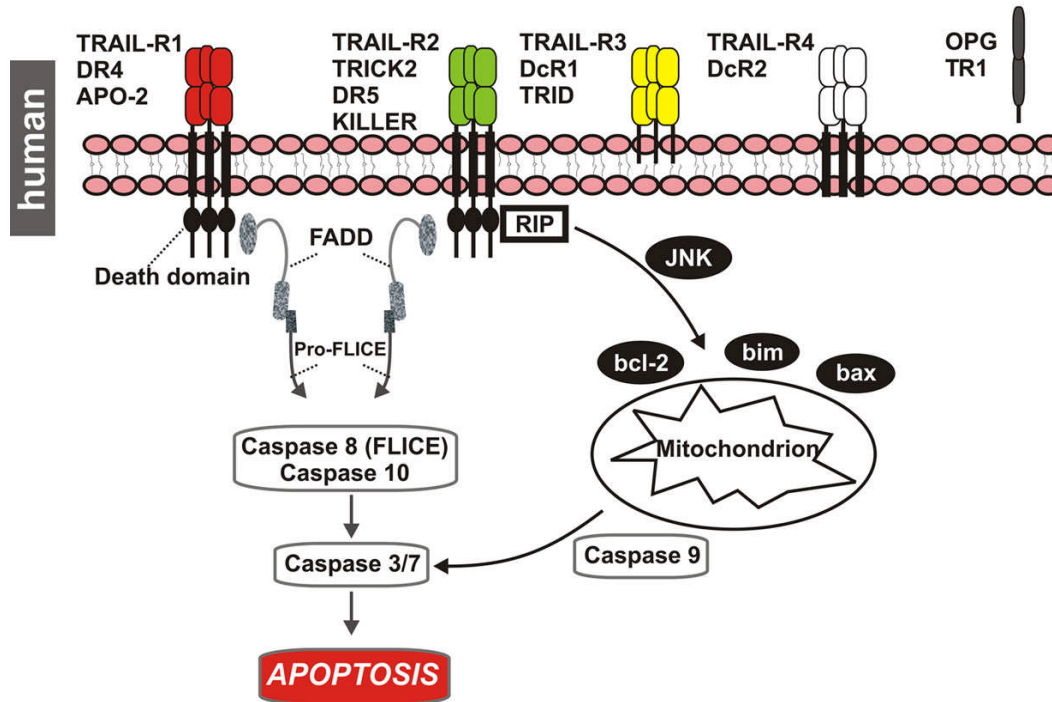
Initially, the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) aroused major interest due to its preferential toxic effect against malignant cells. However, subsequent studies revealed that the TRAIL system, comprising the family of signal-mediating and decoy TRAIL receptors, (i) can also induce death of non-transformed cells, (ii) has potent immunoregulatory functions, and (iii) exhibits a unique expression pattern in the central nervous system (CNS). Indeed, TRAIL is not expressed within the human brain, while apoptosis-inducing TRAIL receptors are found differently distributed on neurons, oligodendrocytes, and astrocytes. These findings rule out a major contribution of TRAIL to the so-called "immune privilege" of the brain, in which local inflammation is limited, although such a role has previously been suggested for the CD95 (Fas) ligand belonging to the same TNF/nerve growth factor (NGF) family. If, under pathologic circumstances, the CNS is inflamed, immune cells such as macrophages and T cells upregulate TRAIL upon activation and use this death ligand as a weapon, not only against tumor cells but also against neurons and oligodendrocytes within the inflamed CNS. In parallel, a profound immunoregulatory impact of TRAIL on activation and proliferation of encephalitogenic T cells outside the brain has also been shown. Thus, these studies have uncovered a complex action of TRAIL on CNS pathology, indicating the possible value of targeted manipulation of the TRAIL system for the treatment of inflammatory neurodegenerative diseases such as multiple sclerosis.

### 2. INTRODUCTION

The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) (1), also named Apo2 ligand (2), is a member of the TNF/nerve growth factor (NGF) superfamily (3) and acts via several receptors thought to induce or block apoptosis (4). Earlier reports emphasized the significance of the TRAIL system for immunological tumor surveillance (5,6). Later, however, TRAIL-mediated death of non-transformed human and murine cells such as hepatocytes (7) and neurons (8,9) suggested a possible implication for further non-cancer-related pathological conditions. Here, we focus on the diverse roles of TRAIL in the course of inflammatory processes of the CNS. According to the studies reviewed, this ligand possesses pronounced effector and immune-regulatory functions which imply beneficial and deleterious effects in neurological diseases such as multiple sclerosis (MS), HIV encephalopathy, stroke, Alzheimer's disease (AD) and primary brain tumors.

### 3. EXPRESSION OF TRAIL AND ITS RECEPTORS ON IMMUNE CELLS AND IN THE CNS

TRAIL is a 281 amino acids long type II transmembrane protein which forms a stable homotrimeric molecule with potent apoptotic activity (5). TRAIL has a calculated molecular mass of 32.5 kDa and shares sequence homology with CD95 (Fas/Apo1) ligand (23.2%), followed by CD40L (20.8%), lymphotoxin (LT)-alpha (20.2%), LT-beta (19.6%), TNF-alpha (19.0%), CD30L and CD27L (15.5%), OX-40L (14.3%), and 4-1BBL (13.7%) in human



**Figure 1.** Human TRAIL receptors and intracellular signal cascade. TRAIL binds to two death-mediating receptors, TRAIL-R1 and TRAIL-R2, and to two non-death receptors (“decoy receptors”), TRAIL-R3 and TRAIL-R4. TRAIL-mediated death occurs upon binding of the trimerized ligand to the receptor, which induces recruitment of the signaling protein FADD (Fas-associated death domain) and pro-caspase 8 (pro-FLICE). Activation of pro-caspase 8 leads to the generation of caspase 8 and subsequent activation of caspase 3, which mediates caspase-activated DNase and apoptotic demise of the cell. Moreover, TRAIL signaling may modulate mitochondrial apoptosis routes via induction of JNK and regulation of the bcl-2 family members bcl-2, bim and bax. The contribution of an additional TRAIL-binding receptor, osteoprotegerin (OPG), to TRAIL-mediated effects *in vivo* has not so far been clarified.

(1,2). Recently, two novel TRAIL splice variants TRAIL-beta and TRAIL-gamma were described in the human system (10). The relevance of these variants is not so far known, but according to the initial report, they may have a diminished apoptotic potential caused by the lack of exon 3 (in TRAIL-beta) or of exons 2 and 3 (in TRAIL-gamma) which implies truncation of the extracellular binding domain. Four membrane-bound receptors for TRAIL have so far been identified (Figure 1). Of these, only TRAIL receptor 1 (TRAIL-R1 or death receptor 4, DR4) (11) and TRAIL receptor 2 (TRAIL-R2, TRICK2, KILLER, DR5) (12) have the capacity to induce caspase-dependent apoptotic cell death, whereas TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2) lack a functional death domain and are considered to act as decoy receptors (13,14). In the murine system, two decoy receptors have recently been reported (15), whereas only one death-mediating TRAIL receptor is so far known, whose highest homology is with the human TRAIL receptor 2 (16) (Figure 2).

### 3.1. Immune cells

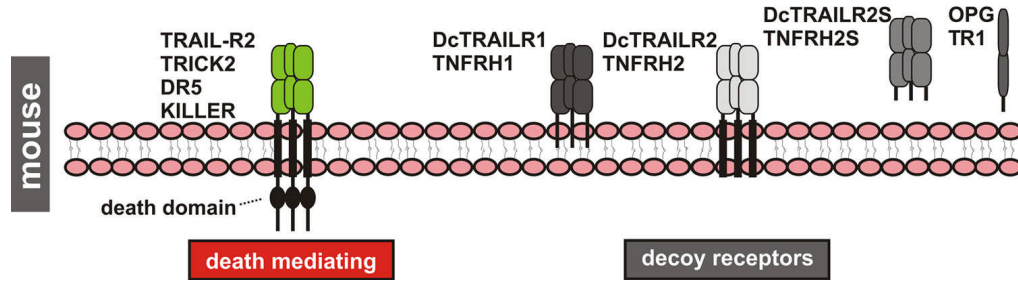
For resting human CD4<sup>+</sup> T cells and CD14<sup>+</sup> macrophages, expression of TRAIL and all TRAIL receptors including the truncated TRAIL-R3 and TRAIL-R4 has been described (17). In contrast, TRAIL expression in resting human B cells has not thus far been detected (18). Upon cell-type specific stimulation, human CD3<sup>+</sup> T cells,

CD19<sup>+</sup> B cells, and CD14<sup>+</sup> macrophages express or upregulate expression of TRAIL (19-21). In human antigen-specific T cell lines, TRAIL-receptors 1 and 2 are down-regulated upon T cell receptor stimulation with agonistic antibodies to CD3 and CD28 whereas TRAIL is up-regulated (17). In addition to the findings in human cells, TRAIL expression was also found in murine macrophages, T and B cells in response to specific stimuli (22-24).

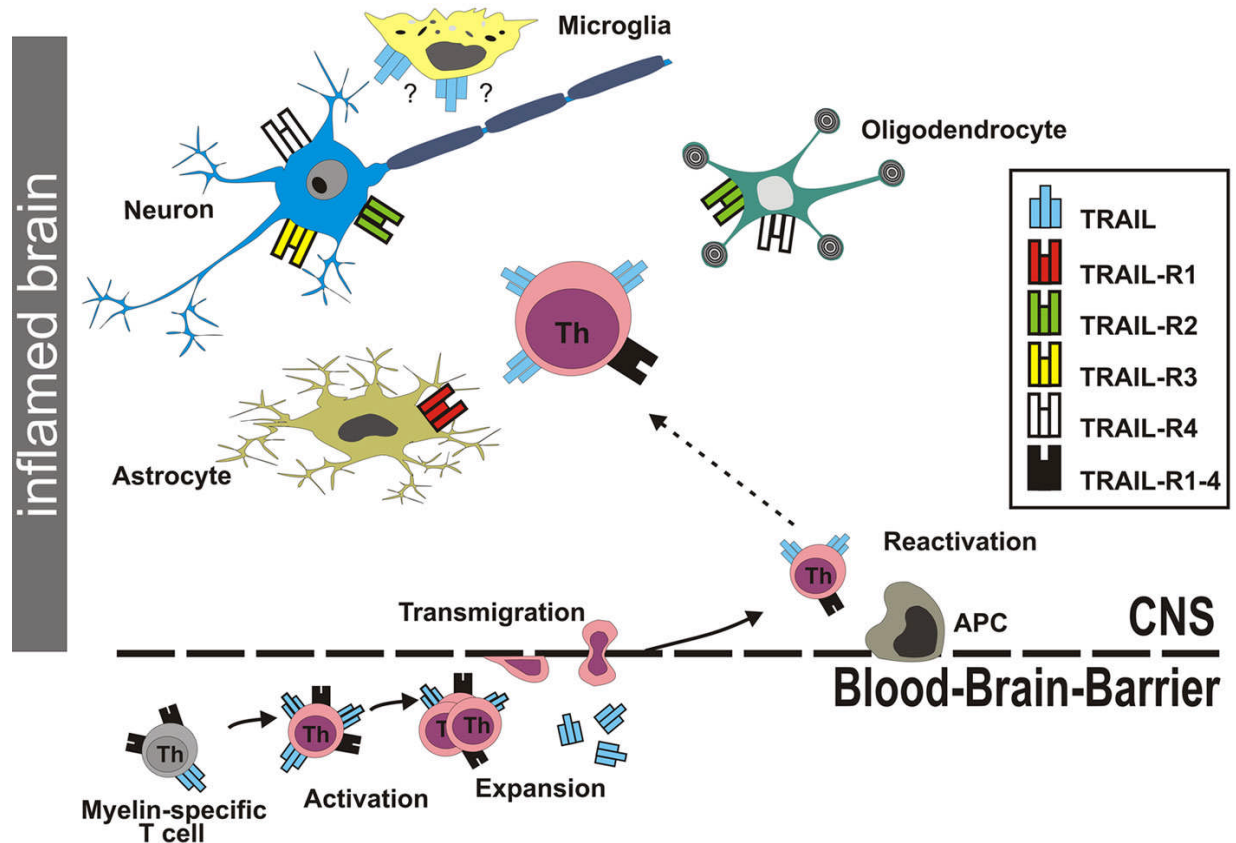
### 3.2. CNS

Previously, members of the TNF family have been shown to contribute to the so-called “immune privilege” of the CNS, as demonstrated for the CD95 system: the CD95 death ligand has been detected on astrocytes in the brain of rodents and humans (25), and such CD95-ligand-bearing astrocytes, which form the first barrier for cells invading the CNS, were later shown to kill T cells via CD95/CD95 ligand interaction (26). Moreover, neurons of the CNS were also shown to express CD95 ligand on their surface and to induce T cell apoptosis (27). Thus, the brain containing mainly postmitotic tissue and requiring a strict control of inflammation to maintain its integrity (28) suppress immune cell invasion by using the CD95 ligand as a weapon. This is similar to other immunologically protected niches of the organism which are either half foreign (the half-paternal placenta), harbour

## Role of TRAIL/TRAIL receptors in central nervous system pathology



**Figure 2.** Mouse TRAIL receptors. In the murine system one death-mediating TRAIL receptor 2 (TRAIL-R2) and two non-death receptors (“decoy receptors”), DcTRAILR1 and DcTRAILR2, including the secreted splice variant DcTRAILR2S have thus far been identified. The death-mediating function of TRAIL-R2 upon binding of the trimerized ligand to the receptor induces recruitment of the signaling protein FADD (Fas-associated death domain) and pro-caspase 8 (pro-FLICE), leading to apoptosis. The additional soluble TRAIL-binding receptor, osteoprotegerin (OPG), is also known in the murine system.



**Figure 3.** Disease model of multiple sclerosis. According to current disease models, myelin-specific T cells become activated outside the CNS and escape endogenous immunoregulatory mechanisms. Guided by adhesion molecules, they transmigrate the blood-brain barrier (BBB), recognize their specific autoantigen (presented by local antigen-presenting cells (APCs) such as dendritic cells located at the perivascular space of the BBB), are reactivated, and finally cause a local Th1-type, i.e. proinflammatory immune response. The inflammatory reaction, directed against the myelin sheath, causes demyelination, axon loss, dysregulated ion channel expression, oligodendrocyte and neuron death, and plaque formation. Antibodies directed against the myelin sheath enhance this effect. Simultaneously, tissue alteration results in the release of new tissue antigens, which in turn can lead to new, autoreactive T cells being established. Following the existing data, TRAIL is involved at two crucial stages: firstly, outside the brain, TRAIL contributes to the regulatory inhibition of myelin-specific T cells by inhibiting overwhelming activation of TRAIL receptor-expressing T cells. Secondly, inside the brain, TRAIL is critically involved in the collateral damage process, as T cells and macrophages/microglia can damage target cells such as neurons and oligodendrocytes in an antigen-independent manner via the TRAIL system. As a basis, these target cells express death-mediating TRAIL receptors on their surface and upregulate them under inflammatory conditions. In contrast to neurons and oligodendrocytes, astrocytes have been shown to be resistant towards TRAIL-mediated apoptosis (80).

germ-line cells (ovary and testis), or contain tissues with limited regenerative capacity (lens and cornea in the anterior chamber of the eye), where a prominent role for the CD95 system has been suggested (29-32). However, in contrast to the CD95 ligand, TRAIL expression has not been found in the human brain under physiological conditions (33). While TRAIL is lacking in the CNS, death-mediating and decoy TRAIL receptors are expressed on brain cells such as microtubuli-associated protein (MAP)2+ neurons (TRAIL-R1, -R3 and -R4), glial fibrillary acid protein (GFAP)+ astrocytes (TRAIL-R1) and proteolipid (PLP)+ oligodendrocytes (TRAIL-R2 and -R4) (33). Interestingly, CD68+ microglia cells neither express TRAIL nor TRAIL receptors when they rest in the normal CNS *in situ*.

The expression pattern of the TRAIL system within the brain is markedly modulated in disease. TRAIL was detected in neuropathological scenarios such as human brain tumors and in brain debris samples from patients with severe penetrating head injuries (34). TRAIL expression is induced on neurons in the brains of patients with AD (35). Moreover, we found upregulation of TRAIL-R2 and TRAIL itself in the inflamed CNS tissue of mice suffering from experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis (MS) (23). In the latter study, TRAIL-R2 upregulation was detected particularly on neurons, explaining the vulnerability of these cells to TRAIL-mediated cytotoxicity (see below). Expression of TRAIL in the inflamed CNS was mainly due to infiltrating and activated immune cells, among them T cells, macrophages, and microglia. Experimentally, TRAIL expression can be induced in murine microglia upon stimulation with interferon (IFN)-gamma or lipopolysaccharide (LPS) *in vitro* (36). Similarly, exposure of fetal brain astrocytes to IFN-gamma has been reported to result in TRAIL expression *in vitro* as well (37).

#### 4. TRAIL FOR TREATMENT OF PRIMARY BRAIN TUMORS

At the time of its discovery, TRAIL was shown to exert potent and quite specific capacities to kill several tumor cell lines *in vitro* and to eliminate tumor growth in experimental cancer models *in vivo*. Toxic effects against normal, i.e. non-transformed tissue were lacking when TRAIL was injected into non-human primates (5,6). For the CNS, similar observations indicated a specific anti-tumor effect of TRAIL: in line with TRAIL mediated glioma cell death *in vitro*, the survival of U87MG human glioma xenograft was decreased in mice through apoptosis induction of transplanted glioma cells without any neurotoxic effect in the surrounding healthy tissue after intratumor TRAIL injection (38). The sensitivity of malignant glioma cells towards TRAIL-mediated apoptosis may be reduced by disease-specific genomic aberrations (39). It can, however, be increased by manipulating the second mitochondria-derived activator of caspase (Smac), involved in the mitochondrial pathway of apoptosis induction (40). In a further exploration of the *in vivo* role of TRAIL in neurooncological conditions, a recent study investigated the expression of TRAIL-R1 and TRAIL-R2

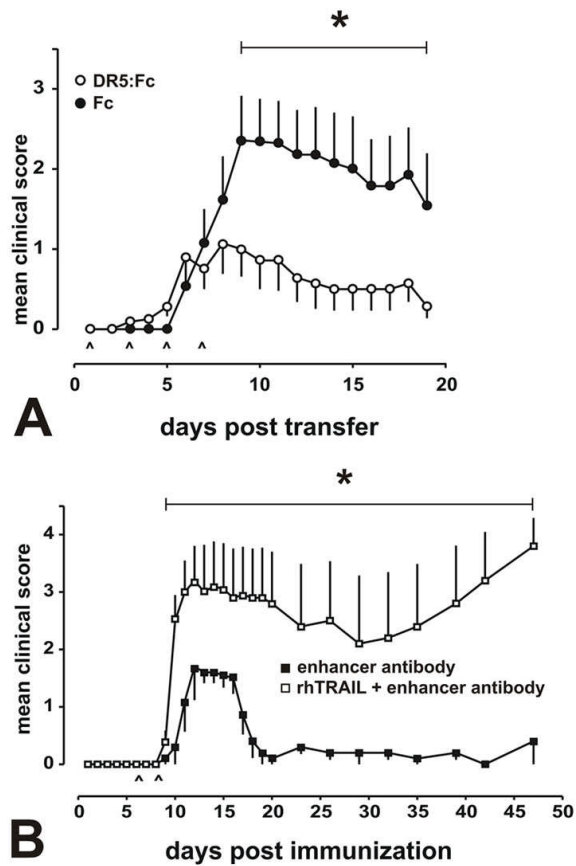
in tumor tissue from 62 patients with glioblastoma multiforme (41). Combined immunohistochemical and clinical analysis revealed that TRAIL-R1 and TRAIL-R2 expression on tumor cells represent independent prognostic factors for survival in such patients. Thus, such TRAIL-based strategies, aiming at enhanced apoptosis of chemotherapy-resistant brain cancer cells, have been proposed as new therapeutic options for primary brain tumors (42). Indeed, the organism uses TRAIL as an endogenous weapon of tumor surveillance, as reflected by the enhanced survival of engrafted tumors in TRAIL-deficient animals (43). As regards an underlying mechanism, we have shown that activated human CD4+ T cells employ TRAIL to selectively kill glioma cell lines, a process which might play an important role in tumor regression (44).

#### 5. DUAL ROLE OF TRAIL IN MULTIPLE SCLEROSIS

##### 5.1. TRAIL as an inflammatory effector molecule responsible for collateral damage

In the latter study (44), however, we also observed that the TRAIL-mediated cytotoxic effect of human T cells was not antigen-specific, i.e. not restricted to specific target structures. Moreover, the apparent selectivity of TRAIL against transformed cells was challenged when an increased sensitivity of human hepatocytes towards certain TRAIL preparations was reported (7,45). Indeed, recent studies indicate the relevance of TRAIL as a general immunological weapon of activated immune cells not only in tumor defense. For example, in arteriosclerosis, which is accepted as a generalized, T cell-mediated inflammatory disease of the vascular system (46,47), carotid artery plaque-infiltrating CD4+ T-cells express TRAIL and induce apoptosis of TRAIL-R2-bearing vascular smooth muscle cells, thus regulating plaque stability and consecutive thrombotic complications (48). Similarly, a critical role for TRAIL was shown for hepatocyte damage in the course of liver inflammation: TRAIL expression by cells of the immune system is required for the development of Con-A and *Listeria*-induced hepatitis, as liver cell death was drastically reduced in TRAIL-deficient mice (49). Taken together, these findings indicate that the immune system employs TRAIL not only as a powerful weapon of tumor surveillance, but also for antigen-unspecific killing of non-transformed target cells, especially in an inflammatory context.

Initial *in vitro* studies showed that soluble TRAIL can induce death of non-transformed brain cells such as neurons (8) and oligodendrocytes (50,51). Considering the upregulation of TRAIL by activated human lymphocytes (17,44), we speculated about a potential role of TRAIL in the course of MS, the most common chronic inflammatory CNS disease in Western countries characterized by the presence of inflammatory plaques (52). Animal experiments and human studies suggest that MS is a T cell-mediated multiphase autoimmune disease of the CNS (53-55), in which myelin-specific CD4+ Th1 cells attack their target antigen, the myelin sheath, within the CNS (Figure 3). However, during the last decade it has become clear that



**Figure 4.** A. Amelioration of autoimmune encephalomyelitis by intracerebral injection of DR5:Fc. Mean clinical disease scores were significantly reduced by blockade of TRAIL through intracerebral injection of DR5:Fc fusion protein (open circles) compared to mice which received Fc fragment only (filled circles) after adoptive transfer of encephalitogenic myelin-specific lymphocytes. Reproduced with permission from Ref. (23) © (2005) Elsevier. B. TRAIL susceptibility of the inflamed CNS. Recombinant human TRAIL (rhTRAIL together with an enhancer antibody for multimerization; open squares) or enhancer antibody alone (filled squares) were injected intracisternally prior to and at the onset of disease during actively induced EAE. Mild active EAE was significantly deteriorated by delivery of recombinant human TRAIL into the CNS. Reproduced with permission from Ref. (23) © (2005) Elsevier.

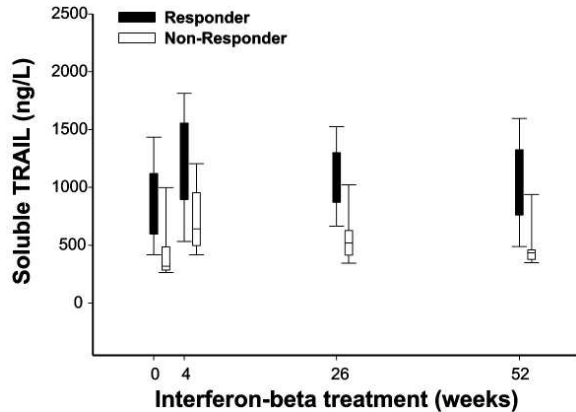
neuronal pathology involving axonal transection and neuronal death plays a pivotal role in disease pathology and critically contributes to irreversible disability of patients (56). Data from the MS animal model EAE supported the conclusion that axonal damage and neuronal death are an integral part of the disease processes and occur at early stages of pathology (57,58). By intracerebral injection of a soluble TRAIL receptor which blocks deleterious TRAIL actions, we observed rescue of animals from EAE-related disability (23) (Figure 4A). This treatment did not influence the peripheral immune response, including T cell proliferation and cytokine release, but resulted in a

significant protection of the CNS from immune cell-mediated damage, i.e. neuronal apoptosis and myelin loss. Furthermore, transfer of myelin-specific TRAIL-deficient T cells into wildtype recipients led to a significantly attenuated disease score, indicating that brain-invading myelin-specific T cells devoid of TRAIL are significantly reduced in their capacity to induce CNS damage.

Interestingly, we were unable to detect any cytotoxic effects from two different TRAIL preparations applied into the brains of healthy animals. This finding is supported by the above mentioned neurooncological study in which local treatment with TRAIL into a xenograft brain tumor affected only the tumor cells and not the surrounding normal CNS tissue (38). Quantitative real-time gene expression analysis for TRAIL-R2 and TRAIL showed that in EAE, both molecules were significantly upregulated in the CNS prior to and during the peak phase of EAE (23). Accordingly, soluble TRAIL injected into the cerebrospinal fluid of animals with ongoing mild EAE enhanced disease severity (Figure 4B), while healthy animals were not affected. This indicates that TRAIL receptor levels under normal conditions are insufficient to mediate a significant death signal, which is only achieved in the course of inflammation. A similar inflammatory sensitization of the target tissue to harmful TRAIL effects was also observed in ConA-mediated experimental hepatitis, while this study showed an upregulation of TRAIL-expression in the liver including constitutively expressed TRAIL-R2. In this model, hepatitis cell death was diminished in TRAIL-deficient mice, whereas transfer of TRAIL-expressing liver mononuclear cells was sufficient to renew hepatic cell death (49).

**5.2. TRAIL as a critical regulator of autoimmune T cell activation**

According to our observations, inflammation itself was not greatly altered by intracerebral TRAIL blockade and even rather enhanced locally in early stages of intervention. An initial increase in T cell activity in the brain following CNS-restricted TRAIL blockade is in line with the anti-inflammatory capacity of TRAIL, as previously reported for the peripheral immune system (59-61). Indeed, in T cells, TRAIL does not induce apoptosis (17,62), but inhibits proliferation *in vitro* and *in vivo* (60,63). Mechanistically, TRAIL inhibits activation of T cells by blocking calcium influx through store-operated calcium release-activated calcium channels, IFN-gamma/IL-4 production, and proliferation. TRAIL-induced hypoproliferation of T cells could be attributed to the down-regulation of the cyclin-dependent kinase 4, indicating a G1 arrest of the cell cycle (60). Interestingly, ligands of the TNF superfamily may themselves transmit signals to the cell after engaging their receptors. These anti-inflammatory properties may explain why systemic (intraperitoneal) injection of soluble neutralizing TRAIL receptor leads to exacerbation of murine collagen-induced arthritis (63), autoimmune diabetes (64,65), and EAE (59). An alternative explanation is the fact that TRAIL, along with other ligands of the TNF superfamily, may itself transmit signals back into the cell, engaging its receptors (66). Recently, we also showed an important link between



**Figure 5.** Soluble TRAIL protein concentrations in patients with multiple sclerosis during IFN beta-1a therapy. Reproduced with permission from Ref. (61) © (2003).

TRAIL expression in PBMCs from untreated patients and a potentially successful immunomodulatory therapy, supporting the regulatory role of this death ligand. Detailed analysis of our data showed that clinical responders to IFN-beta treatment can be distinguished from non-responders by early and sustained induction of TRAIL mRNA in peripheral immune cells, pointing to TRAIL as an early surrogate marker of therapeutic success (61) (Figure 5). A further study reported that activated T cells from IFN-beta-treated MS patients express higher levels of TRAIL than untreated patients, supporting the view that *in vivo* exposure to IFN-beta modulates the TRAIL system in MS (67).

## 6. TRAIL IN OTHER NEUROLOGICAL DISEASES

In addition to the role of TRAIL in immune cell-mediated collateral damage in EAE (23), several studies have suggested a prominent role for TRAIL in other diseases of the CNS (68). Neuropathological studies have shown that HIV encephalopathy, a neurodegenerative disease, is not directly mediated by HIV, as neurons are fairly resistant to HIV infection (69). In contrast, it was shown that HIV-infected macrophages infiltrate the brain and induce a diffuse inflammatory reaction characterized by local astrocyte and microglia activation. As a result widespread synaptic and dendritic damage occurs, paving the way for final neuronal demise (70). Moreover, in human macrophages, HIV infection leads to marked and persistent upregulation of TRAIL *in vitro* (71). Indeed, a recent study reported the critical contribution of TRAIL expressed by HIV-infected macrophages in a humanized mouse model of HIV encephalopathy (72). Here, neutralizing TRAIL blocked neuronal apoptosis *in vivo*, while blockade of TNF-alpha or CD95 ligand had no effects. Indeed, supporting these experimental findings, two independent histopathological studies recently found TRAIL-expressing macrophages in the direct vicinity of apoptotic cortical neurons in patients with HIV encephalopathy (73,74). Moreover, the critical contribution of TRAIL has recently been suggested in stroke (75). In ischemic brain injury, the blood flow to the brain is

suddenly inhibited by occlusion of brain arteries, leading to reduced delivery of oxygen and thus causing acute damage to brain cells (mainly vulnerable neurons) within the ischemic core zone. Interestingly, within the area surrounding the ischemic core zone, the penumbra or peri-infarct zone, surviving brain cells are exposed to harmful secondary processes, such as excitotoxicity and inflammation (76). Here, adhesion molecules are upregulated and inflammatory immune cells invade the penumbra, contributing to brain damage (77). In experimental rodent models of ischemia, a relevant upregulation of both CD95 ligand and TRAIL was detected in the apoptotic brain areas (75). Using the immunomodulator, tacrolimus (FK506, fujimycin), inflammatory changes within the postischemic brain were blocked, which led to a downregulation of these death ligands and was followed by a significant protection from ischemic neurodegeneration in these settings (75). In AD, TRAIL expression was found in direct proximity to amyloid plaques within affected brain regions (35), and TRAIL itself was recently demonstrated to be involved in [beta]-amyloid (A[beta])-induced neurotoxicity *in vitro* (78,79). However, while these results indicate the involvement of TRAIL in AD, functional data in experimental *in vivo* models have thus far been lacking. Based on our increasing knowledge of inflammation in stroke and primary neurodegenerative diseases like AD, future experimental work should seek to clarify the precise role of TRAIL-mediated neuronal damage in stroke and primary neurodegenerative diseases like AD, as has already been suggested for MS. Both for stroke and AD, it has yet to be clarified whether the destructive role of TRAIL is mediated by immune cells (56).

## 7. CONCLUSION

Taken together, the studies reviewed here reveal the involvement of TRAIL in diverse CNS pathologies. There is no doubt as to the critical contribution of this death ligand to irreversible CNS destruction, especially in the course of inflammatory brain disease. One possible scenario is that, under inflammatory conditions, TRAIL-bearing immune cells cross the blood brain-barrier and, after local reactivation, destroy through interaction with TRAIL receptor-expressing neurons and oligodendrocytes (Figure 3). The specificities of the TRAIL system discussed – the upregulation of TRAIL on CNS infiltrating immune cells such as autoreactive T cells or macrophages, the resistance of these T cells towards TRAIL-mediated apoptosis, and the absence of TRAIL expression in the brain – indicate that this system is not involved in the elimination of T cells from the brain. Thus, in contrast to the TNF and CD95 system, local blockade of TRAIL signaling in the brain does not lead to unwanted disease exacerbation. Since the pivotal impact of inflammation in classical neurodegenerative diseases has recently been recognized (56), therapeutic inhibition of TRAIL-mediated damage may have a broader therapeutic application than to MS alone. In addition to the contribution of TRAIL to CNS destruction mentioned above, the immunoregulatory properties of TRAIL may also, by impairing the function or viability of autoreactive T lymphocytes, be useful for

therapeutic intervention, especially in the disease pathogenesis of MS, in which peripheral T cell activation constitutes an important step. Thus, despite the complexity of the TRAIL system, the specificities of this system and the peculiarities of the immune-privileged CNS could allow for carefully targeted modulation of TRAIL receptor-TRAIL interactions as a possible therapeutic strategy in CNS diseases such as brain tumors and MS.

**8. ACKNOWLEDGMENTS**

We thank Alistair Noon for reading the manuscript as a native English speaker. The original work was supported by the Institute for Multiple Sclerosis Research (IMSF) Goettingen/ Gemeinnuetzige Hertie Foundation, the German Research Foundation (SFB 507), the German Federal Ministry of Education and Research (BMBF).

**9. REFERENCES**

1. S. R. Wiley, K. Schooley, P. J. Smolak, W. S. Din, C. P. Huang, J. K. Nicholl, G. R. Sutherland, T. D. Smith, C. Rauch, C. A. Smith and R. G. Goodwin: Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 3, 673-682 (1995)

2. R. M. Pitti, S. A. Marsters, S. Ruppert, C. J. Donahue, A. Moore and A. Ashkenazi: Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J. Biol. Chem.* 271, 12687-12690 (1996)

3. O. Aktas, T. Prozorovski and F. Zipp: Death ligands and autoimmune demyelination. *Neuroscientist* 12, 305-316 (2006)

4. M. A. Degli-Esposti: To die or not to die - the quest of the TRAIL receptors. *J. Leukoc. Biol.* 65, 535-542 (1999)

5. A. Ashkenazi, R. C. Pai, S. Fong, S. Leung, D. A. Lawrence, S. A. Marsters, C. Blackie, L. Chang, A. E. McMurtrey, A. Hebert, L. DeForge, I. L. Koumenis, D. Lewis, L. Harris, J. Bussiere, H. Koeppen, Z. Shahrokh and R. H. Schwall: Safety and antitumor activity of recombinant soluble Apo2 ligand. *J. Clin. Invest* 104, 155-162 (1999)

6. H. Walczak, R. E. Miller, K. Ariail, B. Gliniak, T. S. Griffith, M. Kubin, W. Chin, J. Jones, A. Woodward, T. Le, C. Smith, P. Smolak, R. G. Goodwin, C. T. Rauch, J. C. Schuh and D. H. Lynch: Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat. Med.* 5, 157-163 (1999)

7. M. Jo, T.-H. Kim, D.-W. Seol, J. E. Esplen, K. Dorko, T. R. Billiar and S. C. Strom: Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. *Nat. Med.* 45, 564-567 (2000)

8. R. Nitsch, I. Bechmann, R. A. Deisz, D. Haas, T. N. Lehmann, U. Wendling and F. Zipp: Human brain-cell death induced by tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL) *Lancet* 356, 827-828 (2000)

9. O. Aktas, T. Prozorovski, A. Smorodchenko, N. E. Savaskan, R. Lauster, P.-M. Kloetzel, C. Infante-Duarte, S. Brocke and F. Zipp: Green tea epigallocatechin-3-gallate mediates T cellular NF- $\kappa$ B inhibition and exerts neuroprotection in autoimmune encephalomyelitis. *J. Immunol.* 173, 5794-5800 (2004)

10. A. Krieg, T. Krieg, M. Wenzel, M. Schmitt, U. Ramp, B. Fang, H. E. Gabbert, C. D. Gerharz and C. Mahotka: TRAIL-beta and TRAIL-gamma: two novel splice variants of the human TNF-related apoptosis-inducing ligand (TRAIL) without apoptotic potential. *Br. J. Cancer* 88, 918-927 (2003)

11. G. Pan, K. O'Rourke, A. M. Chinnaiyan, R. Gentz, R. Ebner, J. Ni and V. M. Dixit: The receptor for the cytotoxic ligand TRAIL. *Science* 276, 111-113 (1997)

12. H. Walczak, M. A. Degli-Esposti, R. S. Johnson, P. J. Smolak, J. Y. Waugh, N. Boiani, M. S. Timour, M. J. Gerhart, K. A. Schooley, C. A. Smith, R. Goodwin and C. Rauch: TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. *EMBO J.* 16, 5386-5397 (1997)

13. M. A. Degli-Esposti, W. C. Dougall, P. J. Smolak, J. Y. Waugh, C. A. Smith and R. Goodwin: The novel receptor TRAIL-R4 induces NF-kappaB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. *Immunity* 7, 813-820 (1997)

14. M. A. Degli-Esposti, P. J. Smolak, H. Walczak, J. Y. Waugh, C. P. Huang, R. F. DuBose, R. Goodwin and C. A. Smith: Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *J. Exp. Med.* 186, 1165-1170 (1997)

15. P. Schneider, D. Olson, A. Tardivel, B. Browning, A. Lugovskoy, D. Gong, M. Dobles, S. Hertig, K. Hofmann, H. Van Vlijmen, Y. M. Hsu, L. C. Burkly, J. Tschopp and T. S. Zheng: Identification of a new murine tumor necrosis factor receptor locus that contains two novel murine receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) *J. Biol. Chem.* 278, 5444-5454 (2003)

16. G. S. Wu, T. F. Burns, Y. Zhan, E. S. Alnemri and W. S. El-Deiry: Molecular cloning and functional analysis of the mouse homologue of the KILLER/DR5 tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptor. *Cancer Res.* 59, 2770-2775 (1999)

17. U. Wendling, H. Walczak, J. Dörr, C. Jacobi, M. Weller, P. H. Kramer and F. Zipp: Expression of TRAIL receptors in human autoreactive and foreign antigen-specific T cells. *Cell Death Differ.* 7, 637-644 (2000)

18. S. Zhao, Z. Asgary, Y. Wang, R. Goodwin, M. Andreeff and A. Younes: Functional expression of TRAIL by lymphoid and myeloid tumour cells. *Br. J. Haematol.* 106, 827-832 (1999)

19. M. J. Martinez-Lorenzo, A. Anel, S. Gamon, I. Monleon, P. Lasierra, L. Larrad, A. Pineiro, M. A. Alava and J. Naval: Activated human T cells release bioactive Fas ligand and APO2 ligand in microvesicles. *J. Immunol.* 163, 1274-1281 (1999)

20. T. S. Griffith, S. R. Wiley, M. Z. Kubin, L. M. Sedger, C. R. Maliszewski and N. A. Fanger: Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL. *J. Exp Med.* 189, 1343-1354 (1999)

21. S. Ehrlich, C. Infante-Duarte, B. Seeger and F. Zipp: Regulation of soluble and surface-bound TRAIL in human T cells, B cells, and monocytes. *Cytokine* 24, 244-253 (2003)

22. S. M. Mariani and P. H. Kramer: Surface expression of TRAIL/Apo-2 ligand in activated mouse T and B cells. *Eur. J. Immunol.* 28, 1492-1498 (1998)

23. O. Aktas, A. Smorodchenko, S. Brocke, C. Infante-Duarte, U. S. Toppoff, J. Vogt, T. Prozorovski, S. Meier,

- V. Osmanova, E. Pohl, I. Bechmann, R. Nitsch and F. Zipp: Neuronal Damage in Autoimmune Neuroinflammation Mediated by the Death Ligand TRAIL. *Neuron* 46, 421-432 (2005)
24. G. E. Diehl, H. H. Yue, K. Hsieh, A. A. Kuang, M. Ho, L. A. Morici, L. L. Lenz, D. Cado, L. W. Riley and A. Winoto: TRAIL-R as a negative regulator of innate immune cell responses. *Immunity*. 21, 877-889 (2004)
25. I. Bechmann, G. Mor, J. Nilsen, M. Eliza, R. Nitsch and F. Naftolin: FasL (CD95L, Apo1L) is expressed in the normal rat and human brain: evidence for the existence of an immunological brain barrier. *Glia* 27, 62-74 (1999)
26. I. Bechmann, B. Steiner, U. Gimsa, G. Mor, S. Wolf, M. Beyer, R. Nitsch and F. Zipp: Astrocyte-induced T cell elimination is CD95 ligand dependent. *J. Neuroimmunol.* 132, 60-65 (2002)
27. A. Flugel, F. W. Schwaiger, H. Neumann, I. Medana, M. Willem, H. Wekerle, G. W. Kreutzberg and M. B. Graeber: Neuronal FasL induces cell death of encephalitogenic T lymphocytes. *Brain Pathol.* 10, 353-364 (2000)
28. I. Bechmann: Failed central nervous system regeneration: a downside of immune privilege? *Neuromolecular Med.* 7, 217-228 (2005)
29. D. Bellgrau, D. Gold, H. Selawry, J. Moore, A. Franzusoff and R. C. Duke: A role for CD95 ligand in preventing graft rejection. *Nature* 377, 630-632 (1995)
30. T. S. Griffith, T. Brunner, S. M. Fletcher, D. R. Green and T. A. Ferguson: Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 270, 1189-1192 (1995)
31. T. S. Griffith and T. A. Ferguson: The role of FasL-induced apoptosis in immune privilege. *Immunol. Today* 18, 240-244 (1997)
32. G. Mor, L. S. Gutierrez, M. Eliza, F. Kahyaoglu and A. Arici: Fas-fas ligand system-induced apoptosis in human placenta and gestational trophoblastic disease. *Am. J. Reprod. Immunol.* 40, 89-94 (1998)
33. J. Dörr, I. Bechmann, S. Waiczies, O. Aktas, H. Walczak, P. H. Krammer, R. Nitsch and F. Zipp: Lack of tumor necrosis factor-related apoptosis-inducing ligand but presence of its receptors in the human brain. *J. Neurosci.* 22, RC209 (2002)
34. S. Frank, U. Kohler, G. Schackert and H. K. Schackert: Expression of TRAIL and its receptors in human brain tumors. *Biochem. Biophys. Res. Commun.* 257, 454-459 (1999)
35. D. Uberti, G. Cantarella, F. Facchetti, A. Cafici, G. Grasso, R. Bernardini and M. Memo: TRAIL is expressed in the brain cells of Alzheimer's disease patients. *NeuroReport* 15, 579-581 (2004)
36. S. Genc, S. Kizildag, K. Genc, H. Ates and N. Atabay: Interferon gamma and lipopolysaccharide upregulate TNF-related apoptosis-inducing ligand expression in murine microglia. *Immunol. Lett.* 85, 271-274 (2003)
37. J. Lee, J. S. Shin, J. Y. Park, D. Kwon, S. J. Choi, S. J. Kim and I. H. Choi: p38 mitogen-activated protein kinase modulates expression of tumor necrosis factor-related apoptosis-inducing ligand induced by interferon-gamma in fetal brain astrocytes. *J. Neurosci. Res.* 74, 884-890 (2003)
38. W. Roth, S. Isenmann, U. Naumann, S. Kugler, M. Bahr, J. Dichgans, A. Ashkenazi and M. Weller: Locoregional Apo2L/TRAIL eradicates intracranial human malignant glioma xenografts in athymic mice in the absence of neurotoxicity. *Biochem. Biophys. Res. Commun.* 265, 479-483 (1999)
39. Y. C. Li, C. C. Tzeng, J. H. Song, F. J. Tsia, L. J. Hsieh, S. J. Liao, C. H. Tsai, E. G. Van Meir, C. Hao and C. C. Lin: Genomic alterations in human malignant glioma cells associate with the cell resistance to the combination treatment with tumor necrosis factor-related apoptosis-inducing ligand and chemotherapy. *Clin. Cancer Res.* 12, 2716-2729 (2006)
40. S. Fulda, W. Wick, M. Weller and K.-M. Debatin: Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. *Nat. Med.* 8, 808-815 (2002)
41. J. M. Kuijlen, J. J. Mooij, I. Platteel, E. W. Hoving, W. T. van der Graaf, M. M. Span, H. Hollema and W. F. den Dunnen: TRAIL-receptor expression is an independent prognostic factor for survival in patients with a primary glioblastoma multiforme. *J. Neurooncol.* 78, 161-171 (2006)
42. J. P. Steinbach and M. Weller: Apoptosis in Gliomas: Molecular Mechanisms and Therapeutic Implications. *J. Neurooncol.* 70, 247-256 (2004)
43. L. M. Sedger, M. B. Glaccum, J. C. Schuh, S. T. Kanaly, E. Williamson, N. Kayagaki, T. Yun, P. Smolak, T. Le, R. Goodwin and B. Gliniak: Characterization of the in vivo function of TNF-alpha-related apoptosis-inducing ligand, TRAIL/Apo2L, using TRAIL/Apo2L gene-deficient mice. *Eur. J. Immunol.* 32, 2246-2254 (2002)
44. J. Dörr, S. Waiczies, U. Wendling, B. Seeger and F. Zipp: Induction of TRAIL-mediated glioma cell death by human T cells. *J. Neuroimmunol.* 122, 117-124 (2002)
45. D. Lawrence, Z. Shahrokh, S. Marsters, K. Achilles, D. Shih, B. Mounho, K. Hillan, K. Totpal, L. DeForge, P. Schow, J. Hooley, S. Sherwood, R. Pai, S. Leung, L. Khan, B. Gliniak, J. Bussiere, C. A. Smith, S. S. Strom, S. Kelley, J. A. Fox, D. Thomas and A. Ashkenazi: Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions. *Nat. Med.* 7, 383-385 (2001)
46. G. K. Hansson, J. Holm and L. Jonasson: Detection of activated T lymphocytes in the human atherosclerotic plaque. *Am. J. Pathol.* 135, 169-175 (1989)
47. R. Ross: Atherosclerosis--an inflammatory disease. *N. Engl. J. Med.* 340, 115-126 (1999)
48. K. Sato, A. Niessner, S. L. Kopecky, R. L. Frye, J. J. Goronzy and C. M. Weyand: TRAIL-expressing T cells induce apoptosis of vascular smooth muscle cells in the atherosclerotic plaque. *J. Exp. Med.* 203, 239-250 (2006)
49. S. J. Zheng, P. Wang, G. Tsabary and Y. H. Chen: Critical roles of TRAIL in hepatic cell death and hepatic inflammation. *J. Clin. Invest.* 113, 58-64 (2004)
50. M. Matysiak, A. Jurewicz, D. Jaskolski and K. Selmaj: TRAIL induces death of human oligodendrocytes isolated from adult brain. *Brain* 125, 2469-2480 (2002)
51. A. Jurewicz, M. Matysiak, K. Tybor, L. Kilianek, C. S. Raine and K. Selmaj: Tumor necrosis factor-induced death of adult human oligodendrocytes is mediated by apoptosis inducing factor. *Brain* 128, 2675-2688 (2005)
52. E. M. Frohman, M. K. Racke and C. S. Raine: Multiple sclerosis--the plaque and its pathogenesis. *N. Engl. J. Med.* 354, 942-955 (2006)



53. L. Steinman: Optic neuritis, a new variant of experimental encephalomyelitis, a durable model for all seasons, now in its seventieth year. *J. Exp. Med.* 197, 1065-1071 (2003)
54. D. A. Hafler: Multiple sclerosis. *J. Clin. Invest* 113, 788-794 (2004)
55. M. Sospedra and R. Martin: Immunology of multiple sclerosis. *Annu. Rev. Immunol.* 23, 683-747 (2005)
56. F. Zipp and O. Aktas: The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. *Trends Neurosci* 29, 518-527 (2006)
57. R. Meyer, R. Weissert, R. Diem, M. K. Storck, K. L. de Graaf, B. Kramer and M. Bähr: Acute Neuronal Apoptosis in a Rat Model of Multiple Sclerosis. *J. Neurosci.* 21, 6214-6220 (2001)
58. A. Diestel, O. Aktas, D. Hackel, I. Hake, S. Meier, C. S. Raine, R. Nitsch, F. Zipp and O. Ullrich: Activation of microglial poly(ADP-ribose)-polymerase-1 by cholesterol breakdown products during neuroinflammation: a link between demyelination and neuronal damage. *J. Exp. Med.* 198, 1729-1740 (2003)
59. B. Hilliard, A. Wilmen, C. Seidel, T.-S. L. Liu, R. Göke and Y. Chen: Roles of TNF-related apoptosis-inducing ligand in experimental autoimmune encephalomyelitis. *J. Immunol.* 166, 1314-1319 (2001)
60. J. D. Lünemann, S. Waiczies, S. Ehrlich, U. Wendling, B. Seeger, T. Kamradt and F. Zipp: Death ligand TRAIL induces no apoptosis but inhibits activation of human (auto)antigen-specific T cells. *J. Immunol.* 168, 4881-4888 (2002)
61. K. P. Wandinger, J. D. Lunemann, O. Wengert, J. Bellmann-Strobl, O. Aktas, A. Weber, E. Grundstrom, S. Ehrlich, K. D. Wernecke, H. D. Volk and F. Zipp: TNF-related apoptosis inducing ligand (TRAIL) as a potential response marker for interferon-beta treatment in multiple sclerosis. *Lancet* 361, 2036-2043 (2003)
62. I. Jeremias, I. Herr, T. Boehler and K.-M. Debatin: TRAIL/Apo-2-ligand-induced apoptosis in human T cells. *Eur. J. Immunol.* 28, 143-152 (1998)
63. K. Song, Y. Chen, R. Göke, A. Wilmen, C. Seidel, A. Göke and B. Hilliard: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is an inhibitor of autoimmune inflammation and cell cycle progression. *J. Exp. Med.* 191, 1095-1103 (2000)
64. S. E. Lamhamedi-Cherradi, S. Zheng, R. M. Tisch and Y. H. Chen: Critical roles of tumor necrosis factor-related apoptosis-inducing ligand in type 1 diabetes. *Diabetes* 52, 2274-2278 (2003)
65. Q. S. Mi, D. Ly, S. E. Lamhamedi-Cherradi, K. V. Salojin, L. Zhou, M. Grattan, C. Meagher, P. Zucker, Y. H. Chen, J. Nagle, D. Taub and T. L. Delovitch: Blockade of tumor necrosis factor-related apoptosis-inducing ligand exacerbates type 1 diabetes in NOD mice. *Diabetes* 52, 1967-1975 (2003)
66. A. H. Chou, H. F. Tsai, L. L. Lin, S. L. Hsieh, P. I. Hsu and P. N. Hsu: Enhanced proliferation and increased IFN-gamma production in T cells by signal transduced through TNF-related apoptosis-inducing ligand. *J. Immunol.* 167, 1347-1352 (2001)
67. N. Arbour, E. Rastikerdar, E. McCrea, Y. Lapierre, J. Dorr, A. Bar-Or and J. P. Antel: Upregulation of TRAIL expression on human T lymphocytes by interferon beta and glatiramer acetate. *Mult. Scler.* 11, 652-657 (2005)
68. Y. Huang, N. Erdmann, H. Peng, Y. Zhao and J. Zheng: The role of TNF related apoptosis-inducing ligand in neurodegenerative diseases. *Cell Mol. Immunol.* 2, 113-122 (2005)
69. B. A. Navia, E. S. Cho, C. K. Petito and R. W. Price: The AIDS dementia complex: II. Neuropathology. *Ann. Neurol.* 19, 525-535 (1986)
70. E. Masliah, C. L. Achim, N. Ge, R. DeTeresa, R. D. Terry and C. A. Wiley: Spectrum of human immunodeficiency virus-associated neocortical damage. *Ann. Neurol.* 32, 321-329 (1992)
71. M. Zhang, X. Li, X. Pang, L. Ding, O. Wood, K. Clouse, I. Hewlett and A. I. Dayton: Identification of a potential HIV-induced source of bystander-mediated apoptosis in T cells: upregulation of trail in primary human macrophages by HIV-1 tat. *J. Biomed. Sci.* 8, 290-296 (2001)
72. Y. Miura, N. Misawa, Y. Kawano, H. Okada, Y. Inagaki, N. Yamamoto, M. Ito, H. Yagita, K. Okumura, H. Mizusawa and Y. Koyanagi: Tumor necrosis factor-related apoptosis-inducing ligand induces neuronal death in a murine model of HIV central nervous system infection. *Proc. Natl. Acad. Sci. U. S. A* 100, 2777-2782 (2003)
73. Y. Miura, Y. Koyanagi and H. Mizusawa: TNF-related apoptosis-inducing ligand (TRAIL) induces neuronal apoptosis in HIV-encephalopathy. *J. Med. Dent. Sci.* 50, 17-25 (2003)
74. L. A. Ryan, H. Peng, D. A. Erichsen, Y. Huang, Y. Persidsky, Y. Zhou, H. E. Gendelman and J. Zheng: TNF-related apoptosis-inducing ligand mediates human neuronal apoptosis: links to HIV-1-associated dementia. *J. Neuroimmunol.* 148, 127-139 (2004)
75. A. Martin-Villalba, I. Herr, I. Jeremias, M. Hahne, R. Brandt, J. Vogel, J. Schenkel, T. Herdegen and K.-M. Debatin: CD95 ligand (Fas-L/APO-1L) and tumor necrosis factor-related apoptosis-inducing ligand mediate ischemia-induced apoptosis in neurons. *J. Neurosci.* 19, 3809-3817 (1999)
76. U. Dimagl, C. Iadecola and M. A. Moskowitz: Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci.* 22, 391-397 (1999)
77. G. H. Danton and W. D. Dietrich: Inflammatory mechanisms after ischemia and stroke. *J. Neuropathol. Exp. Neurol.* 62, 127-136 (2003)
78. G. Cantarella, D. Uberti, T. Carsana, G. Lombardo, R. Bernardini and M. Memo: Neutralization of TRAIL death pathway protects human neuronal cell line from beta-amyloid toxicity. *Cell Death. Differ.* 10, 134-141 (2003)
79. D. Uberti, G. Ferrari-Toninelli, S. A. Bonini, I. Samico, M. Benarese, M. Pizzi, L. Benussi, R. Ghidoni, G. Binetti, P. Spano, F. Facchetti and M. Memo: Blockade of the Tumor Necrosis Factor-Related Apoptosis Inducing Ligand Death Receptor DR5 Prevents beta-Amyloid Neurotoxicity. *Neuropsychopharmacology* (2006)
80. J. H. Song, A. Bellail, M. C. Tse, V. W. Yong and C. Hao: Human astrocytes are resistant to Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis. *J. Neurosci* 26, 3299-3308 (2006)

**Key Words:** TRAIL, Multiple Sclerosis, Tumor, Cns, T Cells, Neurons, Oligodendrocytes, Apoptosis, Review

**Role of TRAIL/TRAIL receptors in central nervous system pathology**

**Send correspondence to:** Professor Frauke Zipp, Institute of Neuroimmunology, Neuroscience Research Center, NWFZ 2680, Charité Campus Mitte, 10098 Berlin, Germany; Tel: 49-30-450-539028, Fax: 49-30-450-539906, E-mail: frauke.zipp@charite.de

<http://www.bioscience.org/current/vol12.htm>