Deciphering the Physiology Underlying the Rapid Clinical Effects of Perispinal Etanercept in Alzheimer's Disease

Edward Tobinick*

100 UCLA Medical Plaza, Suites 205-210, Los Angeles, California 90095, USA

Abstract: Excess tumor necrosis factor (TNF) plays a pivotal role in the pathogenesis of Alzheimer's disease(AD). Clinical improvement following perispinal administration of etanercept in patients with Alzheimer's disease and other forms of dementia and brain dysfunction is characteristically evident within minutes. The rapidity and constellation of the clinical effects across multiple domains (cognition, mood, memory, motor function, and attention) suggest they are mediated by non-synaptic signaling mechanisms previously unrecognized for etanercept. These mechanisms likely extend beyond the known roles of TNF as a gliotransmitter that modulates synaptic strength, synaptic scaling, and AMPA receptor trafficking. Preliminary basic science and clinical investigation suggests that perispinal administration of etanercept may lead to its rapid penetration into the cerebrospinal fluid (CSF) within the cerebral ventricles. Diffusion of large molecules into the periventricular brain parenchyma is known to occur, but this process may not be sufficient to explain the rapidity of the clinical effects. There exist populations of cells, including CSF-contacting neurons and modified ependymal cells called tanycytes, that have receptive surfaces in direct contact with the CSF. It is hypothesized that the rapid clinical effects of perispinal etanercept involve non-synaptic signal transduction across the ependymal barrier and into neuronal networks via these CSF-contacting cells. This hypothesis challenges the dogma that penetration of a therapeutic into the cerebral parenchyma through the endothelium of the cerebral vasculature (the so-called blood-brain barrier) is necessary to produce rapid clinical effects in AD. CSF-contacting cells may constitute a therapeutic target for a diverse group of brain, psychiatric and spinal disorders.

Keywords: Alzheimer's f kugcug", cerebrospinal, CSF-contacting, dementia, etanercept, perispinal, tanycytes, TNF.

*"Nature is often willing to whisper her secrets, but
we must be prepared to listen"* (**Jerome Kassirer**)

INTRODUCTION

In science it is not unusual for an unexpected result to be dismissed as an aberration, a mistake, or a misinterpretation, particularly if the unexpected result presents a challenge to an existing scientific paradigm [1-5]. The barriers to acceptance that historically have impeded new scientific ideas are, in the field of Alzheimer's disease (AD) research, multiplied several fold [2]. This is, in large part, due to the multi-billion dollar investment the pharmaceutical industry has made in pursuing the amyloid plaque hypothesis [6]. The unproven dogma that removal of amyloid plaques would result in clinical improvement in AD has, at least until recently, reigned supreme in the Alzheimer world, despite failure upon failure of therapeutics targeted at amyloid, and despite the efforts of a small group of "renegade" researchers who questioned the amyloid hypothesis [6-10]. Removal of amyloid plaque via active immunization, even when successful, did not alter the trajectory of decline into severe dementia [11,12]. In the past several years all amyloid-targeted therapeutics (including AN-1792, bapineuzumab, tramiprosate, tarenfluribil, ELND005 and semagacestat) have failed to meet their endpoints in clinical trials. In several of these clinical trials serious adverse effects were noted in the active

treatment groups, including brain inflammation[1], worsening cognition, or excess mortality [8,13-18].

Acceptance of new paradigms in science and medicine has historically been vigorously resisted, often by the most eminent scientists in the field [1,2]. The concept that excess TNF[2] is centrally involved in the pathogenesis of AD, despite years of accumulating scientific evidence, is only beginning to be considered by mainstream AD scientists [19-55]. The positive clinical effects of etanercept[3] delivered by perispinal administration for treatment of patients with AD and other forms of dementia [45,48-50,56] have undoubtedly been surprising to a research community whose main therapeutic focus for two decades has been on amyloid mechanisms. The clinical results produced by perispinal etanercept in AD, including rapid and sustained clinical improvement in cognition, memory, mood, motor function and attention, often beginning within minutes of the first dose, are unique

---

*Address correspondence to this author at the 100 UCLA Medical Plaza, Suites 205-210, Los Angeles, California 90095, USA Tel: (310) 824-6191; Fax: (310) 824-6196; E-mail: etmd@ucla.edu

[1] The adverse brain effects associated with anti-amyloid immunotherapy may at least in part be due to neuroinflammation. See Minami (2010).
[2] TNF stands for tumor necrosis factor, also commonly referred to as TNF-alpha. TNF is an immune signaling molecule that initiates and amplifies the inflammatory response in a variety of organ systems, including the brain, and, in addition, has numerous additional physiologic effects, including modulation of synaptic transmission.
[3] Etanercept is a biologic recombinant dimeric fusion protein consisting of the extracellular ligand-binding portions of two human p75 TNF receptors linked to the Fc fragment of human IgG1. Etanercept, by binding to TNF and blocking its interaction with cell surface TNF receptors, rapidly neutralizes the physiologic effects of excess TNF.

[46-48,50,51][4]. Six years of clinical experience has confirmed the consistent reproducibility of these results. These results have also been confirmed by physicians from three continents who have been trained by the author and have used perispinal etanercept for their own patients [23,46-51,56]. Beyond the value of this method for patient treatment, the clinical response to perispinal etanercept establishes the existence of novel TNF-mediated pathophysiologic mechanisms operative in Alzheimer's disease [35,46-48,50, 51,57]. Efforts to decipher the physiologic mechanisms underlying the rapid clinical effects of perispinal etanercept have the potential to provide new insights into brain physiology and pathophysiology and are discussed in Section 2 [45-51,56,58-60].

The clinical efficacy of perispinal etanercept does not negate the involvement of amyloid mechanisms in AD. In fact, there is substantial research that has revealed the multiple ways in which TNF and amyloid may interact in AD [22,33,61,62]. Just as a single example, TNF has been demonstrated to mediate the impairment in memory mechanisms produced by both beta-amyloid and amyloid oligomers [34,39,62]. The ability of perispinal etanercept to successfully intervene in AD simply establishes that excess TNF is involved in mechanisms central to AD pathogenesis. This does not imply that excess TNF is necessarily the "cause" of the disease, for TNF-mediated mechanisms may well be downstream from the initiating event, in the same way that TNF-mediated mechanisms may be downstream from the initiating event in rheumatoid arthritis. In neither case is the primary driver of the disease known. Nevertheless neutralization of excess TNF is therapeutically beneficial for both diseases.

In the past two years interest in anti-TNF treatment for Alzheimer's disease (AD) has increased. In 2010 no fewer than three reviews (the first on anti-inflammatory strategies for AD, the second on TNF-mediated signaling in the CNS, and the third on TNF and brain dysfunction) discussed the evidence connecting excess TNF with AD [32,35,61]. A TNF genotype was singled out as the only significant association among 21 candidate genes in a population of 1300 Finnish AD patients and controls [40]. Serum protein-based multiplex biomarker data suggested that a group of inflammatory proteins, including TNF, might help classify AD, concluding that the existence of an inflammatory-related phenotype of AD "may provide targeted therapeutic opportunities for this subset of patients" [63]. In this serum protein study, TNF was one of only two biomarkers that overlapped with a previous study of plasma signaling proteins that reliably predicted AD diagnosis [63,64]. Three separate studies published in 2010 provided data that etanercept may improve cognition in a diverse group of clinical disorders, in addition to AD, that are associated with excess TNF: traumatic brain injury, sarcoidosis, and rheumatoid arthritis [65-67]. Additional basic science studies demonstrated that anti-TNF therapeutics ameliorated AD mechanisms in animal models [22,33,34,39,62,68,69]. Passive anti-amyloid immunization, known to have the potential to cause brain inflammation in humans, was shown to produce elevated CNS TNF levels in

an animal AD model [70]. In a cohort of AD patients with mild to severe dementia those patients with baseline elevated levels of serum TNF experiencing systemic inflammatory events had a nearly ten-fold increase in the rate of cognitive decline over a six month period compared with patients with the lowest quartile of serum TNF at baseline and no systemic inflammatory events [24,71]. Intracerebroventricular administration of infliximab, a chimeric anti-TNF monoclonal antibody rapidly reduced amyloid plaques and tau phosphorylation in APP/PS1 transgenic mice [72]. Increasing and accumulating basic science, clinical, epidemiologic, and genetic data support a central role of excess TNF in the pathogenesis of AD and other neuroinflammatory disorders and has recently been reviewed [19,21,23,37,46-49,52,73]. The growing acceptance of the potential of etanercept to intervene in AD is highlighted by the collaboration of the University of Southampton, the Hampshire Partnership National Health Service Trust and Wyeth in initiating a clinical trial of etanercept in AD[5]. Critical evaluation of perispinal etanercept for AD, however, may arguably best be initiated by attempting to understand the physiological basis underlying the unique rapid therapeutic effects that characteristically occur [48,50, 51,56].

DECIPHERING THE PHYSIOLOGICAL BASIS UNDERLYING THE RAPID EFFECTS OF PERISPINAL ETANERCEPT IN ALZHEIMER'S DISEASE

An unexpected observation may present an opportunity to advance science. The more puzzling the scientific result the greater its potential significance. The clinical effects of perispinal etanercept, when newly observed, or newly read about, are indeed unexpected and puzzling because they are unprecedented. Unraveling their physiological basis is undoubtedly a difficult undertaking but promises to shed new light on the pathogenesis of AD.
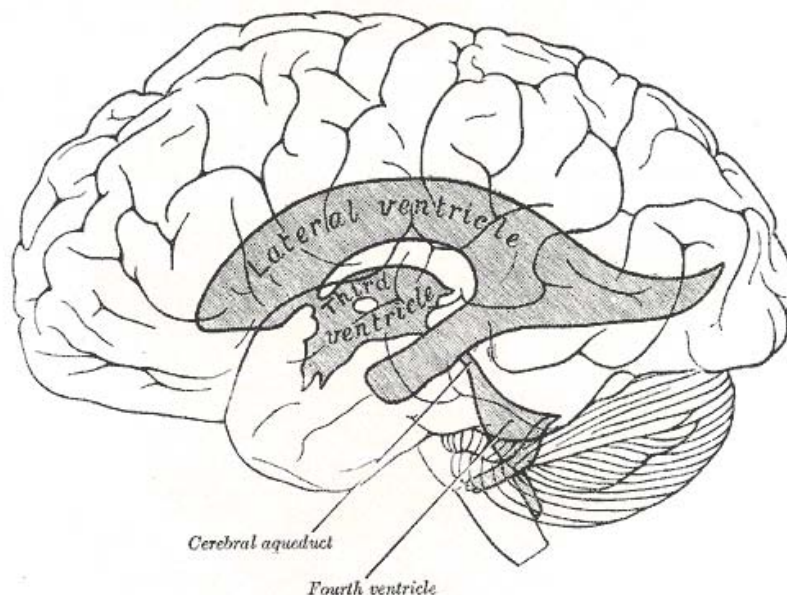
The rapidity of the clinical effects produced by perispinal etanercept is remarkable. Within minutes (sometimes in less than one minute) after perispinal delivery there is characteristically a noticeable clinical improvement in the AD patient. Improvement often increases over the next few hours. Rapid response is the first essential clue to the underlying physiology. Etanercept is thought to be unable to directly cross the blood-brain barrier (BBB) formed by the tight junctions between the endothelial cells of the cerebral vasculature [74]. Clinical effects on the brain within minutes following perispinal etanercept therefore suggest a mechanism that initially bypasses the BBB, such as distribution into the cerebrospinal fluid(CSF) [46-48,60].

The second essential clue to the underlying physiology is the constellation of clinical effects that follow perispinal administration of etanercept in AD. This constellation consists of rapid improvement in multiple disparate domains, including cognition, mood, motor function, memory and attention [45-48,50,51,56]. These are exactly the domains that are regulated by molecules carried through the CSF that produce distant effects via non-synaptic mechanisms [75-77]. The ventricular CSF borders on multiple brain regions[6] Figs. (**1, 2**) [78].

Fig. (1). The cerebral ventricles within the human brain. The cerebral ventricles border on multiple brain regions, including the hippocampus, circumventricular organs, striatum, hypothalamus, thalamus, brain stem, and cerebellum. From Gray's Anatomy, 20th U.S. edition, 1918.

Therefore both the *rapidity* and the *constellation* of the clinical effects produced by perispinal etanercept in AD imply rapid distribution of etanercept into the CSF. Independent and emerging evidence supports this novel concept.

Independent Evidence

Delivery into the CSF is recognized as one way to bypass the BBB [79-81]. Delivery of molecules into the CSF invariably leads to parenchymal brain delivery [75,76,79-85]. There exist multiple pathways for this to occur, as there is no major diffusional barrier between the interstitial fluid bathing neurons, neuroglia and the CSF [75,76,81-87][7]. Experimental results examining the brain distribution of substances following intracerebroventricular (ICV) delivery verify these concepts. ICV delivery can result in pervasive distribution into the brain [81,85]. For example, both interleukin-1 or of interleukin-1 receptor antagonist, large molecules(each with MW 17,000), rapidly penetrate periventricular tissue and spread into the caudoputamen, hypothalamus and amygdala after ICV injection [83]. ICV delivery is a standard method for testing the cerebral effects of drugs or biologics in animal models, and has specifically been used to test the brain effects of anti-TNF biologics in animal AD models [33,34,39,68]. ICV-injection of an anti-TNF-alpha antibody prevented the nitration of proteins in the hippocampus and the impairment of recognition memory induced by beta-amyloid fragments in mice [68]. ICV pre-treatment with a murine anti-TNF antibody improved the cognitive benefits induced by beta-amyloid1-40 in mice [34]. ICV injection of
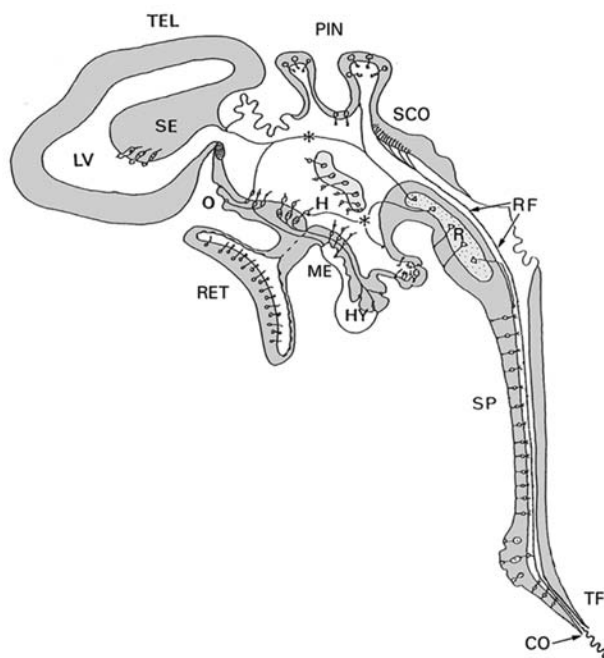
Fig. (2). A schematic diagram of structures and specialized cell types bordering the different parts of the mammalian ventricular system, and in contact with the cerebrospinal fluid (CSF). CSF-contacting neurons are illustrated at periventricular and spinal locations in contact with the CSF. Abbreviations: CO: caudal opening of the central canal of the spinal cord;H: hypothalamic CSF-contacting neurons; HY: Hypophysis; LV: lateral ventricle; ME: median eminence; O: vascular organ of the terminal lamina; PIN: pineal organ; R: raphe nuclei; RET: retina; RF: Reissner's fiber; SE: septal region; SCO: subcommissural organ; SP: medullo-spinal CSF-contacting neurons; TEL: telencephalon; TF: terminal filum. Adapted from Veening and Barendregt HP [75], with original source Vigh *et al.* [104].

Organum vasculosum of the lamina terminalis, and subfornical organ), striatum, hypothalamus, thalamus, brain stem, and cerebellum.
[7] Penetration into the periventricular parenchyma may occur by diffusion through junctions between ependymal cells. Spread more deeply into the parenchyma may occur along perivascular spaces aided by the paravascular fluid circulation. See Veening (2010a).

either infliximab(an anti-TNF antibody) or a TNF antagonist peptide prevented the inhibition of LTP at CA1 synapses caused by ICV injection of beta-amyloid in rats [33,34, 39,68]. Clinically, intracerebroventricular delivery of rituximab(MW 145,000) is effective for the treatment of CNS lymphoma, whereas systemic administration of rituximab is not [88-90]. The reason for this is that large molecules delivered by the conventional systemic routes (intravenous, intramuscular or subcutaneous) are generally limited in their penetration into the CSF to 0.5% or less of their serum levels [84,91]. CSF levels of rituximab, for example, are only approximately 0.1% of matched serum levels after intravenous administration [89].

Subcutaneous vs. Perispinal Administration of Etanercept for AD

As the rituximab data illustrates, penetration of large molecules[8] into the CSF when delivered systemically is generally poor. When administered intravenously to mice, there was no significant entry of etanercept into the brain [74]. A 24 week, randomized, double-blind, placebo-controlled study for treatment of dementia of the Alzheimer type in 12 subjects (nine receiving etanercept 25mg twice per week, three receiving placebo) showed that etanercept was well-tolerated but no apparent clinical benefit was observed, despite reduction of peripheral levels of TNF [92]. Perhaps most convincingly a series of scientific studies examining CSF levels of TNF in AD and related conditions documented that CSF TNF levels were on the average 25-fold higher in patients with AD compared with controls; that serum TNF levels were not higher in patients with AD compared with controls; and that, in a prospective, longitudinal study of patients with mild cognitive impairment (MCI) during a period of four years of enrollment, only MCI patients who progressed to AD at follow-up showed significantly higher CSF levels of TNF than controls [42,43,93]. Taken together, this data not only suggests that TNF is associated with disease progression, it also indicates that production of TNF is intrathecal [42,43,93]. In an *in vivo* basic science model, intracerebroventricular delivery of etanercept was found to result in modulation of neurotransmitters in the hypothalamic paraventricular nucleus, but intraperitoneal delivery did not have the same effect [94]. The evidence available therefore suggests that methods having the potential to improve selective delivery of etanercept to the CSF, such as perispinal administration, will be preferable to subcutaneous administration for treatment of AD [23,34,42,43,45-51,56,58,60, 61, 68,74,91,92].

Emerging Evidence

The invention of etanercept as a neurologic therapeutic was reported more than a decade ago [95-98][9]. In subsequent publications it was documented that patients treated with perispinal etanercept for intractable intervertebral disc-related pain and sciatica exhibited rapid improvement in pain and neurologic deficits, often beginning within minutes, a temporal course of onset similar to that seen in AD [47,48, 95-98]. It later became clear that there existed an essentially forgotten anatomic pathway that could explain these rapid spinal results: carriage of etanercept in the vertebral venous system, the interconnected plexuses of internal and external spinal veins [47,48,59,99-102]. Bi-directional flow within the vertebral venous system, potentially enabling etanercept injected perispinally to rapidly reach the spinal nerve roots, dorsal ganglia, and spinal cord, was the only viable mechanism that could explain the rapid improvement in pain [47,48,59]. Favorable randomized, double-blind placebo-controlled results of epidural etanercept for treatment of sciatica were subsequently published by Johns Hopkins physicians and their colleagues after consultation with this author, originally in 2003 [103].

One of the initial steps in the development of perispinal etanercept as a neurologic therapeutic was the development of evidence that introduction of etanercept locally, such as into the CSF, was a usable and safe treatment method [45,49, 95-97]. Next was the recognition that carriage via the vertebral venous system was an anatomic/physiologic route that could help explain the rapid pattern of effects of perispinal etanercept [45-51,59,60]. Following this, emerging scientific data that the vertebral venous system was a viable route for delivery of etanercept to the cerebral venous system was developed [45-51,59,60]. This required not only uncovering of obscure data that suggested that the vertebral and cerebral venous systems were in anatomic continuity, but also initiation of a clinical trial to provide proof-of-concept regarding the safety and potential efficacy of perispinal etanercept for treatment of AD. These efforts were successful [49,59]. The proof-of-concept trial was begun in 2004 and completed and published in 2006 [49]. Also in 2006, a review of the anatomy and physiology of the cerebral and spinal (Batson's) venous plexuses was published that detailed the evidence of their confluence [59]. In order to emphasize this confluence, a fact seemingly long forgotten, the term "cerebrospinal venous system (CSVS)" was coined [59]. The results of the proof-of-concept trial were presented at international conferences beginning in 2006[10].

In 2007 further data regarding the anatomic distribution of etanercept after perispinal administration was developed. In March 2007 the author wrote the following in an e-mail to a colleague:

- "Since etanercept works instantaneously [on a molecular level] it need only meet with free TNF for an instant to inactivate it. It is my belief that retrograde delivery via the CSVS results in etanercept flow into the cerebral

---

[8] With respect to CSF or BBB penetration, the term "large molecules" is generally used for molecules with a molecular weight (MW) greater than about 500 daltons. Etanercept has a MW of 150,000 daltons.
[9] See also U.S. patents 6,015,557; 6,177,077; 6,419,944; 6,537,549; 6,982,089; 7,214,658; 7,629,311; Australian patent 758,523; and others issued to the author, detailing methods to deliver etanercept and other anti-TNF molecules locally, including, but not limited to, perispinal, epidural, intrathecal, and intracerebroventricular administration, for treatment of Alzheimer's disease, other forms of dementia, sciatica and other neurological disorders.

[10] Conference presentations included the *Days of Molecular Medicine Conference* at the Karolinska Institute in Stockholm and the *7th International Alzheimer's Disease Drug Discovery Conference* in New York City, both in 2006; the *Drug Repositioning Summit* in Boston, the *International Conference on Alzheimer's Disease* in Chicago, and the *Advances in Alzheimer's Disease Management Conference* at the University of Arkansas for Medical Sciences, all in 2008; and the *3rd International Restauracion Neurologica Conference* in Havana, the *World Pharmaceutical Congress* in Philadelphia and the *5th Modern Drug Discovery Conference* in San Diego in 2009.

veins which then, with the benefit of a brief reversal of the usual venous pressure gradient within the cerebral sinuses….allows etanercept to reach brain TNF momentarily. This may occur via reverse flow into the parenchyma/CSF through the choroid plexus/ arachnoid villi…"

The human experiment that was later completed, utilizing nuclear medicine imaging of a radiolabeled molecule, confirmed that perispinal administration in the posterior neck could result in retrograde delivery into the cerebral venous system [48]. A subsequent animal experiment utilizing PET imaging, performed at Stanford in July 2007, provided preliminary proof-of-concept evidence that perispinal administration of radiolabeled etanercept could result in rapid delivery of etanercept into the CSF and the choroid plexus, as previously contemplated Fig. (**3**) [48,60]. These results provided further support for the concept that delivery of etanercept into the ventricular CSF via the cerebrospinal venous system was responsible for the clinical improvement sustained through six months documented in the 2006 pilot trial [46-49,51,59,60]. Subsequent clinical experience has also revealed rapid clinical improvement following perispinal etanercept in patients with frontotemporal dementia, primary progressive aphasia, corticobasal degeneration, stroke and traumatic brain injury [45-51,56,58,95,96].
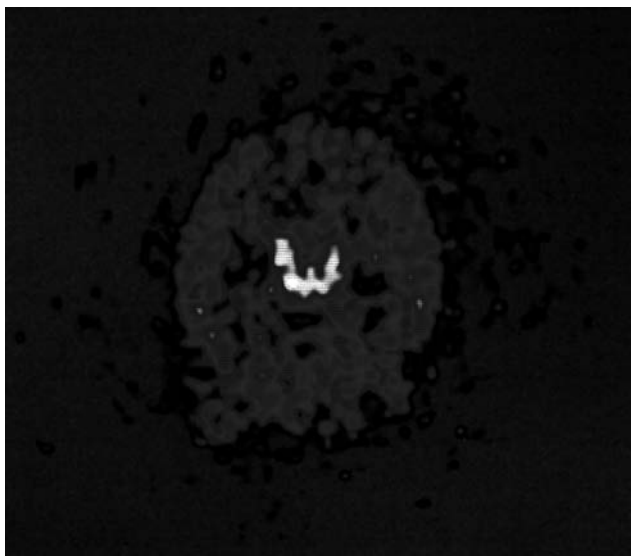
Fig. (3). PET image, transverse section, of a living rat brain following perispinal extrathecal administration of 64Cu-DOTA-etanercept, imaged 5 to 10 minutes following etanercept administration. The distinctive central pattern of brain distribution suggests penetration of 64Cu-DOTA-etanercept into the CSF in the lateral and third ventricles and accumulation within the choroid plexus following perispinal administration. PET=positron emission tomography. Cu=copper. DOTA=1,4,7,10-tetraazadodecane-N,NI,NII,NIII-tetraacetic acid. Adapted from Tobinick EL, Chen K, Chen X. *Rapid intracerebroventricular delivery of Cu-DOTA-etanercept after peripheral administration demonstrated by PET imaging.* BMC Res Notes, 2, 28 (2009).

Physiological Mechanisms that have the Potential to Facilitate the Rapid Effects of Perispinal Etanercept

Known mechanisms exist that facilitate signaling from the CSF across the ependymal barrier. These mechanisms may enable signaling molecules within the CSF to exert widespread and diverse neurologic and psychiatric effects even prior to their diffusion across the ependymal barrier. These mechanisms are as follows:

Rapid Diffusion Through the CSF

There are no barriers to diffusion of molecules throughout the CSF, except for distance. Many periventricular brain structures that mediate distinct functions are nevertheless in close proximity to each other, e.g. the hypothalamus, pituitary, median eminence and other circumventricular organs [104,105]. Within the brain signaling molecules diffuse throughout the ventricles within minutes [75,85,106]. In animal studies (C14)inulin, a large molecule (MW approx. 5,500), distributes in five minutes throughout the ventricles, subarachnoid spaces and cerebral cisterns after injection into the lateral ventricle. Alpha-melanocyte stimulating hormone injected into the lateral ventricle is evident in the cisterna magna CSF in two minutes [85,106]. Because signaling molecules diffusing through the CSF contact structures with diverse functions, a single molecule can exert diverse biologic effects [75,76].

Rapid Signal Transduction into Neuronal Networks via Cerebrospinal-Fluid Contacting Cells

The extreme rapidity of onset of the diverse constellation of neurological effects produced by perispinal etanercept suggests the existence of a physiological mechanism that is capable of transmission of signals from the CSF directly into neuronal networks. Such a mechanism exists: neurons whose dendrites float freely within the CSF, the so-called cerebrospinal fluid-contacting neurons(CSF-CNs) [75,104,105,107-113]. CSF-CNs can be divided into three types according to their distribution: intraependymal neurons that line the walls of the cerebral ventricles and the central canal of the spinal cord; supraependymal cells that are subjacent to the ependyma; and distal CSF-CNs whose cell bodies lie within the parenchyma of the brain but contain processes that extend to the ventricular CSF [113]. CSF-CNs contain receptors for a wide variety of molecules on their dendritic surfaces in contact with the CSF [104,111-115]. CSF-CNs receive, transform, and transmit non-synaptic signals from the CSF to neurons and glia in the parenchyma of the brain [104,111-115]. CSF-CNs are universally present in vertebrates, and constitute a phylogenetically ancient structure that enables communication between distant elements of the nervous system via non-synaptic signals carried in the CSF [104,105, 108-113,115-118]. A marvelous evolutionary hypothesis traces back CSF-CNs from mammals to reptiles; and then to the fluid-contacting neurons of marine organisms, from the CSF-CNs of cyclostomes, and further back to neurons in larval lancelets that contact a central canal enveloping seawater [104]. A parallel phylogenetic search reveals that TNF family signaling molecules have been found in *amphioxus*, the basal chordate; have been implicated in neuronal signaling in a fly, *Drosophila;* and subserve a signaling function in

a marine organism, the crustacean *Daphnia pulex* [119-121][11].

It is hypothesized that CSF-CNs, perhaps with TNF receptors on their CSF-contacting dendritic surfaces, are involved in the signal transduction from the CSF to the cerebral parenchyma necessary to effectuate the rapid effects of perispinal etanercept in AD and stroke. Constitutive expression of p55 (type 1) TNF receptor messenger RNA has, in fact, been detected in the circumventricular organs, choroid plexus, leptomeninges, and in the ependymal lining of the ventricular walls [122]. Moreover a biologic TNF antagonist (soluble TNF receptor type 1) has recently been shown to be capable of affecting the function of a subset of another type of CSF-contacting cell called alpha-tanyctes[12] [123-131].

CSF-CNs and tanycytes may represent new therapeutic targets for intervention in Alzheimer's disease. Additionally, the possible roles of CSF-CNs and/or tanycytes in the rapid effects of perispinal etanercept, and the known diversity of signaling molecules within the CSF, suggest that modulation of the activity of CSF-CNs or tanycytes may represent a therapeutic target for a diverse group of brain, psychiatric and spinal disorders, including neurodegenerative diseases; mood disorders; opiate and nicotine addiction and withdrawal; autism; schizophrenia; stroke and traumatic brain and spinal cord injury.

Glial-Neuronal Signaling Modulation by TNF

Modulation of synaptic function by TNF, including modulation of synaptic function across neuronal networks, has been established [132-136]. TNF is one of the small group of molecules, called gliotransmitters, that are released by glia and serve to modulate neuronal function[13] [75,137-143]. Brain functioning requires coordinated activity of neurons and glia, with glia actively modulating synaptic activity [139-146]. A single astrocyte may make contact with over 100,000 synapses, so a change in activity of even a single astrocyte can have widespread neuronal effects[139,147]. A single cortical neuron may be in contact with 60,000 synapses [148]. Thus it is perhaps not surprising that it has been demonstrated that stimulation of even a single neuron can have behavioral effects [149].

Synthesis of this physiology, including the massive interconnected nature of neurons and glia in the brain [137,138,140,150,151], with the clinical results produced by perispinal etanercept leads to a plausible hypothesis that modulation of the activity of even a small population of CSF-CNs by TNF could produce clinical changes; and that delivery of etanercept into the CSF, even without penetration

across the blood-brain barrier[14], may produce rapid and diverse clinical effects via signals transduced from the CSF into neuronal networks.

A proposed mechanism to explain the rapid onset of the clinical effects of perispinal administration of etanercept in AD is therefore summarized as follows [45,46,48,50,51,59,60]:

1) Perispinal administration of etanercept may result in rapid delivery of etanercept into the CSF within the cerebral ventricles, producing a rapid change in CSF TNF bioactivity [47,48,51];

2) Changes in TNF bioactivity in the CSF so produced may affect the activity of populations of CSF-CNs or other CSF-contacting cells by binding to TNF receptors on their CSF-contacting surfaces;

3) Changes in the signaling activity of these TNF-sensitive CSF-contacting cells may modulate cerebral glial/ neuronal network activity and thereby produce rapid clinical effects Fig. (**4**).

The use of perispinal etanercept for treatment of Alzheimer's disease, stroke, and disc-related pain as cited [45-51,56,58,95-97] involves unique extrathecal methods of administration that were developed by the author specifically for these applications. One challenge is the unfamiliarity of practicing physicians with the novel method of delivery; biologics have traditionally been administered systemically. An additional challenge in dementia is the lack of established biomarkers for diagnosis and the overlapping clinical presentation of patients with AD, Lewy Body Dementia, frontotemporal dementia, and mixed dementia. Although the long-term clinical experience to date using perispinal etanercept for AD and disc-related pain is favorable and the long-term safety data for etanercept for its approved indications is also favorable, clinical experience using etanercept off-label for additional disorders remains limited and therefore the long-term safety profile for these additional indications is not yet defined [48,152]. These factors combine to indicate the necessity of physician training prior to use of perispinal etanercept in patient treatment or clinical study.

CONCLUSION

Clinical improvement following perispinal administration of etanercept in patients with Alzheimer's disease and other forms of dementia, stroke and brain dysfunction is characteristically evident within minutes. The rapidity and constellation of the clinical effects across multiple domains (cognition, mood, memory, motor function, and attention) suggest they are mediated by non-synaptic signaling mechanisms previously unrecognized for etanercept. These mechanisms likely extend beyond the known roles of TNF as a gliotransmitter that modulates synaptic strength, synaptic scaling, and AMPA receptor trafficking. Preliminary basic science and clinical investigation suggests that perispinal administration of etanercept may lead to its rapid penetration into the cere-

---

[11]These facts, that both TNF family neuronal signaling and CSF-CNs are phylogenetically ancient, lead to the speculation that perhaps TNF family molecules are involved in CSF-CN signaling in a variety of organisms, including humans.
[12]*Tanycytes* are specialized ependymal cells lining the cerebral ventricles that possess long basilar processes that extend into the cerebral parenchyma. Tanycytes can be subdivided into at least four different subtypes based upon their morphological and physiological features: alpha-1, alpha-2, beta-1, and beta-2. Alpha-tanycytes have been shown to have direct projections into the neuropil of the arcuate and ventromedial nuclei.
[13] Known gliotransmitters include adenosine, ATP, D-serine, GABA, glutamate, and TNF.

---

[14] The less rapid effects of perispinal etanercept in AD may also involve etanercept delivered into the extracellular fluid and brain parenchyma, or through etanercept's effects on ECF or parenchymal TNF levels.
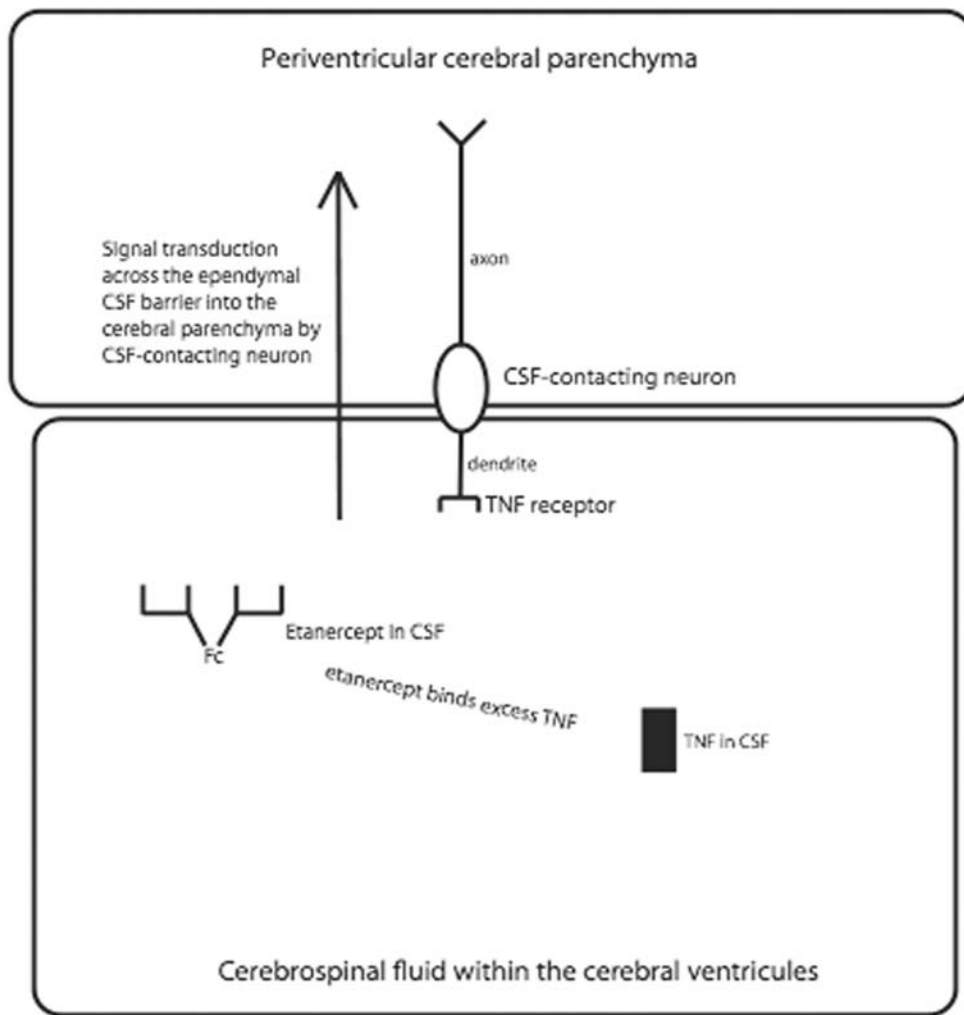
Fig. (4). Proposed mechanism underlying rapid cerebral effects of perispinal etanercept. Etanercept delivered into the CSF within the cerebral ventricles may produce a rapid change in CSF TNF bioactivity, affecting TNF binding to TNF receptors on the CSF-contacting surface of cells in contact with the CSF, such as tanycytes or CSF-contacting neurons, resulting in signal transduction across the ependymal barrier into the cerebral parenchyma and rapid clinical and behavioral effects.

brospinal fluid (CSF) within the cerebral ventricles. Diffusion of large molecules into the periventricular brain parenchyma is known to occur, but this process may not be sufficient to explain the rapidity of the clinical effects. It is hypothesized that the rapid clinical effects of perispinal etanercept involve non-synaptic signal transduction from the CSF across the ependymal barrier and into neuronal networks via CSF-contacting cells, including CSF-contacting neurons and tanycytes. This hypothesis challenges the dogma that penetration of a therapeutic into the cerebral parenchyma through the endothelium of the cerebral vasculature (the so-called blood- brain barrier) is necessary to produce rapid clinical effects in Alzheimer's disease.

Basic science and clinical evidence suggests that methods that have the potential to improve selective delivery of etanercept to the CSF, such as perispinal administration, may be preferable to subcutaneous administration for treatment of Alzheimer's disease. CSF-contacting cells are a potential therapeutic target for a diverse group of brain psychiatric, and spinal disorders, through modulation of their signaling

activity. Characterization of the non-synaptic receptors present on CSF-contacting cells may lead to new therapeutic approaches for brain disorders. For diseases for which current therapies are inadequate, challenges to existing paradigms may be necessary. This discovery process may be engendered by careful clinical observation and synthesis of physiologic, anatomic, and phylogenetic data developed in other scientific disciplines.

CONFLICT OF INTEREST

The author has multiple issued U.S. and foreign patents and patent applications including U.S. patents 6,015,557; 6,177,077; 6,419,944; 6,537,549; 6,982,089; 7,214,658; 7,629,311; Australian patent 758,523; and others detailing methods to deliver etanercept and other anti-TNF molecules locally, including, but not limited to, perispinal, epidural, intrathecal, and intracerebroventricular administration, for treatment of Alzheimer's disease, other forms of dementia, sciatica and other neurological disorders.

ACKNOWLEDGEMENTS

REFERENCES

[1]   Kuhn TS. The Structure of Scientific Revolutions (The University of Chicago Press., 1962).

[2]   Wolinsky H. Paths to acceptance. The advancement of scientific knowledge is an uphill struggle against 'accepted wisdom'. EMBO Rep 9(5): 416-418 (2008).

[3]   Ellis H. Ignaz Semmelweis: tragic pioneer in the prevention of puerperal sepsis. Br J Hosp Med (Lond) 69(6): 358 (2008).

[4]   Lang L. Barry Marshall 2005 Nobel laureate in medicine and physiology. Gastroenterology 129(6): 1813-1814 (2005).

[5]   Sobel RK. Barry Marshall. A gutsy gulp changes medical science. US News World Rep 131(7): 59 (2001).

[6]   Sheridan C. J&J's billion dollar punt on anti-amyloid antibody. Nat Biotechnol 27(8): 679-681 (2009).

[7]   Smith MA, Atwood CS, Joseph JA, Perry G. Predicting the failure of amyloid-beta vaccine. Lancet 359(9320): 1864-1865 (2002).

[8]   Soscia SJ, Kirby JE, Washicosky KJ, *et al.* The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. PLoS One 5(3): e9505 (2010).

[9]   Lee HG, Casadesus G, Zhu X, Takeda A, Perry G, Smith MA. Challenging the amyloid cascade hypothesis: senile plaques and amyloid-beta as protective adaptations to Alzheimer disease. Ann N Y Acad Sci 1019: 1-4 (2004).

[10]   Joseph J, Shukitt-Hale B, Denisova NA, Martin A, Perry G, Smith MA. Copernicus revisited: amyloid beta in Alzheimer's disease. Neurobiol Aging 22(1): 131-146 (2001).

[11]   Maarouf C, Daugs I, Kokjohn T, *et al.* The biochemical aftermath of anti-amyloid immunotherapy. Mol Neurodegener 5: 39 (2010).

[12]   Holmes C, Boche D, Wilkinson D, *et al.* Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet 372(9634): 216-223 (2008).

[13]   Wilcock GK. Bapineuzumab in Alzheimer's disease: where now? Lancet Neurol 9(2): 134-136 (2010).

[14]   Vellas B. Tarenflurbil for Alzheimer's disease: a "shot on goal" that missed. Lancet Neurol 9(3): 235-237 (2010).

[15]   Talan J. New study theorizes that amyloid beta protein is an antimicrobial peptide: a shift in the thinking about Alzheimer disease? Neurology Today 10(9): 22 (2010).

[16]   Robinson SR, Bishop GM, Lee HG, Munch G. Lessons from the AN 1792 Alzheimer vaccine: lest we forget. Neurobiol Aging 25(5): 609-615 (2004).

[17]   Boche D, Denham N, Holmes C, Nicoll JA. Neuropathology after active Abeta42 immunotherapy: implications for Alzheimer's disease pathogenesis. Acta Neuropathol 120(3): 369-384 (2010).

[18]   Salloway S, Sperling R, Gilman S, *et al.* A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. Neurology 73(24): 2061-2070 (2009).

[19]   Alvarez V, Mata IF, Gonzalez P, *et al.* Association between the TNFalpha-308 A/G polymorphism and the onset-age of Alzheimer disease. Am J Med Genet 114(5): 574-577 (2002).

[20]   Buchhave P, Zetterberg H, Blennow K, Minthon L, Janciauskiene S, Hansson O. Soluble TNF receptors are associated with Abeta metabolism and conversion to dementia in subjects with mild cognitive impairment. Neurobiol Aging 31(11): 1877-1884 (2010).

[21]   Di Bona D, Candore G, Franceschi C, *et al.* Systematic review by meta-analyses on the possible role of TNF-alpha polymorphisms in association with Alzheimer's disease. Brain Res Rev 61(2): 60-68 (2009).

[22]   Giuliani F, Vernay A, Leuba G, Schenk F. Decreased behavioral impairments in an Alzheimer mice model by interfering with TNF-alpha metabolism. Brain Res Bull 80(4-5): 302-308 (2009).

[23]   Griffin WS. Perispinal etanercept: potential as an Alzheimer therapeutic. J Neuroinflammation 5: 3 (2008).

[24]   Holmes C, Cunningham C, Zotova E, *et al.* Systemic inflammation and disease progression in Alzheimer disease. Neurology 73(10): 768-774 (2009).

[25]   Hu NW, Klyubin I, Anwy R, Rowan MJ. GluN2B subunit-containing NMDA receptor antagonists prevent A{beta}-mediated synaptic plasticity disruption *in vivo*. Proc Natl Acad Sci USA 106(48): 20504-20509 (2009).

[26]   Janelsins MC, Mastrangelo MA, Oddo S, LaFerla FM, Federoff HJ, Bowers WJ. Early correlation of microglial activation with enhanced tumor necrosis factor-alpha and monocyte chemoattractant protein-1 expression specifically within the entorhinal cortex of triple transgenic Alzheimer's disease mice. J Neuroinflammation 2: 23 (2005).

[27]   Janelsins MC, Mastrangelo MA, Park KM, *et al.* Chronic neuron-specific tumor necrosis factor-alpha expression enhances the local inflammatory environment ultimately leading to neuronal death in 3xTg-AD mice. Am J Pathol 173(6): 1768-1782 (2008).

[28]   Laws SM, Perneczky R, Wagenpfeil S, *et al.* TNF polymorphisms in Alzheimer disease and functional implications on CSF beta-amyloid levels. Hum Mutat 26(1): 29-35 (2005).

[29]   Lio D, Annoni G, Licastro F, *et al.* Tumor necrosis factor-alpha -308A/G polymorphism is associated with age at onset of Alzheimer's disease. Mech Ageing Dev 127(6): 567-571 (2006).

[30]   McAlpine FE, Lee JK, Harms AS, *et al.* Inhibition of soluble TNF signaling in a mouse model of Alzheimer's disease prevents pre-plaque amyloid-associated neuropathology. Neurobiol Dis 34(1): 163-177 (2009).

[31]   McCoy MK, Tansey MG. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. J Neuroinflammation 5: 45 (2008).

[32]   McNaull BB, Todd S, McGuinness B, Passmore AP. Inflammation and anti-inflammatory strategies for Alzheimer's disease--a mini-review. Gerontology 56(1): 3-14 (2010).

[33]   Medeiros R, Figueiredo CP, Pandolfo P, *et al.* The role of TNF-alpha signaling pathway on COX-2 upregulation and cognitive decline induced by beta-amyloid peptide. Behav Brain Res 209(1): 165-173 (2010).

[34]   Medeiros R, Prediger RD, Passos GF, *et al.* Connecting TNF-alpha signaling pathways to iNOS expression in a mouse model of Alzheimer's disease: relevance for the behavioral and synaptic deficits induced by amyloid beta protein. J Neurosci 27(20): 5394-5404 (2007).

[35]   Park KM, Bowers WJ. Tumor necrosis factor-alpha mediated signaling in neuronal homeostasis and dysfunction. Cell Signal 22(7): 977-983 (2010).

[36]   Perry RT, Collins JS, Wiener H, Acton R, Go RC. The role of TNF and its receptors in Alzheimer's disease. Neurobiol Aging 22(6): 873-883 (2001).

[37]   Ramos EM, Lin MT, Larson EB, *et al.* Tumor necrosis factor alpha and interleukin 10 promoter region polymorphisms and risk of late-onset Alzheimer disease. Arch Neurol 63(8): 1165-1169 (2006).

[38]   Rogers J. The inflammatory response in Alzheimer's disease. J Periodontol 79(8): 1535-1543 (2008).

[39]   Rowan MJ, Klyubin I, Wang Q, Hu NW, Anwyl R. Synaptic memory mechanisms: Alzheimer's disease amyloid beta-peptide-induced dysfunction. Biochem Soc Trans 35(5): 1219-1223 (2007).

[40]   Sarajarvi T, Helisalmi S, Antikainen L, *et al.* An association study of 21 potential Alzheimer's disease risk genes in a Finnish population. J Alzheimers Dis 21(3): 763-767 (2010).

[41]   Tan ZS, Beiser AS, Vasan RS, *et al.* Inflammatory markers and the risk of Alzheimer disease: the Framingham Study. Neurology 68(22): 1902-1908 (2007).

[42]   Tarkowski E, Andreasen N, Tarkowski A, Blennow K. Intrathecal inflammation precedes development of Alzheimer's disease. J Neurol Neurosurg Psychiatry 74(9): 1200-1205 (2003).

[43]   Tarkowski E, Blennow K, Wallin A, Tarkowski A. Intracerebral production of tumor necrosis factor-alpha, a local neuroprotective agent, in Alzheimer disease and vascular dementia. J Clin Immunol 19(4): 223-230 (1999).

[44]   Teeling JL, Perry VH. Systemic infection and inflammation in acute CNS injury and chronic neurodegeneration: underlying mechanisms. Neuroscience 158(3): 1062-1073 (2009).

[45]   Tobinick E. Perispinal etanercept for treatment of Alzheimer's disease. Curr Alzheimer Res 4(5): 550-552 (2007).

[46]   Tobinick E. Tumour necrosis factor modulation for treatment of Alzheimer's disease: rationale and current evidence. CNS Drugs 23(9): 713-725 (2009).

[47]   Tobinick E. Perispinal etanercept for neuroinflammatory disorders. Drug Discov Today 14(3-4): 168-177 (2009).

[48]   Tobinick E. Perispinal etanercept: a new therapeutic paradigm in neurology. Expert Review of Neurotherapeutics 10(6): 985-1002 (2010).

[49]   Tobinick E, Gross H, Weinberger A, Cohen H. TNF-alpha modulation for treatment of Alzheimer's disease: a 6-month pilot study. MedGenMed 8(2): 25 (2006).

[50]   Tobinick EL, Gross H. Rapid improvement in verbal fluency and aphasia following perispinal etanercept in Alzheimer's disease. BMC Neurol 8: 27 (2008).

[51]   Tobinick EL, Gross H. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. J Neuroinflammation 5: 2 (2008).

[52]   Tweedie D, Sambamurti K, Greig NH. TNF-alpha inhibition as a treatment strategy for neurodegenerative disorders: new drug candidates and targets. Curr Alzheimer Res 4(4): 378-385 (2007).

[53]   Van Eldik LJ, Thompson WL, Ralay Ranaivo H, Behanna HA, Martin Watterson D. Glia proinflammatory cytokine upregulation as a therapeutic target for neurodegenerative diseases: function-based and target-based discovery approaches. Int Rev Neurobiol 82: 277-296 (2007).

[54]   Wang Y. P4-266: Modification of synaptic plasticity by TNF and sphingomyelinase: Implications for cognitive impairment in Alzheimer's disease. Alzheimers Dement 4(4): T749 (2008).

[55]   McAfoose J, Baune BT. Evidence for a cytokine model of cognitive function. Neurosci Biobehav Rev 33(3): 355-366 (2009).

[56]   Tobinick E. Perispinal etanercept produces rapid improvement in primary progressive aphasia: identification of a novel, rapidly reversible TNF-mediated pathophysiologic mechanism. Medscape J Med 10(6): 135 (2008).

[57]   Ferguson AR, Christensen RN, Gensel JC, et al. Cell death after spinal cord injury is exacerbated by rapid TNF alpha-induced trafficking of GluR2-lacking AMPARs to the plasma membrane. J Neurosci 28(44): 11391-11400 (2008).

[58]   Tobinick E. Rapid improvement of chronic stroke deficits after perispinal etanercept: three consecutive cases. CNS Drugs 25(2): 145-155 (2011).

[59]   Tobinick E, Vega CP. The cerebrospinal venous system: anatomy, physiology, and clinical implications. MedGenMed 8(1): 53 (2006).

[60]   Tobinick EL, Chen K, Chen X. Rapid intracerebroventricular delivery of Cu-DOTA-etanercept after peripheral administration demonstrated by PET imaging. BMC Res Notes 2: 28 (2009).

[61]   Clark IA, Alleva LM, Vissel B. The roles of TNF in brain dysfunction and disease. Pharmacol Ther 128(3): 519-548 (2010).

[62]   Wang Q, Wu J, Rowan MJ, Anwyl R. Beta-amyloid inhibition of long-term potentiation is mediated via tumor necrosis factor. Eur J Neurosci 22(11), 2827-2832 (2005).

[63]   O'Bryant SE, Xiao G, Barber R, et al. A serum protein-based algorithm for the detection of Alzheimer disease. Arch Neurol 67(9): 1077-1081 (2010).

[64]   Ray S, Britschgi M, Herbert C, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. Nat Med 13(11): 1359-1362 (2007).

[65]   Chen YM, Chen HH, Lan JL, Chen DY. Improvement of cognition, a potential benefit of anti-TNF therapy in elderly patients with rheumatoid arthritis. Joint Bone Spine 77(4): 366-367 (2010).

[66]   Chio CC, Lin JW, Chang MW, et al. Therapeutic evaluation of etanercept in a model of traumatic brain injury. J Neurochem 115(4): 921-929 (2010).

[67]   Elfferich MD, Nelemans PJ, Ponds RW, De Vries J, Wijnen PA, Drent M. Everyday cognitive failure in sarcoidosis: the prevalence and the effect of anti-TNF-alpha treatment. Respiration 80(3): 212-219 (2010).

[68]   Alkam T, Nitta A, Mizoguchi H, et al. Restraining tumor necrosis factor-alpha by thalidomide prevents the amyloid beta-induced impairment of recognition memory in mice. Behav Brain Res, 189(1), 100-106 (2008).

[69]   Gorlovoy P, Larionov S, Pham TT, Neumann H. Accumulation of tau induced in neurites by microglial proinflammatory mediators. FASEB J 23(8): 2502-2513 (2009).

[70]   Minami SS, Sidahmed E, Aid S, et al. Therapeutic versus neuroinflammatory effects of passive immunization is dependent on Abeta/amyloid burden in a transgenic mouse model of Alzheimer's disease. J Neuroinflammation 7(1): 57 (2010).

[71]   Perry VH. Contribution of systemic inflammation to chronic neurodegeneration. Acta Neuropathol 120(3): 277-286 (2010).

[72]   Shi J, Shen W, Chen J, et al. Anti-TNF-alpha reduces amyloid plaques and tau phosphorylation and induces CD11c-positive dendritic-like cells in the APP/PS1 transgenic mouse brain. Brain Research 1368: 239-247 (2011).

[73]   Frankola KA, Greig NH, Luo W, Tweedie D. Targeting TNF-Alpha to Elucidate and Ameliorate Neuroinflammation in Neurodegenerative Diseases. CNS Neurol Disord Drug Targets [Epub ahead of print] (2011).

[74]   Banks WA, Plotkin SR, Kastin AJ. Permeability of the blood-brain barrier to soluble cytokine receptors. Neuroimmunomodulation 2(3): 161-165 (1995).

[75]   Veening JG, Barendregt HP. The regulation of brain states by neuroactive substances distributed via the cerebrospinal fluid; a review. Cerebrospinal Fluid Res 7: 1 (2010a).

[76]   Veening JG, de Jong T, Barendregt HP. Oxytocin-messages via the cerebrospinal fluid: behavioral effects; a review. Physiol Behav 101(2): 193-210 (2010).

[77]   Vizi ES, Fekete A, Karoly R, Mike A. Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment. Br J Pharmacol 160(4): 785-809 (2010).

[78]   Strakowski SM, DelBello MP, Zimmerman ME, et al. Ventricular and periventricular structural volumes in first- versus multiple-episode bipolar disorder. Am J Psychiatry 159(11): 1841-1847 (2002).

[79]   Alam MI, Beg S, Samad A, et al. Strategy for effective brain drug delivery. Eur J Pharm Sci 40(5): 385-403 (2010).

[80]   Potschka H. Targeting the brain--surmounting or bypassing the blood-brain barrier. Handb Exp Pharmacol (197): 411-431 (2010).

[81]   Johanson CE, Duncan JA, Stopa EG, Baird A. Enhanced prospects for drug delivery and brain targeting by the choroid plexus-CSF route. Pharm Res 22(7): 1011-1037 (2005).

[82]   Johanson CE, Duncan JA, 3rd, Klinge PM, Brinker T, Stopa EG, Silverberg GD. Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. Cerebrospinal Fluid Res 5: 10 (2008).

[83]   Konsman JP, Tridon V, Dantzer R. Diffusion and action of intracerebroventricularly injected interleukin-1 in the CNS. Neuroscience 101(4): 957-967 (2000).

[84]   Proescholdt MG, Chakravarty S, Foster JA, Foti SB, Briley EM, Herkenham M. Intracerebroventricular but not intravenous interleukin-1beta induces widespread vascular-mediated leukocyte infiltration and immune signal mRNA expression followed by brain-wide glial activation. Neuroscience 112(3): 731-749 (2002).

[85]   Proescholdt MG, Hutto B, Brady LS, Herkenham M. Studies of cerebrospinal fluid flow and penetration into brain following lateral ventricle and cisterna magna injections of the tracer [14C]inulin in rat. Neuroscience 95(2): 577-592 (2000).

[86]   Zheng W, Chodobski A. The blood-cerebrospinal fluid barrier (Chapman & Hall/CRC, 2005).

[87]   Bruni JE. Ependymal development, proliferation, and functions: a review. Microsc Res Tech 41(1): 2-13 (1998).

[88]   Rubenstein JL, Combs D, Rosenberg J, et al. Rituximab therapy for CNS lymphomas: targeting the leptomeningeal compartment. Blood 101(2): 466-468 (2003).

[89]   Rubenstein JL, Fridlyand J, Abrey L, et al. Phase I study of intraventricular administration of rituximab in patients with recurrent CNS and intraocular lymphoma. J Clin Oncol 25(11): 1350-1356 (2007).

[90]   Feugier P, Virion JM, Tilly H, et al. Incidence and risk factors for central nervous system occurrence in elderly patients with diffuse large-B-cell lymphoma: influence of rituximab. Ann Oncol 15(1): 129-133 (2004).

[91]   Banks WA. Are the extracellular [correction of extracelluar] pathways a conduit for the delivery of therapeutics to the brain? Curr Pharm Des 10(12): 1365-1370 (2004).

[92]   Bohac D, Burke W, Cotter R, Zheng J, Potter J, Gendelman H. A 24-week randomized, double-blind, placebo-controlled study of the efficacy and tolerability of TNFR: Fc (etanercept) in the treatment of dementia of the Alzheimer type. Neurobiol Aging 23(S1): 315 (2002).

[93]   Tarkowski E, Liljeroth AM, Minthon L, Tarkowski A, Wallin A, Blennow K. Cerebral pattern of pro- and anti-inflammatory cytokines in dementias. Brain Res Bull 61(3): 255-260 (2003).

[94]   Kang YM, He RL, Yang LM, et al. Brain tumour necrosis factor-alpha modulates neurotransmitters in hypothalamic paraventricular nucleus in heart failure. Cardiovasc Res 83(4): 737-746 (2009).

[95] Tobinick EL, Britschgi-Davoodifar S. Perispinal TNF-alpha inhibition for discogenic pain. Swiss Med Wkly 133(11-12): 170-177 (2003).

[96] Tobinick E, Davoodifar S. Efficacy of etanercept delivered by perispinal administration for chronic back and/or neck disc-related pain: a study of clinical observations in 143 patients. Curr Med Res Opin 20(7): 1075-1085 (2004).

[97] Tobinick EL. Targeted etanercept for treatment-refractory pain due to bone metastasis: two case reports. Clin Ther 25(8): 2279-2288 (2003).

[98] Tobinick EL. Targeted etanercept for discogenic neck pain: uncontrolled, open-label results in two adults. Clin Ther 25(4): 1211-1218 (2003).

[99] Batson OV. The vertebral vein system. Caldwell lecture, 1956. Am J Roentgenol Radium Ther Nucl Med 78(2): 195-212 (1957).

[100] Batson OV. The function of the vertebral veins and their role in the spread of metastases. 1940. Clin Orthop Relat Res (312): 4-9 (1995).

[101] Groen RJ, du Toit DF, Phillips FM, *et al.* Anatomical and pathological considerations in percutaneous vertebroplasty and kyphoplasty: a reappraisal of the vertebral venous system. Spine 29(13): 1465-1471 (2004).

[102] Groen RJ, Groenewegen HJ, van Alphen HA, Hoogland PV. Morphology of the human internal vertebral venous plexus: a cadaver study after intravenous Araldite CY 221 injection. Anat Rec 249(2): 285-294 (1997).

[103] Cohen SP, Bogduk N, Dragovich A, *et al.* Randomized, double-blind, placebo-controlled, dose-response, and preclinical safety study of transforaminal epidural etanercept for the treatment of sciatica. Anesthesiology 110(5): 1116-1126 (2009).

[104] Vigh B, Manzano e Silva MJ, Frank CL, *et al.* The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. Histol Histopathol 19(2): 607-628 (2004).

[105] Vigh-Teichmann I, Vigh B. The system of cerebrospinal fluid-contacting neurons. Arch Histol Jpn 46(4): 427-468 (1983).

[106] De Rotte AA, Bouman HJ, van Wimersma Greidanus TB. Relationships between alpha-MSH levels in blood and in cerebrospinal fluid. Brain Res Bull 5(4): 375-381 (1980).

[107] Vigh B, Vigh-Teichmann I. Actual problems of the cerebrospinal fluid-contacting neurons. Microsc Res Tech 41(1): 57-83 (1998).

[108] Vigh B, Vigh-Teichmann I, Manzano e Silva MJ, van den Pol AN. Cerebrospinal fluid-contacting neurons of the central canal and terminal ventricle in various vertebrates. Cell Tissue Res 231(3): 615-621 (1983).

[109] Vigh-Teichmann I, Vigh B. The cerebrospinal fluid-contacting neuron: a peculiar cell type of the central nervous system. Immunocytochemical aspects. Arch Histol Cytol 52(S): 195-207 (1989).

[110] Mitchell JA. Scanning electron microscopy of brain ventricular surfaces: a bibliography. Scan Electron Microsc 3: 474-484 (1980).

[111] Sano Y, Ueda S, Yamada H, Takeuchi Y, Goto M, Kawata M. Immunohistochemical demonstration of serotonin-containing CSF-contacting neurons in the submammalian paraventricular organ. Histochemistry 77(4): 423-430 (1983).

[112] Xiao M, Ding J, Wu L, *et al.* The distribution of neural nitric oxide synthase-positive cerebrospinal fluid-contacting neurons in the third ventricular wall of male rats and coexistence with vasopressin or oxytocin. Brain Res 1038(2): 150-162 (2005).

[113] Zhang LC, Zeng YM, Ting J, Cao JP, Wang MS. The distributions and signaling directions of the cerebrospinal fluid contacting neurons in the parenchyma of a rat brain. Brain Res 989(1): 1-8 (2003).

[114] Stoeckel ME, Uhl-Bronner S, Hugel S, *et al.* Cerebrospinal fluid-contacting neurons in the rat spinal cord, a gamma-aminobutyric acidergic system expressing the P2X2 subunit of purinergic receptors, PSA-NCAM, and GAP-43 immunoreactivities: light and electron microscopic study. J Comp Neurol 457(2): 159-174 (2003).

[115] Vigh B, Vigh-Teichmann I. Cytochemistry of CSF-contacting neurons and pinealocytes. Prog Brain Res 91: 299-306 (1992).

[116] Blahser S, Vigh-Teichmann I, Ueck M. Cerebrospinal fluid-contacting neurons and other somatostatin-immunoreactive perikarya in brains of tadpoles of Xenopus laevis. Cell Tissue Res 224(3): 693-697 (1982).

[117] Melendez-Ferro M, Perez-Costas E, Villar-Cheda B, *et al.* Ontogeny of gamma-aminobutyric acid-immunoreactive neuronal populations in the forebrain and midbrain of the sea lamprey. J Comp Neurol 446(4): 360-376 (2002).

[118] Vigh B, Vigh-Teichmann I, Aros B. Special dendritic and axonal endings formed by the cerebrospinal fluid contacting neurons of the spinal cord. Cell Tissue Res 183(4)541-552 (1977).

[119] Wilson KH. The genome sequence of the protostome Daphnia pulex encodes respective orthologues of a neurotrophin, a Trk and a p75NTR: evolution of neurotrophin signaling components and related proteins in the bilateria. BMC Evol Biol 9: 243 (2009).

[120] Yuan S, Liu T, Huang S, *et al.* Genomic and functional uniqueness of the TNF receptor-associated factor gene family in amphioxus, the basal chordate. J Immunol 183(7): 4560-4568 (2009).

[121] Babcock DT, Landry C, Galko MJ. Cytokine signaling mediates UV-induced nociceptive sensitization in Drosophila larvae. Curr Biol 19(10): 799-806 (2009).

[122] Nadeau S, Rivest S. Effects of circulating tumor necrosis factor on the neuronal activity and expression of the genes encoding the tumor necrosis factor receptors (p55 and p75) in the rat brain: a view from the blood-brain barrier. Neuroscience 93(4): 1449-1464 (1999).

[123] Peruzzo B, Pastor FE, Blazquez JL, *et al.* A second look at the barriers of the medial basal hypothalamus. Exp Brain Res 132(1): 10-26 (2000).

[124] Sanchez E, Singru PS, Wittmann G, *et al.* Contribution of TNF-alpha and nuclear factor-kappaB signaling to type 2 iodothyronine deiodinase activation in the mediobasal hypothalamus after lipopolysaccharide administration. Endocrinology 151(8): 3827-3835 (2010).

[125] Akmayev IG, Popov AP. Morphological aspects of the hypothalamic-hypophyseal system. VII. The tanycytes: Their relation to the hypophyseal adrenocorticotrophic function. An ultrastructural study. Cell Tissue Res 180(2): 263-282 (1977).

[126] Bruni JE, Clattenburg RE, Millar E. Tanycyte ependymal cells in the third ventricle of young and adult rats: a Golgi study. Anat Anz 153(1): 53-68 (1983).

[127] Rodriguez EM, Blazquez JL, Pastor FE, *et al.* Hypothalamic tanycytes: a key component of brain-endocrine interaction. Int Rev Cytol 247: 89-164 (2005).

[128] Sharp PJ. Tanycyte and vascular patterns in the basal hypothalamus of Coturnix quail with reference to their possible neuroendocrine significance. Z Zellforsch Mikrosk Anat 127(4): 552-569 (1972).

[129] Wagner HJ, Pilgrim C. Extracellular and transcellular transport of horseradish peroxidase (HRP) through the hypothalamic tanycyte ependyma. Cell Tissue Res 152(4): 477-491 (1974).

[130] Horstmann E. Die Faserlia des Selachiergehirns. Z. Zellforsch, 39: 588-617 (1954).

[131] Pilgrim C. Transport function of hypothalamic tanycyte ependyma: how good is the evidence? Neuroscience 3(3): 277-283 (1978).

[132] Beattie EC, Stellwagen D, Morishita W, *et al.* Control of synaptic strength by glial TNFalpha. Science 295(5563): 2282-2285 (2002).

[133] Beattie MS, Ferguson AR, Bresnahan JC. AMPA-receptor trafficking and injury-induced cell death. Eur J Neurosci 32(2): 290-7 (2010).

[134] Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J Neurosci 25(12): 3219-3228 (2005).

[135] Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF-alpha. Nature 440(7087): 1054-1059 (2006).

[136] Tancredi V, D'Arcangelo G, Grassi F, *et al.* Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. Neurosci Lett 146(2): 176-178 (1992).

[137] Fields RD, Burnstock G. Purinergic signalling in neuron-glia interactions. Nat Rev Neurosci 7(6): 423-436 (2006).

[138] Fields RD, Stevens B. ATP: an extracellular signaling molecule between neurons and glia. Trends Neurosci 23(12): 625-633 (2000).

[139] Halassa MM, Fellin T, Haydon PG. The tripartite synapse: roles for gliotransmission in health and disease. Trends Mol Med 13(2): 54-63 (2007).

[140] Halassa MM, Fellin T, Haydon PG. Tripartite synapses: roles for astrocytic purines in the control of synaptic physiology and behavior. Neuropharmacology 57(4): 343-346 (2009).

[141] Oliet SH, Piet R, Poulain DA, Theodosis DT. Glial modulation of synaptic transmission: Insights from the supraoptic nucleus of the hypothalamus. Glia 47(3): 258-267 (2004).

[142] Wieronska JM, Pilc A. Metabotropic glutamate receptors in the tripartite synapse as a target for new psychotropic drugs. Neurochem Int 55(1-3): 85-97 (2009).

[143] Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32(8): 421-431 (2009).

[144] Bains JS, Oliet SH. Glia: they make your memories stick! Trends Neurosci 30(8): 417-424 (2007).

[145] Lalo U, Andrew J, Palygin O, Pankratov Y. Ca2+-dependent modulation of GABAA and NMDA receptors by extracellular ATP: implication for function of tripartite synapse. Biochem Soc Trans, 37(Pt 6): 1407-1411 (2009).

[146] Machado-Vieira R, Manji HK, Zarate CA. The role of the tripartite glutamatergic synapse in the pathophysiology and therapeutics of mood disorders. Neuroscientist 15(5): 525-539 (2009).

[147] Bushong EA, Martone ME, Jones YZ, Ellisman MH. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J Neurosci 22(1): 183-192 (2002).

[148] DeFelipe J, Farinas I. The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. Prog Neurobiol 39(6): 563-607 (1992).

[149] Houweling AR, Brecht M. Behavioural report of single neuron stimulation in somatosensory cortex. Nature 451(7174): 65-68 (2008).

[150] Bullock TH, Bennett MV, Johnston D, Josephson R, Marder E, Fields RD. Neuroscience. The neuron doctrine, redux. Science 310(5749): 791-793 (2005).

[151] Lopez-Munoz F, Boya J, Alamo C. Neuron theory, the cornerstone of neuroscience, on the centenary of the Nobel Prize award to Santiago Ramon y Cajal. Brain Res Bull 70(4-6): 391-405 (2006).

[152] Gottlieb AB, Gordon K, Giannini EH, *et al.* Clinical trial safety and mortality analyses in patients receiving etanercept across approved indications. J Drugs Dermatol 10(3): 289-300 (2011).