Published in final edited form as:

Trends Neurosci. 2008 October ; 31(10): 504–511. doi:10.1016/j.tins.2008.07.005.

Targeting TNF- α receptors for neurotherapeutics

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Tumor necrosis factor α : a matter of life and death

Tumor necrosis factor α (TNF- α) is a proinflammatory cytokine implicated in a variety of peripheral inflammatory human diseases including rheumatoid arthritis, asthma, ankylosing spondylitis, cancer and neurodegenerative disorders such as Alzheimer's disease (AD). TNF- α is generated as a membrane-bound polypeptide precursor that is processed by proteolysis. TNF- α synthesis and secretion are controlled by TNF- α converting enzyme (TACE). The secreted form then homotrimerizes to form the active TNF- α ligand. TNF- α binds with high affinity to two types of transmembrane-spanning receptors, TNFR1 and TNFR2, to exert most of its actions (Figure 1). These receptors belong to the larger TNFR superfamily [1]. Although TNFR1 is more ubiquitous, both TNFR1 and TNFR2 are present on virtually all cell types, except, most notably, red blood cells. Indicative of its name, TNF- α has long been associated with induction of apoptotic cell death. However, considerable evidence now exists that TNF- α also exerts converse actions such as cellular proliferation, differentiation and neuroprotection.

Considerable evidence has demonstrated that TNF- α acts as a potent neuroinflammatory agent within the central nervous system (CNS) and has been linked to disorders such as AD and Parkinson's disease (PD). However, as with other cytokines within the CNS, pleiotropic activities are often seen, that is, TNF- α mediates neuroprotective actions as well as the more typically observed apoptotic actions [2–4]. Exactly how the same molecule can exert these converse effects and how this diversity is exploited physiologically is presently unclear. This crucial topic is the major discussion point of this article. Recent advances in our appreciation of how transmembrane receptor systems can be compartmentalized within discrete signaling microdomains might shed light upon the potential mechanisms by which TNF- α 's protean nature is generated. One hypothetical model which could account for such controlled signaling diversity is the generation of multiple stable receptor states in specific microdomains that show a predilection to generate discrete signaling outputs. We will describe evidence demonstrating the functional flexibility of TNF- α and how different TNF- α receptor states could explain its pleiotropic activity within the CNS.

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TNF- α activity in the brain: two sides of one coin

In this section we describe how a single ligand, TNF- α , can act in different regions of the brain in multiple ways to control a diverse array of physiological processes; an in-depth understanding of the underlying signaling mechanisms might facilitate our ability to selectively control discrete aspects of TNF- α function. The production and secretion of inflammatory cytokines are processes associated with both neuronal and peripheral immunity. The CNS possesses its own immune system which includes microglia. When activated, microglia secrete multiple factors that can ensure neuronal survival, but are also able to initiate apoptosis of fatally damaged neurons through the secretion and production of inflammatory precursors such as TNF- α [5]. It has been demonstrated that overstimulation of microglia, through amyloid β -peptide (A β) deposits [6] or as a result of severe head trauma, causes an excessive secretion of TNF- α , which leads to neuronal cell apoptosis. Severe inflammation within the CNS can prove fatal for neurons and can be induced by severe head trauma in various neurological disorders including PD, multiple sclerosis (MS), AD and amyotrophic lateral sclerosis [7]. By illuminating several examples, we will demonstrate that in most physiological scenarios, however, TNF- α readily demonstrates a protean signaling nature, namely both classically apoptotic or the more recent neuroprotective capacity.

TNF- α and neurotoxicity

Although initially designated as a cytotoxic agent, TNF- α has been shown to have clear protective activity with respect to neurotoxic insults [8,9]. There are many mechanisms by which TNF- α can exert a neuroprotective action, including stimulation of neuronal plasticity; NF- κ B activation; induction of antiapoptotic factors; intracellular calcium buffering; and stimulation of neurotrophic factor release from astroglia [10–13]. With regard to disorders such as AD, TNF- α exerts a particularly strong neuroprotective action against hippocampal insults such as NMDA or nitric oxide excitotoxicity [14,15]. Mice deficient in TNF- α and its receptors are also significantly more sensitive to hippocampal excitotoxic and ischemic injury [16,17]. Interestingly, though, and further underlining the duality of TNF- α activity, mice lacking both TNF- α receptors (but not individual receptors) are protected against dopaminergic neurotoxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [18]. It appears that TNF receptors in various brain regions are linked to specific signaling actions, for example TNF receptor deficiency in the striatum attenuates MPTP-induced toxicity whereas a similar lack enhances the vulnerability of hippocampal neurons. Moreover, TNFR1 and TNFR2 can exert regenerative effects after injury in a tissue-specific manner. Hence, striatal injury repair was shown to be specifically via TNFR1, whereas hippocampal injury repair was TNFR2 mediated despite the equal expression of TNFR1 and TNFR2 in both brain regions [19]. It is therefore evident that in multiple experimental paradigms, TNF- α acts as a multifunctional agent at TNFR1 or TNFR2.

TNF- α and acute neurodegeneration

Not only can TNF- α be acutely neuroprotective, but preconditioning with TNF- α protects neurons against ischemic injury, potentially through decreasing microglial activation [20]. Pharmacological neutralization of TNF- α has been reported to reduce damage to neurons and improve recovery in animal stroke models [21], providing support for a detrimental role for endogenous TNF- α , at least in ischemic brain injury. Other studies, though, have provided considerable evidence that TNF- α can prevent neuronal death by activating NF- κ B, eventually upregulating expression of antioxidant enzymes and antiapoptotic Bcl-2 family members [22]. For example: TNF receptor-deficient mice exhibit increased vulnerability to excitotoxic brain injury associated with a blunted antioxidant enzyme response [16]; TNF- α protects neurons from being damaged by A β -induced damage in animal models of AD [10,23]; and TNF- α mediates ischemic and inflammatory preconditioning in stroke models [24,25]. Hence,

TNF- α receptor activation in neurons promotes their survival, whereas activation of TNF- α receptors in macrophages and microglia promotes neuronal death indirectly by inducing the production of neurotoxic substances.

TNF- α and myelination

The contrasting protective or toxic effects of TNF- α have been demonstrated particularly well in models of demyelination. These models attempt to mimic pathologies in disorders such as MS. TNF- α mediates myelin and oligodendrocyte damage not only *in vitro* [26,27] but also *in vivo*. For example, transgenic mice with a CNS-driven overexpression of TNF- α develop spontaneous demyelination [28]. In addition, elevated levels of TNF- α protein are detected in the serum and cerebrospinal fluid of MS patients [29] and by resident and infiltrating cells [30] at sites of CNS injury. In experimental autoimmune encephalomyelitis (EAE), anti-TNF- α treatment prevents initiation of pathology and ameliorates the progression of established disease [31,32]. Besides directly induced cell death, TNF- α also triggers a variety of indirect effects during the course of an autoimmune CNS inflammation. For example, TNF- α regulates glutamate transport capacity in astrocytes [33], which leads to the accumulation of glutamate, increasing excitotoxic damage to oligodendrocytes and neurons. These findings are contrasted by studies utilizing TNF- α protein knockout mice stimulated by myelin oligodendrocyte glycoprotein (MOG) peptide for EAE induction. TNF- α -deficient mice display an increase in disease severity, and the magnitude of demyelination in these mice is either variable [34] or enhanced in comparison to controls [35]. These findings parallel those of clinical therapies aimed at the blockade of TNF- α protein in MS [36], which have shown little efficacy or have even worsened symptoms in some patients [37]. In addition, TNF- α promotes apoptosis particularly in autoreactive T cells [38] and can enhance CNS remyelination [39].

TNF- α receptor signaling in the brain

To understand how these diverse effects of TNF- α are created, one must consider the most simple mechanism that dictates signaling specificity, namely receptor activation. In addition, discussion of the complex nature of the protein–protein interactions involved in TNFR function is germane to our proposed concept of the existence of multiple states of TNF- α receptors, organized into entities that have a specific signaling predilection.

As mentioned previously, TNF- α propagates its actions via stimulation of two transmembrane receptors, TNFR1 or TNFR2 [1,40] (Figure 1). TNFR1 is expressed in almost all tissues, whereas TNFR2 is tightly regulated and associated mainly with the immune system [41]. The type 2 receptor is only activated by membrane-bound TNF, whereas the type 1 receptor is activated by either membrane-bound TNF or soluble TNF [42]. The receptors differ from one another, in that TNFR1 contains an intracellular death domain (DD), responsible mainly for apoptosis, whereas TNFR2 seems to be neuroprotective and has no DD [23,43,44]. Although each receptor mediates distinct cellular responses, there are considerable overlaps of their signaling capacities with respect to downstream cellular events [45,46]. The receptors themselves associate with a large array of intracellular binding proteins that generate the discrete downstream signals. Activation of TNFR1 initiates the formation of one of two intracellular complexes, complex I or complex II. Complex I is made up of RIP1 (receptor-interacting protein 1), TRAF2 (TNFR-associated factor 2), cIAP-1 (inhibitor of apoptosis protein 1) and TRADD (TNFR-associated DD) and is associated with cell survival through the activation of I- κ B kinase and NF- κ B [47–49]. If this complex is not stimulated, complex II, consisting of TRADD, RIP1 and FADD (Fas-associated DD), is incorporated by the activated TNFR1. This complex recruits and activates procaspase 8, leading to the release of the active p18/p12 fragments which activate other caspases to promote apoptosis [50]. TNFR2 is able to directly interact with TRAF2, mediating the recruitment of cIAP-1 and cIAP-2, which inhibit the activation of caspase 8, through their BIR domain, and ultimately apoptosis. cIAP-1 and

cIAP-2 can act as ubiquitin ligases involved in the degradation of caspase 3 and caspase 7 [40,41,51]. cIAP1 is also responsible for the ubiquitination of TRAF2, and it is because of this that TNFR2 has been associated with TNFR1-induced cell death for two reasons. First, TNFR1 and TNFR2 compete for TRAF2 and the associated cIAP-1 and cIAP-2 proteins. Increased activation of TNFR2 depletes TRAF2 and cIAP-1 necessary for complex I formation, thereby promoting cell death [41]. Second, cIAP-1-induced ubiquitination of TRAF2 can enhance receptor competition for the remaining cytoplasmic pool of TRAF2, cIAP-1 and cIAP-2 protein [41]. Which signaling complex (I or II) is activated will depend on the cell's fate and therefore requires tight regulation and/or molecular separation. Signaling complex formation can also be controlled by membrane microenvironment, such as lipid rafts [41,47,48], NF- κ B activity [52] and JNK stimulation [53].

Exploring the protean nature of TNF- α activity: potential mechanisms

Understanding how TNF- α 's actions are controlled by circumstance and location in the CNS might potentially yield novel therapeutic agents that could possess signal-specific actions. The tissue- or cell-specific 'packaging' and expression of the TNF receptors into discrete, differential signaling entities is one hypothetical mechanism by which the protean nature of TNF- α is mediated. Under normal conditions, TNF- α receptors are expressed in neurons and vascular endothelial cells primarily, yet under traumatic or inflammatory conditions they are expressed in astrocytes and microglia [34]. The balance of the degree of receptor expression and the location has been shown to play a crucial role in the resultant action, protective or destructive, of TNF- α in the CNS [54–56]. In addition to these mechanisms of TNF- α functional plasticity, evidence from the study of other transmembrane receptor systems (G-protein-coupled receptors; GPCRs) suggests that molecular interactions between the receptors and their downstream signaling systems might be crucial for determining ligand selection and function. To fully appreciate this potential new role for receptor preassembly in controlling pharmacology, we will first discuss how receptor system complexity might be generated.

TNF receptorsomes: a hypothesis for functional specificity

TNF- α receptors have unique structural attributes that couple them directly to signaling pathways for cell proliferation, survival and differentiation. Thus, they have assumed prominent roles in the organization of tissues and transient signaling microenvironments. The formation of specific signaling forms of TNF receptors can be generated at three distinct levels: (i) receptor-interacting proteins; (ii) organization of the TNF receptors in distinct plasma membrane microdomain environments; and (iii) association of the receptor systems with more distal signaling entities (Figure 2). TNF- α ligands exist as both membrane-embedded 'pro' as well as cleaved, soluble 'mature' forms [57]. The activity of these TNF ligands can vary between these embedded and soluble forms; for example, soluble TNF is less active than membrane-embedded TNF- α at TNFR2/p75 [42]. The 25–30% amino acid similarity between TNF-like ligands is largely confined to internal aromatic residues responsible for trimer assembly. The external surfaces of ligand trimers show little sequence similarity, which accounts for receptor selectivity. Certain ligands and receptors in the TNF/TNFR superfamily can bind more than one partner with high affinity, thereby enhancing regulatory flexibility and complexity [57]. After ligand binding, the receptor cytoplasmic tails form a 3:3 internal complex with signaling proteins such as TRAF2 or FADD [58]. Hence, ligand binding and signal complex formation involve stoichiometrically defined protein complexes with threefold symmetry.

A common recent theme in cell signaling is that the preassembly of cell-signaling cascades might be required for selectivity of function (for a review, see Ref. [59]; Figure 2). In addition to this it seems that the structural interaction between signaling complexes and transmembrane receptors has the capacity to control ligand and signaling selectivity of the receptor [60,61]

and predetermine the eventual functional outcome of the receptor activation event. The association of transmembrane receptors with additional protein factors, creating a higher-order receptor structure, has been described as a 'receptorsome.' As many forms of transmembrane receptor systems appear to demonstrate analogy in their signaling functions, it might be possible therefore that systems as distinct as the TNFR family and the GPCRs both exist in receptorsomes. The signaling and physiological profile of GPCRs seems to be highly flexible from tissue to tissue and even perhaps between different cellular compartments. This flexibility is, in part, endowed to the receptor via its stable interaction with transmembrane and cytoplasmic scaffolding structures and signaling proteins. Many downstream cell signaling factors are preassembled into discrete signaling units, and these are often stably associated with the receptor itself at the plasma membrane. These associations create the stable higher-order receptorsome structures. The nature and stoichiometry of the multiple accessory proteins associated with receptors in these structures can create *de facto* distinct receptors with singular pharmacological profiles. It has been recently demonstrated that such distinct receptor structures can be pharmacologically targeted in a selective manner [62].

TNFRs can preassemble into complexes on the cell surface before ligand binding [62,63]. The formation of oligomers, possibly trimers, in the absence of ligand requires the N-terminal end of the receptor, including the first cysteine-rich domain (CRD) of TNFR1 and TNFR2. This region, termed the PLAD (pre-ligand assembly domain), is necessary and sufficient for the self-assembly. Parallel dimer structures resulting from the crystallization of the unliganded TNFR1 ectodomain show extensive contacts in the PLAD region [57]. The PLAD is distinct from the ligand binding domain and the unliganded complex. PLAD interactions are highly specific and usually only receptor homotrimers are formed; however, 'transplanting' the TNFR1 PLAD onto TNFR2 allows it to enter TNFR1 complexes. Receptor preassembly appears to be essential for ligand binding and signal transmission. Interestingly, even the TNFR ligands themselves can also preassemble into trimers on the cell surface, and several reports suggest that membrane-anchored ligands can send 'reverse' signals into the ligand-bearing cells when they contact their receptor, introducing the possibility of multidirectional signal transduction [64,65]. The nature of interacting partners with TNF receptors clearly has a role in determining the eventual cellular outcome of receptor stimulation [1,66]. For example, interaction of the activated receptor with TRADD transduces signals through FADD and FLICE (FADD-like interleukin 1 β -converting enzyme) to mediate apoptotic caspase mechanisms or interaction with members of the TRAF family that mediate alteration of NF- κ B activity that can be either protective [10] or neurotoxic [67]. These complex and diverse cellular outcomes are probably controlled by the presence of multiple additional protein factors with the stable complexes physically associated with the receptors, for example, the specificity of action upon NF- κ B is dictated by TRAF-interacting proteins. I-TRAF, TRIP, A20 and BRE (brain and reproductive organ expressed) are just several proteins that form complexes with TRAF2 and prevent it from stimulating NF- κ B. The relative association levels of these proteins, and their tissue-specific expression therefore, would provide facile means of creating selective signaling forms of the TNF receptors, protective or apoptotic. It has also been shown that in different tissues, TNF- α activation of apoptotic (striatum [67,68]) or neuroprotective (hippocampus [69]) pathways can be determined by functional interactions with TNF receptor systems. Not only are intracellular molecular determinants important for dictating the nature of TNF- α signaling, the ability of CNS architecture to change with selective microglial activation at sites of injury might have dramatic effects upon the nature of TNF receptor signaling; for example, the specific concentrations of certain inflammatory cytokines might vary temporally and spatially during the injury process [70,71]. The availability, therefore, of distinct ligand pools might allow discrete TNF- α receptor structures to be preferentially stimulated by specific ligand subsets to ensure a particular downstream event. The hypothetical presence of different TNF- α receptor signaling states, created by stable interactions with intracellular accessory proteins which possess a signaling predilection, might facilitate the

eventual design of therapeutic agents to target these entities and generate selective signaling. The creation of these states by stable protein–protein interactions might constrain the receptor to both selective signal transduction cascade interactions but could also present a distinct extracellular aspect to circulating ligands. The importance of a potential strong and functionally relevant connectivity between transduction cascade and receptor pharmacology demands that we approach therapeutic design with both ends of the signaling transduction cascade in mind. We should consider strongly that, as with GPCR signaling, it might be possible in the future to eventually create signal-selective therapies.

TNF-targeted therapeutics: an opportunity for neurodegenerative disease treatment

We propose that gaining a deeper understanding of the molecular relationships between receptors and their transduction cascades could facilitate the creation of new TNF- α -system-targeted therapeutics which could aid disorders such as AD or MS. As with most therapeutic strategies, multiple angles of attack are desirable. We shall briefly outline some specific current and future targets for therapeutics that target TNF- α signaling and their potential weaknesses. As we propose an important functional link between signal transduction cascade and receptor function, we shall consider both these aspects for therapeutic intervention.

Signal transduction targets

TNFR1-mediated JNK activation is associated with apoptosis [72]. By inhibiting certain regulatory pathways associated with JNK activation, cell survival could be favored. One potential target is X-linked inhibitor of apoptosis protein (XIAP), a member of the inhibitor of apoptosis proteins family of caspase blockers, upregulated by NF- κ B [73]. XIAP seems to be an effective target against apoptosis, as it inhibits caspase-mediated cell death. Inhibiting the formation of reactive oxygen species (ROS) by ferritin heavy chain is another mechanism whereby JNK can be deactivated [74]. It also appears that regulators of ROS can control TNF- α actions, albeit in a complex manner. Manganese SOD (MnSOD) overexpression has been demonstrated to attenuate TNF- α -mediated toxicity in some systems [75], whereas in other paradigms MnSOD overexpression affords little protective activity against TNF- α actions [76]. The differences between these actions might be linked to the prevailing cellular levels/activity of NF- κ B and free iron [76]. NF- κ B activation can induce Gadd45 β expression. This growth arrest and development protein attenuates TNFR1-mediated JNK activation through the inhibition of MKK7/JNKK2 [77]. Therefore, tissue-selective activation of NF- κ B or Gadd45b might also facilitate neuroprotective mechanisms. Specific inhibition of caspases, activated by TNF- α as a result of traumatic brain injury, would be another way of maintaining cell longevity. Chemical inhibitors of caspase 3, both specific [78,79] and broad-spectrum [80], have shown promise in preventing TNF- α -mediated apoptosis *in vitro* and *in vivo*.

Signal-selective TNF- α receptor targeting: future promise?

With the multitude of proteins that can directly interact with TNFR1/2, it is unlikely that the tissue and subcellular expression of TNFR1/2 is identical across regions of the brain or different parts of cells. It is also therefore unlikely that the specific signaling preferences/ranges of these different receptor states are also identical, suggesting that some TNFR1/2s could possess a specific signaling preference (see Figure 2). Therefore, such hypothetical differences might present a tractable possibility for therapeutic intervention in the future. Using the pleiotropic nature of the TNF receptor system, it might be possible to develop therapeutic strategies to prevent crossover into nondesirable TNF- α actions. A strong preference for distinct active TNF receptor structural states to interact with downstream signaling cascades that possess a preference for apoptotic or protective events might facilitate the creation of TNF-mimetic agents that can distinguish between these multiple TNF receptor active states. It has recently

been made evident that contrary to existing dogma, the flux of 'signals' across a transmembrane receptor is reversible. Hence, the nature of the protein complexes (primary structural and signaling complexes) and the microenvironment (e.g. lipid raft) not only dictate the functional outcome of receptor activation but might also structurally constrain the receptor, favoring preferential interactions with certain ligands. The tissue-specific expression of receptor-interacting proteins and the local availability and concentration of inflammatory cytokines therefore might dictate the eventual nature of TNF- α receptor signaling. A greater understanding of the relationship between TNF receptorsome structure and ligand-binding specificity might facilitate the creation of cytokine xenobiotics with selective protective actions.

Due to the huge impact of TNF receptor modulation on many disorders, the potential benefits of specific, targeted TNF receptor therapeutics even for CNS diseases seem likely. TNF antagonistic agents have been developed which control previously resistant inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease [81,82]. TNF-related therapeutic agents are also being targeted against widespread human diseases such as atherosclerosis, osteoporosis, autoimmune disorders, allograft rejection and cancer [83]. One novel receptor-based therapeutic model has recently been pioneered by Sedger and colleagues [84], who demonstrated that M-T2, a leporipoxvirus TNF receptor homolog, is able to inhibit apoptosis in primary rabbit lymphocytes. M-T2 forms a heterocomplex with TNFR1, thereby inhibiting intracellular signaling, without affecting TNFR1 surface expression or impeding TNF- α binding. Thus, through the creation of an alternative TNF receptor complex, the activity of the endogenous ligands has been modified to favor a beneficial outcome. Three major therapeutic approaches that target TNF signaling are at various stages of development. One approach is to develop agents, such as thalidomide analogs [85,86], that inhibit the production/release of TNF. Another approach which has moved to clinical trials for sepsis and related severe acute systemic inflammatory conditions is to administer antibodies that capture TNF [87,88]. A third approach is to develop TNF receptor antagonists that might include blocking peptides or low molecular weight agents [89,90]. The important issue with the current approaches which involve suppressing TNF production/release, capturing TNF with antibodies or even blocking receptor activation is that they all will inhibit both the detrimental and beneficial effects of TNF and so will have side effects associated with inhibition of the repair- and neuron survival-promoting actions of TNF. Therefore, targeting components of downstream signaling scaffolds or specific TNF receptor conformations associated with a particular downstream pathway provides the opportunity to block the pathogenic component of TNF signaling while preserving the beneficial component.

Conclusion

Even though promising, the direct inhibition of TNF- α and treatment with TNF- α cascade blockers have so far largely failed in the treatment of CNS disorders. An in-depth understanding of the multiple molecular interactions that control system output might be crucial for the future. The composition and stoichiometry of directly interacting proteins with the TNF- α receptor could play a central role in the outcome of TNF signaling. The complex stoichiometry of receptors with intracellular accessory proteins also raises the possibility that trauma-induced receptor expression might yield the creation of receptors with a distinctive function compared to preexisting receptors.

The inherent molecular complexity of the whole TNFR signaling system might facilitate the generation of many distinct structural active or inactive states of the same receptor, each with the capacity to display divergent signaling and ligand binding preferences. Once we can understand how this complicated receptor system is expressed in cells, an expanded pharmacopeia of TNF-related agents can be rationally designed to exploit this powerful

nervous system modulator. Even though the complexity of the TNF receptor–ligand system is daunting, especially with the added level of complexity of tissue-specific expression patterns, it is this complexity that might facilitate the creation of signal-selective therapeutics possessing minimal deleterious side effects.

Acknowledgements

This research was supported by the Intramural Research Program of the NIH, National Institute on Aging. The authors have no conflicts of scientific interest with respect to the manuscript.

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Figure 1.

Multiple forms of TNF receptor (TNFR) signaling activity are mediated via intracellular protein complex assembly. Through interaction with the transmembrane TNFR, the trimeric TNF ligands can control the eventual survival or induce cellular apoptosis via distinct and complex signaling pathways. Central to the distinction between these opposing actions is the resultant nature of the activity of the pivotal NF-κB protein. TNF-mediated activation of TNFR1 modulates a multitude of processes resulting in the eventual regulation of cell fate between survival and death via apoptosis (a). In contrast to TNFR1, activation of TNFR2 has a primarily cytoprotective effect via the activation of NF-κB (b). In parallel with other transmembrane receptor systems, such as receptor tyrosine kinase or G-protein-coupled receptors, a productive interaction with the cellular endocytic machinery plays a key role in this cell-fate determination process. The importance of such interactions demonstrates the additional texture given to receptor signaling by the complex interaction of transmembrane receptors with accessory proteins. The coherent orchestration of the multiple downstream processes entrained by TNF receptor activation is controlled by the nature and stoichiometry of protein–protein interactions of signaling factors with the receptor itself. Given the number of molecules involved in these downstream signaling complexes, a large number of potential combinations of these complexes might exist, thus providing a potential mechanism for encryption of the TNF ligand signal. The precise nature and composition of these multimolecular receptor units is therefore a crucial target for therapeutic investigation.

Figure 2.

A model for how multiple receptor states might control physiology, disease and responses to pharmacotherapeutics. The stable interaction with multiple and distinct proteins, which themselves can show tissue-specific or pathological state-specific expression, with the TNF receptor might yield the *de facto* creation of multiple substates of the same receptor form. Each substate will potentially have preferences for certain types of signaling events and might even possess differential affinities for extracellular TNF ligands. This effect upon TNFR structure would be mediated by a retrograde conformational alteration of the extracellular aspect of the receptor by the stable interaction of distinct intracellular signaling complexes. As extracellular ligands transmit their signal through extracellular to intracellular conformational changes, the cytosolic accessory proteins might exert a similar action through a conformational shift transmitting from the intracellular to the extracellular region of the receptor [59]. These different stable receptor substates might be differentially expressed between tissues or altered in their representation by disease or pathological processes. The creation of these distinct receptor forms by a selective and specific set of protein–protein interactions might explain the diverse functional responses to the same ligand, namely cell preservation or death, and might facilitate the eventual creation of tissue- and response-specific TNF-based neurotherapeutics.