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(54) Title: TREATMENT OF TRAUMATIC BRAIN INJURY

(57) Abstract: A method for treating a subject suffering from traumatic brain injury, includes: administering to the subject a therapeutically effective amount of a selective inhibitor of solTNF- α , whereby the subject is treated. In some embodiments, the selective inhibitor of solTNF- α includes a DN- TNF- α protein and/or a nucleic acid encoding the DN-TNF- α protein. In some embodiments, the DN-TNF- α protein includes XPR01595.



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TREATMENT OF TRAUMATIC BRAIN INJURY

TECHNICAL FIELD

[0001] The invention is directed to a method for medical treatment of a subject patient suffering from traumatic brain injury (TBI).

[0002] More particularly, the invention is directed to a method for treating traumatic brain injury (TBI) in a subject patient by administering a selective inhibitor of soluble tumor necrosis factor alpha (solTNF- α), and more particularly, wherein the selective inhibitor of solTNF- α includes a dominant negative tumor necrosis factor alpha (DN-TNF- α) protein or a nucleic acid encoding the DN-TNF- α protein.

BACKGROUND ART

[0003] Traumatic brain injury (TBI) is currently recognized as any disruption in the normal function of the brain that is caused by a bump, blow, or jolt to the head, or penetrating head injury. TBI can result when the head suddenly and violently hits an object or when an object pierces the skull and enters brain tissue. Symptoms of a TBI can be mild, moderate or severe, depending on the extent of damage to the brain. Mild cases may result in a brief change in mental state or consciousness, while severe cases may result in extended periods of unconsciousness, coma or even death.

[0004] It is estimated that about 1.7 million incidences of TBI occur in the U.S. every year. Approximately 5.3 million people live with a disability caused by TBI in the U.S. alone. These numbers are greatly expanded when considering the impact of TBI worldwide.

[0005] A computed tomography scan (CT or CAT scan) is the gold standard for the radiological assessment of a TBI patient. A CT scan is easy to perform and is an excellent test for detecting the presence of blood and fractures, which are the most crucial lesions to identify in medical trauma cases. Plain x-rays of the skull are recommended by some as a way to evaluate patients with only mild neurological dysfunction. However, most centers have readily available CT scanning, which is a more accurate test and therefore preferable to x-ray.

[0006] Many patients with moderate or severe head injuries are taken directly from the emergency room to the operating room. In many cases, surgery is performed to remove a large hematoma or contusion that is significantly compressing the brain or raising the pressure within the skull.

[0007] Those who survive a TBI may develop conditions that can be debilitating, long-lasting or even permanent.

[0008] Currently, there is no medication that can be given to mitigate the more serious damage that often occurs as a result of TBI, for example and without limitation, medication that prevents nerve damage or promotes nerve healing after TBI.

SUMMARY OF INVENTION

Technical Problem

[0009] Without pharmacotherapy intervention for mitigating the impact of TBI on a subject patient, a serious or potentially fatal outcome can occur.

Solution to Problem

[0010] The solution to the aforementioned problem, and other problems that would be appreciated by one having skill in the art, includes, *inter alia*: a method for treating a patient suffering from traumatic brain injury, comprising: administering to the patient a therapeutically effective amount of a selective inhibitor of solTNF- α , such as a DN-TNF- α protein and/or a nucleic acid encoding the DN-TNF- α protein, including the biologic known as XPRO1595. In other aspects, the solution may include a composition comprising a DN-TNF α protein and/or a nucleic acid encoding the DN-TNF- α protein for use in a method of treating traumatic brain injury in a patient, the method comprising: administering to the patient a therapeutically effective amount of the DN-TNF α protein and/or a nucleic acid encoding the DN-TNF- α protein, whereby the patient is treated.

[0011] Other features and aspects concerning the invention and/or solutions to the problems will be recognized by one having skill in the art upon a thorough review of the appended details and descriptions, in particular when reviewed in conjunction with the enclosed drawings.

Advantageous Effects of Invention

[0012] It was surprisingly discovered that selective neutralization of soluble tumor necrosis factor -alpha (solTNF α), by systemic administration of a DN-TNF- α protein following TBI in mice, achieved: (i) significantly reduced cortical and hippocampal inflammation, (ii) improved cortical sparing and associated neurological outcome, (iii) mitigation of injury induced by pathophysiological changes in the hippocampus, and (iv) attenuation of cognitive impairment, pain- and depressive-like behaviors. This discovery provides a reasonable basis to conclude that the solution as described herein, more particularly, a method for treating a patient suffering from traumatic brain injury, comprising: administering to the patient a therapeutically

effective amount of a selective inhibitor of solTNF- α , such as a DN-TNF- α protein and/or a nucleic acid encoding the DN-TNF- α protein, may be useful, with proper regulatory approval and subject to validation in clinical trials, for application in a human subject suffering from TBI.

BRIEF DESCRIPTION OF DRAWINGS

[0013] FIG. 1A shows the nucleic acid sequence of human TNF- α (SEQ ID NO:1). An additional six histidine codons, located between the start codon and the first amino acid, are underlined.

[0014] FIG. 1B shows the amino acid sequence of human TNF- α (SEQ ID NO:2) with an additional 6 histidines (underlined) between the start codon and the first amino acid. Amino acids changed in exemplary TNF- α variants are shown in bold.

[0015] FIG. 1C shows the amino acid sequence of human TNF- α (SEQ ID NO:3).

[0016] FIG. 2 shows the positions and amino acid changes in certain TNF- α variants.

[0017] Figures 3(A-B) show inflammatory response in the uninjured cortex two-weeks following TBI, wherein negligible GFAP protein (astrocytes) is expressed, independent of treatment.

[0018] Figure 3C shows inflammatory response in the injured cortex two-weeks following TBI, wherein injury upregulates GFAP protein (astrogliosis).

[0019] Figure 3D shows inflammatory response in the injured cortex two-weeks following TBI, wherein injury upregulates GFAP (astrogliosis), but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0020] Figure 3E shows inflammatory response in the injured hippocampus two-weeks following TBI, wherein injury upregulates GFAP (astrogliosis).

[0021] Figure 3F shows inflammatory response in the injured hippocampus two-weeks following TBI, wherein injury upregulates GFAP (astrogliosis), but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0022] Figures 3(G-H) show inflammatory response in the uninjured cortex two-weeks following TBI, wherein negligible IBA-1 protein (microglial reactivity) is expressed, independent of treatment.

[0023] Figure 3I shows inflammatory response in the injured cortex two-weeks following TBI, wherein injury upregulates IBA-1 (microglial reactivity).

[0024] Figure 3J shows inflammatory response in the injured cortex two-weeks following TBI, wherein injury upregulates IBA-1 (microglial reactivity), but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0025] Figure 3K shows inflammatory response in the injured hippocampus two-weeks following TBI, wherein injury upregulates IBA-1 (microglial reactivity).

[0026] Figure 3L shows inflammatory response in the injured hippocampus two-weeks following TBI, wherein injury upregulates IBA-1 (microglial reactivity), but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0027] Figure 3M shows glial reactivity (peri-lesional GFAP) in the ipsilateral and contralateral hemisphere following TBI.

[0028] Figure 3N shows glial reactivity (hippocampal GFAP) in the ipsilateral and contralateral hemisphere following TBI.

[0029] Figure 3O shows glial reactivity (peri-lesional IBA-1) in the ipsilateral and contralateral hemisphere following TBI.

[0030] Figure 3P shows glial reactivity (hippocampal IBA-1) in the ipsilateral and contralateral hemisphere following TBI.

[0031] Figure 4A shows the amount of cortical tissue loss after TBI.

[0032] Figure 4B shows systemic XPRO1595 treatment reduces the amount of cortical tissue loss relative to Vehicle treatment.

[0033] Figure 4C shows a plot summarizing spared cortical tissue according to that of Figures 4A and 4B.

[0034] Figure 4D shows a plot illustrating time to fall (seconds) pre- and post- TBI injury; wherein when assessed on the accelerating rotarod, the injured mice systemically treated with XPRO1595 take longer to fall off the rotating rod, and therefore have better motor-function than Vehicle-treated injured mice.

[0035] Figure 4E shows XPRO1595-treated injured mice also performed better than Vehicle-treated injured mice, after XPRO1595 or Vehicle was infused into the lateral ventricles of mice immediately following TBI.

[0036] Figure 5A shows Thy1-YFP mouse hippocampus showing dentate gyrus (DG), CA1, CA2 and CA3 regions.

[0037] Figure 5B shows neurons from CA1 region in naïve mice show extensive dendritic spines, whereas TBI promotes spine regression.

[0038] Figure 5C shows spine regression due to TBI reduced post-synaptic protein expression (PSD-95), which was rescued by XPRO1595 treatment.

- [0039] Figure 5D shows XPRO1595 rescued LTP in hippocampal slices.
- [0040] Figure 5E shows doublecortin immunoreactivity reveals long, thin and straight primary dendrites, within the dentate gyrus, which degenerate (promotion of beading) in vehicle-treated injured mice, but wherein systemic treatment with XPRO1595 attenuates this upregulation
- [0041] Figure 5F shows XPRO1595 treatment significantly reduced the amount of hippocampal dendritic beading, compared to vehicle-treated injured mice
- [0042] Figure 6A shows a plot indicating cognitive abilities in the Morris water maze test (latency (seconds) to find the platform) in both vehicle- and XPRO1595-treated mice post-TBI (day 7 thru day 11). XPRO1595 treatment improves learning.
- [0043] Figure 6B shows a plot indicating cognitive abilities in the Morris water maze test (time in platform quadrant (seconds)) in both vehicle- and XPRO1595-treated mice post-TBI (day 7 thru day 11). XPRO1595 treatment improves learning.
- [0044] Figure 6C shows a plot indicating cognitive abilities in the Morris water maze probe trial (time in platform quadrant (seconds)) in both vehicle- and XPRO1595-treated mice post-TBI (day 11 and day 14). XPRO1595 treatment has a tendency to improve learning.
- [0045] Figure 6D shows a plot indicating cognitive abilities in the Morris water maze probe trial (number of quadrants entered (integer)) in both vehicle- and XPRO1595-treated mice post-TBI (day 11 and day 14).
- [0046] Figure 6E shows a plot indicating depressive-like behavior (% sucrose preference) in vehicle- and XPRO1595-treated mice post-TBI (baseline injury, and days 3, 7, and 14). XPRO1595 treatment prevents the onset of depressive-like behavior following TBI.
- [0047] Figure 6F shows a plot indicating hindpaw mechanical hypersensitivity (threshold) in sham- and TBI-, vehicle- and XPRO1595-treated mice post-TBI (baseline injury, and days 3, 7, and 14). XPRO1595 treatment prevents the progression of hindpaw hypersensitivity following TBI.
- [0048] Figure 7A shows a plot indicating hindpaw mechanical hypersensitivity (% baseline) for both vehicle- and XPRO1595-treatment (ventricle infusion) in mice both pre-injury and post-injury (day 17).
- [0049] Figure 7B shows a comparison of contralateral and ipsilateral images for investigating neural activation in somatosensory cortex by labeling c-Fos protein using fluorescent IHC two weeks post-TBI in vehicle- and XPRO1595-treated mice (ventricle infusion).

DESCRIPTION OF EMBODIMENTS

[0050] Disclosed herein is the novel and unexpected finding that selective neutralization of soluble tumor necrosis factor -alpha (solTNF α), by systemic administration of a DN-TNF- α protein following TBI in mice, achieved: (i) significantly reduced cortical and hippocampal inflammation, (ii) improved cortical sparing and associated neurological outcome, (iii) mitigation of injury induced by pathophysiological changes in the hippocampus, and (iv) attenuation of cognitive impairment, pain- and depressive-like behaviors. A method that applies this unexpected finding is disclosed, and comprises the step of: administering, to a subject suffering from a traumatic brain injury, a therapeutically effective amount of a selective inhibitor of solTNF- α , such as a DN-TNF- α protein or a nucleic acid encoding the DN-TNF- α protein, for example, the DN-TNF- α protein known as XPRO1595.

Selective inhibitors of Soluble Tumor Necrosis Factor

[0051] Proteins with TNF- α antagonist activity, and nucleic acids encoding these proteins, were previously discovered which function to inhibit or otherwise neutralize the soluble form of TNF- α (solTNF- α) without inhibiting transmembrane TNF- α (tmTNF- α); collectively these proteins and nucleic acids encoding these proteins are herein collectively referred to as “selective inhibitors of solTNF- α ”.

[0052] Examples of selective inhibitors of solTNF- α are disclosed in US 7,056,695; US 7,101,974; US 7,144,987; US 7,244,823; US 7,446,174; US 7,662,367; and US 7,687,461; the entire contents of each of which is hereby incorporated by reference.

[0053] Preferred selective inhibitors of solTNF- α may be dominant negative TNF- α proteins, referred to herein as “DNTNF- α ,” “DN-TNF- α proteins,” “TNF α variants,” “TNF α variant proteins,” “variant TNF- α ,” “variant TNF- α ,” and the like. By “variant TNF- α ” or “TNF- α proteins” is meant TNF α or TNF- α proteins that differ from the corresponding wild type protein by at least 1 amino acid. Thus, a variant of human TNF- α is compared to SEQ ID NO:1 (nucleic acid including codons for 6 histidines), SEQ ID NO:2 (amino acid including 6 N-terminal histidines) or SEQ ID NO:3 (amino acid without 6 N-terminal histidines). DN-TNF- α proteins are disclosed in detail in U.S. Pat. No. 7,446,174, which is incorporated herein in its entirety by reference. As used herein variant TNF- α or TNF- α proteins include TNF- α monomers, dimers or trimers. Included within the definition of “variant TNF- α ” are competitive inhibitor TNF- α variants. While certain variants as described herein, one having skill in the art will understand that other variants may be made while retaining the function of inhibiting soluble but not transmembrane TNF- α .

[0054] Thus, the proteins useful in various aspects of the invention are antagonists of wild type TNF- α . By “antagonists of wild type TNF- α ” is meant that the variant TNF- α protein inhibits or significantly decreases at least one biological activity of wild-type TNF- α .

[0055] In a preferred embodiment the variant is antagonist of soluble TNF- α , but does not significantly antagonize transmembrane TNF- α , e.g., DN-TNF- α protein as disclosed herein inhibits signaling by soluble TNF- α , but not transmembrane TNF- α . By “inhibits the activity of TNF- α ” and grammatical equivalents is meant at least a 10% reduction in wild-type, soluble TNF- α , more preferably at least a 50% reduction in wild-type, soluble TNF- α activity, and even more preferably, at least 90% reduction in wild-type, soluble TNF- α activity. Preferably there is an inhibition in wild-type soluble TNF- α activity in the absence of reduced signaling by transmembrane TNF- α . In a preferred embodiment, the activity of soluble TNF- α is inhibited while the activity of transmembrane TNF- α is substantially and preferably completely maintained.

[0056] The TNF proteins useful in various embodiments of the invention have modulated activity as compared to wild type proteins. In a preferred embodiment, variant TNF- α proteins exhibit decreased biological activity (e.g. antagonism) as compared to wild type TNF- α , including but not limited to, decreased binding to a receptor (p55, p75 or both), decreased activation and/or ultimately a loss of cytotoxic activity. By “cytotoxic activity” herein refers to the ability of a TNF- α variant to selectively kill or inhibit cells. Variant TNF- α proteins that exhibit less than 50% biological activity as compared to wild type are preferred. More preferred are variant TNF- α proteins that exhibit less than 25%, even more preferred are variant proteins that exhibit less than 15%, and most preferred are variant TNF- α proteins that exhibit less than 10% of a biological activity of wild-type TNF- α . Suitable assays include, but are not limited to, caspase assays, TNF- α cytotoxicity assays, DNA binding assays, transcription assays (using reporter constructs), size exclusion chromatography assays and radiolabeling/immuno-precipitation, and stability assays (including the use of circular dichroism (CD) assays and equilibrium studies), according to methods known in the art.

[0057] In one embodiment, at least one property critical for binding affinity of the variant TNF- α proteins is altered when compared to the same property of wild type TNF- α and in particular, variant TNF- α proteins with altered receptor affinity are preferred. Particularly preferred are variant TNF- α with altered affinity toward oligomerization to wild type TNF- α . Thus, the invention makes use of variant TNF- α proteins with altered binding affinities such that the variant TNF- α proteins will preferentially oligomerize with wild type TNF- α , but do not substantially interact with wild type TNF receptors, i.e., p55, p75. “Preferentially” in this

case means that given equal amounts of variant TNF- α monomers and wild type TNF- α monomers, at least 25% of the resulting trimers are mixed trimers of variant and wild type TNF- α , with at least about 50% being preferred, and at least about 80-90% being particularly preferred. In other words, it is preferable that the variant TNF- α proteins implemented in embodiments of the invention have greater affinity for wild type TNF- α protein as compared to wild type TNF- α proteins. By “do not substantially interact with TNF receptors” is meant that the variant TNF- α proteins will not be able to associate with either the p55 or p75 receptors to significantly activate the receptor and initiate the TNF signaling pathway(s). In a preferred embodiment, at least a 50% decrease in receptor activation is seen, with greater than 50%, 75%, 80-90% being preferred.

[0058] In some embodiments, the variants of the invention are antagonists of both soluble and transmembrane TNF- α . However, as described herein, preferred variant TNF- α proteins are antagonists of the activity of soluble TNF- α but do not substantially affect the activity of transmembrane TNF- α . Thus, a reduction of activity of the heterotrimers for soluble TNF- α is as outlined above, with reductions in biological activity of at least 10%, 25, 50, 75, 80, 90, 95, 99 or 100% all being preferred. However, some of the variants outlined herein comprise selective inhibition; that is, they inhibit soluble TNF- α activity but do not substantially inhibit transmembrane TNF- α . In these embodiments, it is preferred that at least 80%, 85, 90, 95, 98, 99 or 100% of the transmembrane TNF- α activity is maintained. This may also be expressed as a ratio; that is, selective inhibition can include a ratio of inhibition of soluble to transmembrane TNF- α . For example, variants that result in at least a 10:1 selective inhibition of soluble to transmembrane TNF- α activity are preferred, with 50:1, 100:1, 200:1, 500:1, 1000:1 or higher find particular use in the invention. Thus, one embodiment utilizes variants, such as double mutants at positions 87/145 as outlined herein, that substantially inhibit or eliminate soluble TNF- α activity (for example by exchanging with homotrimeric wild-type to form heterotrimers that do not bind to TNF- α receptors or that bind but do not activate receptor signaling) but do not significantly affect (and preferably do not alter at all) transmembrane TNF- α activity. Without being bound by theory, the variants exhibiting such differential inhibition allow the decrease of inflammation without a corresponding loss in immune response.

[0059] In one embodiment, the affected biological activity of the variants is the activation of receptor signaling by wild type TNF- α proteins. In a preferred embodiment, the variant TNF- α protein interacts with the wild type TNF- α protein such that the complex comprising the variant TNF- α and wild type TNF- α has reduced capacity to activate (as

outlined above for “substantial inhibition”), and in preferred embodiments is incapable of activating, one or both of the TNF receptors, i.e. p55 TNF-R (TNFR1) or p75 TNF-R (TNFR2). In a preferred embodiment, the variant TNF- α protein is a variant TNF- α protein that functions as an antagonist of wild type TNF- α . Preferably, the variant TNF- α protein preferentially interacts with wild type TNF- α to form mixed trimers with the wild type protein such that receptor binding does not significantly occur and/or TNF- α signaling is not initiated. By mixed trimers is meant that monomers of wild type and variant TNF- α proteins interact to form heterotrimeric TNF- α . Mixed trimers may comprise 1 variant TNF- α protein:2 wild type TNF- α proteins, 2 variant TNF- α proteins:1 wild type TNF- α protein. In some embodiments, trimers may be formed comprising only variant TNF- α proteins.

[0060] The variant TNF- α antagonist proteins implemented in embodiments of the invention are highly specific for TNF- α antagonism relative to TNF-beta antagonism. Additional characteristics include improved stability, pharmacokinetics, and high affinity for wild type TNF- α . Variants with higher affinity toward wild type TNF- α may be generated from variants exhibiting TNF- α antagonism as outlined above.

[0061] Similarly, variant TNF- α proteins, for example are experimentally tested and validated in *in vivo* and in *in vitro* assays. Suitable assays include, but are not limited to, activity assays and binding assays. For example, TNF- α activity assays, such as detecting apoptosis via caspase activity can be used to screen for TNF- α variants that are antagonists of wild type TNF- α . Other assays include using the Sytox green nucleic acid stain to detect TNF-induced cell permeability in an Actinomycin-D sensitized cell line. As this stain is excluded from live cells, but penetrates dying cells, this assay also can be used to detect TNF- α variants that are agonists of wild-type TNF- α . By “agonists of wild type TNF- α ” is meant that the variant TNF- α protein enhances the activation of receptor signaling by wild type TNF- α proteins. Generally, variant TNF- α proteins that function as agonists of wild type TNF- α are not preferred. However, in some embodiments, variant TNF- α proteins that function as agonists of wild type TNF- α protein are preferred. An example of an NF kappaB assay is presented in Example 7 of U.S. Pat. No. 7,446,174, which is expressly incorporated herein by reference.

[0062] In a preferred embodiment, binding affinities of variant TNF- α proteins as compared to wild type TNF- α proteins for naturally occurring TNF- α and TNF receptor proteins such as p55 and p75 are determined. Suitable assays include, but are not limited to, e.g., quantitative comparisons comparing kinetic and equilibrium binding constants, as are known in the art. Examples of binding assays are described in Example 6 of U.S. Pat. No. 7,446,174, which is expressly incorporated herein by reference.

[0063] In a preferred embodiment, the variant TNF- α protein has an amino acid sequence that differs from a wild type TNF- α sequence by at least 1 amino acid, with from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 amino acids all contemplated, or higher. Expressed as a percentage, the variant TNF- α proteins of the invention preferably are greater than 90% identical to wild-type, with greater than 95, 97, 98 and 99% all being contemplated. Stated differently, based on the human TNF- α sequence of FIG. 1B (SEQ ID NO:2) excluding the N-terminal 6 histidines, as shown in FIG. 1C (SEQ ID NO:3), variant TNF- α proteins have at least about 1 residue that differs from the human TNF- α sequence, with at least about 2, 3, 4, 5, 6, 7 or 8 different residues. Preferred variant TNF- α proteins have 3 to 8 different residues.

[0064] A % amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the “longer” sequence in the aligned region. The “longer” sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored). In a similar manner, “percent (%) nucleic acid sequence identity” with respect to the coding sequence of the polypeptides identified is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the coding sequence of the cell cycle protein. A preferred method utilizes the BLASTN module of WU-BLAST-2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively.

[0065] TNF- α proteins may be fused to, for example, other therapeutic proteins or to other proteins such as Fc or serum albumin for therapeutic or pharmacokinetic purposes. In this embodiment, a TNF- α protein implemented in embodiments of the invention is operably linked to a fusion partner. The fusion partner may be any moiety that provides an intended therapeutic or pharmacokinetic effect. Examples of fusion partners include but are not limited to Human Serum Albumin, a therapeutic agent, a cytotoxic or cytotoxic molecule, radionucleotide, and an Fc, etc. As used herein, an Fc fusion is synonymous with the terms “immunoadhesin”, “Ig fusion”, “Ig chimera”, and “receptor globulin” as used in the prior art (Chamow et al., 1996, Trends Biotechnol 14:52-60; Ashkenazi et al., 1997, Curr Opin Immunol 9:195-200, both incorporated by reference). An Fc fusion combines the Fc region of an immunoglobulin with the target-binding region of a TNF- α protein, for example. See for example U.S. Pat. Nos. 5,766,883 and 5,876,969, both of which are hereby incorporated by reference.

[0066] In a preferred embodiment, the variant TNF- α proteins comprise variant residues selected from the following positions 21, 23, 30, 31, 32, 33, 34, 35, 57, 65, 66, 67, 69, 75, 84, 86, 87, 91, 97, 101, 111, 112, 115, 140, 143, 144, 145, 146, and 147. Preferred amino acids for each position, including the human TNF- α residues, are shown in FIG. 2. Thus, for

example, at position 143, preferred amino acids are Glu, Asn, Gln, Ser, Arg, and Lys; etc. Preferred changes include: V1M, Q21C, Q21 R, E23C, R31C, N34E, V91E, Q21R, N30D, R31C, R31I, R31D, R31E, R32D, R32E, R32S, A33E, N34E, N34V, A35S, D45C, L57F, L57W, L57Y, K65D, K65E, K65I, K65M, K65N, K65Q, K65T, K65S, K65V, K65W, G66K, G66Q, Q67D, Q67K, Q67R, Q67S, Q67W, Q67Y, C69V, L75E, L75K, L75Q, A84V, S86Q, S86R, Y87H, Y87R, V91E, I97R, I97T, C101A, A111R, A111E, K112D, K112E, Y115D, Y115E, Y115F, Y115H, Y115I, Y115K, Y115L, Y115M, Y115N, Y115Q, Y115R, Y115S, Y115T, Y115W, D140K, D140R, D143E, D143K, D143L, D143R, D143N, D143Q, D143R, D143S, F144N, A145D, A145E, A145F, A145H, A145K, A145M, A145N, A145Q, A145R, A145S, A145T, A145Y, E146K, E146L, E146M, E146N, E146R, E146S and S147R. These may be done either individually or in combination, with any combination being possible. However, as outlined herein, preferred embodiments utilize at least 1 to 8, and preferably more, positions in each variant TNF- α protein.

[0067] In an additional aspect, the invention provides TNF- α variants selected from the group consisting of XENP268 XENP344, XENP345, XENP346, XENP550, XENP551, XENP557, XENP1593, XENP1594, and XENP1595 as outlined in Example 3 OF U.S. Pat. No. 7,662,367, which is incorporated herein by reference.

[0068] In an additional aspect, the invention makes use of methods of forming a TNF- α heterotrimer *in vivo* in a mammal comprising administering to the mammal a variant TNF- α molecule as compared to the corresponding wild-type mammalian TNF- α , wherein said TNF- α variant is substantially free of agonistic activity.

[0069] In an additional aspect, the invention makes use of methods of screening for selective inhibitors comprising contacting a candidate agent with a soluble TNF- α protein and assaying for TNF- α biological activity; contacting a candidate agent with a transmembrane TNF- α protein and assaying for TNF- α biological activity, and determining whether the agent is a selective inhibitor. The agent may be a protein (including peptides and antibodies, as described herein) or small molecules.

[0070] In a further aspect, the invention makes use of variant TNF- α proteins that interact with the wild type TNF- α to form mixed trimers incapable of activating receptor signaling. Preferably, variant TNF- α proteins with 1, 2, 3, 4, 5, 6 and 7 amino acid changes are used as compared to wild type TNF- α protein. In a preferred embodiment, these changes are selected from positions 1, 21, 23, 30, 31, 32, 33, 34, 35, 57, 65, 66, 67, 69, 75, 84, 86, 87, 91, 97, 101, 111, 112, 115, 140, 143, 144, 145, 146 and 147. In an additional aspect, the non-naturally occurring variant TNF- α proteins have substitutions selected from the group of

substitutions consisting of: V1M, Q21C, Q21R, E23C, N34E, V91E, Q21R, N30D, R31C, R31I, R31D, R31E, R32D, R32E, R32S, A33E, N34E, N34V, A35S, D45C, L57F, L57W, L57Y, K65D, K65E, K65I, K65M, K65N, K65Q, K65T, K65S, K65V, K65W, G66K, G66Q, Q67D, Q67K, Q67R, Q67S, Q67W, Q67Y, C69V, L75E, L75K, L75Q, A84V, S86Q, S86R, Y87H, Y87R, V91E, I97R, I97T, C101A, A111R, A111E, K112D, K112E, Y115D, Y115E, Y115F, Y115H, Y115I, Y115K, Y115L, Y115M, Y115N, Y115Q, Y115R, Y115S, Y115T, Y115W, D140K, D140R, D143E, D143K, D143L, D143R, D143N, D143Q, D143R, D143S, F144N, A145D, A145E, A145F, A145H, A145K, A145M, A145N, A145Q, A145R, A145S, A145T, A145Y, E146K, E146L, E146M, E146N, E146R, E146S and S147R.

[0071] In another preferred embodiment, substitutions may be made either individually or in combination, with any combination being possible. Preferred embodiments utilize at least one, and preferably more, positions in each variant TNF- α protein. For example, substitutions at positions 31, 57, 69, 75, 86, 87, 97, 101, 115, 143, 145, and 146 may be combined to form double variants. In addition, triple, quadruple, quintuple and the like, point variants may be generated.

[0072] In one aspect the invention makes use of TNF- α variants comprising the amino acid substitutions A145R/I97T. In one aspect, the invention provides TNF- α variants comprising the amino acid substitutions V1M, R31C, C69V, Y87H, C101A, and A145R. In a preferred embodiment, this variant is PEGylated.

[0073] In a preferred embodiment the variant is XPRO1595, a PEGylated protein comprising V1M, R31C, C69V, Y87H, C101A, and A145R mutations relative to the wild type human sequence, also referred to as "XPro".

[0074] For purposes of the present invention, the areas of the wild type or naturally occurring TNF- α molecule to be modified are selected from the group consisting of the Large Domain (also known as II), Small Domain (also known as I), the DE loop, and the trimer interface. The Large Domain, the Small Domain and the DE loop are the receptor interaction domains. The modifications may be made solely in one of these areas or in any combination of these areas. The Large Domain preferred positions to be varied include: 21, 30, 31, 32, 33, 35, 65, 66, 67, 111, 112, 115, 140, 143, 144, 145, 146 and/or 147. For the Small Domain, the preferred positions to be modified are 75 and/or 97. For the DE Loop, the preferred position modifications are 84, 86, 87 and/or 91. The Trimer Interface has preferred double variants including positions 34 and 91 as well as at position 57. In a preferred embodiment, substitutions at multiple receptor interaction and/or trimerization domains may be combined. Examples include, but are not limited to, simultaneous substitution of amino acids at the large and small

domains (e.g. A145R and I97T), large domain and DE loop (A145R and Y87H), and large domain and trimerization domain (A145R and L57F). Additional examples include any and all combinations, e.g., I97T and Y87H (small domain and DE loop). More specifically, these variants may be in the form of single point variants, for example K112D, Y115K, Y115I, Y115T, A145E or A145R. These single point variants may be combined, for example, Y115I and A145E, or Y115I and A145R, or Y115T and A145R or Y115I and A145E; or any other combination.

[0075] Preferred double point variant positions include 57, 75, 86, 87, 97, 115, 143, 145, and 146; in any combination. In addition, double point variants may be generated including L57F and one of Y115I, Y115Q, Y115T, D143K, D143R, D143E, A145E, A145R, E146K or E146R. Other preferred double variants are Y115Q and at least one of D143N, D143Q, A145K, A145R, or E146K; Y115M and at least one of D143N, D143Q, A145K, A145R or E146K; and L57F and at least one of A145E or E146R; K65D and either D143K or D143R, K65E and either D143K or D143R, Y115Q and any of L75Q, L57W, L57Y, L57F, I97R, I97T, S86Q, D143N, E146K, A145R and I97T, A145R and either Y87R or Y87H; N34E and V91E; L75E and Y115Q; L75Q and Y115Q; L75E and A145R; and L75Q and A145R.

[0076] Further, triple point variants may be generated. Preferred positions include 34, 75, 87, 91, 115, 143, 145 and 146. Examples of triple point variants include V91E, N34E and one of Y115I, Y115T, D143K, D143R, A145R, A145E E146K, and E146R. Other triple point variants include L75E and Y87H and at least one of Y115Q, A145R. Also, L75K, Y87H and Y115Q. More preferred are the triple point variants V91E, N34E and either A145R or A145E.

[0077] Variant TNF- α proteins may also be identified as being encoded by variant TNF- α nucleic acids. In the case of the nucleic acid, the overall homology of the nucleic acid sequence is commensurate with amino acid homology but takes into account the degeneracy in the genetic code and codon bias of different organisms. Accordingly, the nucleic acid sequence homology may be either lower or higher than that of the protein sequence, with lower homology being preferred. In a preferred embodiment, a variant TNF- α nucleic acid encodes a variant TNF- α protein. As will be appreciated by those in the art, due to the degeneracy of the genetic code, an extremely large number of nucleic acids may be made, all of which encode the variant TNF- α proteins of the present invention. Thus, having identified a particular amino acid sequence, those skilled in the art could make any number of different nucleic acids, by simply modifying the sequence of one or more codons in a way which does not change the amino acid sequence of the variant TNF- α .

[0078] In one embodiment, the nucleic acid homology is determined through hybridization studies. Thus, for example, nucleic acids which hybridize under high stringency to the nucleic acid sequence shown in FIG. 1A (SEQ ID NO:1) or its complement and encode a variant TNF- α protein is considered a variant TNF- α gene. High stringency conditions are known in the art; see for example Maniatis et al., *Molecular Cloning: A Laboratory Manual*, 2d Edition, 1989, and *Short Protocols in Molecular Biology*, ed. Ausubel, et al., both of which are hereby incorporated by reference. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, “Overview of principles of hybridization and the strategy of nucleic acid assays” (1993), incorporated by reference. Generally, stringent conditions are selected to be about 5-10° C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m , 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C. for short probes (e.g. 10 to 50 nucleotides) and at least about 60° C for long probes (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. In another embodiment, less stringent hybridization conditions are used; for example, moderate or low stringency conditions may be used, as are known in the art; see Maniatis and Ausubel, *supra*, and Tijssen, *supra*. In addition, nucleic acid variants encode TNF- α protein variants comprising the amino acid substitutions described herein. In one embodiment, the TNF- α variant encodes a polypeptide variant comprising the amino acid substitutions A145R/197T. In one aspect, the nucleic acid variant encodes a polypeptide comprising the amino acid substitutions V1M, R31C, C69V, Y87H, C101A, and A145R, or any 1, 2, 3, 4 or 5 of these variant amino acids.

[0079] The variant TNF- α proteins and nucleic acids of the present invention are recombinant. As used herein, “nucleic acid” may refer to either DNA or RNA, or molecules which contain both deoxy- and ribonucleotides. The nucleic acids include genomic DNA, cDNA and oligonucleotides including sense and anti-sense nucleic acids. Such nucleic acids may also contain modifications in the ribose-phosphate backbone to increase stability and half-

life of such molecules in physiological environments. The nucleic acid may be double stranded, single stranded, or contain portions of both double stranded or single stranded sequence. As will be appreciated by those in the art, the depiction of a single strand (“Watson”) also defines the sequence of the other strand (“Crick”); thus, the sequence depicted in FIG. 1A (SEQ ID NO:1) also includes the complement of the sequence. By the term “recombinant nucleic acid” is meant nucleic acid, originally formed *in vitro*, in general, by the manipulation of nucleic acid by endonucleases, in a form not normally found in nature. Thus, an isolated variant TNF- α nucleic acid, in a linear form, or an expression vector formed *in vitro* by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention.

[0080] By “vector” is meant any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

[0081] It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e. using the *in vivo* cellular machinery of the host cell rather than *in vitro* manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention.

[0082] Similarly, a “recombinant protein” is a protein made using recombinant techniques, i.e. through the expression of a recombinant nucleic acid as depicted above. A recombinant protein is distinguished from naturally occurring protein by at least one or more characteristics. For example, the protein may be isolated or purified away from some or all of the proteins and compounds with which it is normally associated in its wild-type host, and thus may be substantially pure. For example, an isolated protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of the total protein in a given sample. A substantially pure protein comprises at least about 75% by weight of the total protein, with at least about 80% being preferred, and at least about 90% being particularly preferred. The definition includes the production of a variant TNF- α protein from one organism in a different organism or host cell. Alternatively, the protein may be made at a significantly higher concentration than is normally seen, through the use of an inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. Furthermore, all of the variant TNF- α proteins outlined herein are in a form not normally found

in nature, as they contain amino acid substitutions, insertions and deletions, with substitutions being preferred, as discussed below.

[0083] Also included within the definition of variant TNF- α proteins of the present invention are amino acid sequence variants of the variant TNF- α sequences outlined herein and shown in the Figures. That is, the variant TNF- α proteins may contain additional variable positions as compared to human TNF- α . These variants fall into one or more of three classes: substitutional, insertional or deletional variants.

[0084] Amino acid substitutions are typically of single residues; insertions usually will be on the order of from about 1 to 20 amino acids, although considerably larger insertions may be tolerated. Deletions range from about 1 to about 20 residues, although in some cases deletions may be much larger.

[0085] Using the nucleic acids disclosed herein, which encode a variant TNF- α protein, a variety of expression vectors are made. The expression vectors may be either self-replicating extrachromosomal vectors or vectors which integrate into a host genome. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the variant TNF- α protein. The term “control sequences” refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0086] Nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation.

[0087] In a preferred embodiment, when the endogenous secretory sequence leads to a low level of secretion of the naturally occurring protein or of the variant TNF- α protein, a replacement of the naturally occurring secretory leader sequence is desired. In this embodiment, an unrelated secretory leader sequence is operably linked to a variant TNF- α encoding nucleic acid leading to increased protein secretion. Thus, any secretory leader sequence resulting in enhanced secretion of the variant TNF- α protein, when compared to the secretion of TNF- α and its secretory sequence, is desired. Suitable secretory leader sequences that lead to the secretion of a protein are known in the art. In another preferred embodiment, a

secretory leader sequence of a naturally occurring protein or a protein is removed by techniques known in the art and subsequent expression results in intracellular accumulation of the recombinant protein.

[0088] Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. The transcriptional and translational regulatory nucleic acid will generally be appropriate to the host cell used to express the fusion protein; for example, transcriptional and translational regulatory nucleic acid sequences from *Bacillus* are preferably used to express the fusion protein in *Bacillus*. Numerous types of appropriate expression vectors, and suitable regulatory sequences are known in the art for a variety of host cells.

[0089] In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In a preferred embodiment, the regulatory sequences include a promoter and transcriptional start and stop sequences. Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention. In a preferred embodiment, the promoters are strong promoters, allowing high expression in cells, particularly mammalian cells, such as the CMV promoter, particularly in combination with a Tet regulatory element.

[0090] In addition, the expression vector may comprise additional elements. For example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences that flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

[0091] In addition, in a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well

known in the art and will vary with the host cell used. A preferred expression vector system is a retroviral vector system such as is generally described in PCT/US97/01019 and PCT/US97/01048, both of which are hereby incorporated by reference. In a preferred embodiment, the expression vector comprises the components described above and a gene encoding a variant TNF- α protein. As will be appreciated by those in the art, all combinations are possible and accordingly, as used herein, the combination of components, comprised by one or more vectors, which may be retroviral or not, is referred to herein as a “vector composition”.

[0092] A number of viral based vectors have been used for gene delivery. See for example U.S. Pat. No. 5,576,201, which is expressly incorporated herein by reference. For example, retroviral systems are known and generally employ packaging lines which have an integrated defective provirus (the “helper”) that expresses all of the genes of the virus but cannot package its own genome due to a deletion of the packaging signal, known as the psi sequence. Thus, the cell line produces empty viral shells. Producer lines can be derived from the packaging lines which, in addition to the helper, contain a viral vector, which includes sequences required in cis for replication and packaging of the virus, known as the long terminal repeats (LTRs). The gene of interest can be inserted in the vector and packaged in the viral shells synthesized by the retroviral helper. The recombinant virus can then be isolated and delivered to a subject. (See, e.g., U.S. Pat. No. 5,219,740.) Representative retroviral vectors include but are not limited to vectors such as the LHL, N2, LNSAL, LSHL and LHL2 vectors described in e.g., U.S. Pat. No. 5,219,740, incorporated herein by reference in its entirety, as well as derivatives of these vectors. Retroviral vectors can be constructed using techniques well known in the art. See, e.g., U.S. Pat. No. 5,219,740; Mann et al. (1983) Cell 33:153-159.

[0093] Adenovirus based systems have been developed for gene delivery and are suitable for delivery according to the methods described herein. Human adenoviruses are double-stranded DNA viruses that enter cells by receptor-mediated endocytosis. These viruses are particularly well suited for gene transfer because they are easy to grow and manipulate and they exhibit a broad host range *in vivo* and *in vitro*.

[0094] Adenoviruses infect quiescent as well as replicating target cells. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis. The virus is easily produced at high titers and is stable so that it can be purified and stored. Even in the replication-competent form, adenoviruses cause only low-level morbidity and are not associated with human malignancies. Accordingly, adenovirus vectors have been developed which make use of these

advantages. For a description of adenovirus vectors and their uses see, e.g., Haj-Ahmad and Graham (1986) *J. Virol.* 57:267-274; Bett et al. (1993) *J. Virol.* 67:5911-5921; Mittereder et al. (1994) *Human Gene Therapy* 5:717-729; Seth et al. (1994) *J. Virol.* 68:933-940; Barr et al. (1994) *Gene Therapy* 1:51-58; Berkner, K. L. (1988) *BioTechniques* 6:616-629; Rich et al. (1993) *Human Gene Therapy* 4:461-476.

[0095] In a preferred embodiment, the viral vectors used in the subject methods are AAV vectors. By an "AAV vector" is meant a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. Typical AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes, but retain functional flanking ITR sequences. Functional ITR sequences are necessary for the rescue, replication and packaging of the AAV virion. An AAV vector includes at least those sequences required in cis for replication and packaging (e.g., functional ITRs) of the virus. The ITRs need not be the wild-type nucleotide sequences, and may be altered, e.g., by the insertion, deletion or substitution of nucleotides, so long as the sequences provide for functional rescue, replication and packaging. For more on various AAV serotypes, see for example Cearley et al., *Molecular Therapy*, 16:1710-1718, 2008, which is expressly incorporated herein in its entirety by reference.

[0096] AAV expression vectors may be constructed using known techniques to provide as operatively linked components in the direction of transcription, control elements including a transcriptional initiation region, the DNA of interest and a transcriptional termination region. The control elements are selected to be functional in a thalamic and/or cortical neuron. Additional control elements may be included. The resulting construct, which contains the operatively linked components is bounded (5' and 3') with functional AAV ITR sequences.

[0097] By "adeno-associated virus inverted terminal repeats" or "AAV ITRs" is meant the art-recognized regions found at each end of the AAV genome which function together in cis as origins of DNA replication and as packaging signals for the virus. AAV ITRs, together with the AAV rep coding region, provide for the efficient excision and rescue from, and integration of a nucleotide sequence interposed between two flanking ITRs into a mammalian cell genome.

[0098] The nucleotide sequences of AAV ITR regions are known. See, e.g., Kotin, R. M. (1994) *Human Gene Therapy* 5:793-801; Berns, K. I. "Parvoviridae and their Replication" in *Fundamental Virology*, 2nd Edition, (B. N. Fields and D. M. Knipe, eds.) for the AAV-2 sequence. As used herein, an "AAV ITR" need not have the wild-type nucleotide sequence depicted, but may be altered, e.g., by the insertion, deletion or substitution of nucleotides.

Additionally, the AAV ITR may be derived from any of several AAV serotypes, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. Furthermore, 5' and 3' ITRs which flank a selected nucleotide sequence in an AAV vector need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, i.e., to allow for excision and rescue of the sequence of interest from a host cell genome or vector, and to allow integration of the heterologous sequence into the recipient cell genome when AAV Rep gene products are present in the cell.

[0099] Suitable DNA molecules for use in AAV vectors will include, for example, a gene that encodes a protein that is defective or missing from a recipient subject or a gene that encodes a protein having a desired biological or therapeutic effect (e.g., an enzyme, or a neurotrophic factor). The artisan of reasonable skill will be able to determine which factor is appropriate based on the neurological disorder being treated.

[0100] The selected nucleotide sequence is operably linked to control elements that direct the transcription or expression thereof in the subject *in vivo*. Such control elements can comprise control sequences normally associated with the selected gene. Alternatively, heterologous control sequences can be employed. Useful heterologous control sequences generally include those derived from sequences encoding mammalian or viral genes. Examples include, but are not limited to, the SV40 early promoter, mouse mammary tumor virus LTR promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, synthetic promoters, hybrid promoters, and the like. In addition, sequences derived from nonviral genes, such as the murine metallothionein gene, will also find use herein. Such promoter sequences are commercially available.

[0101] Once made, the TNF- α protein may be covalently modified. For instance, a preferred type of covalent modification of variant TNF- α comprises linking the variant TNF- α polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol ("PEG"), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337, incorporated by reference. These nonproteinaceous polymers may also be used to enhance the variant TNF- α 's ability to disrupt receptor binding, and/or *in vivo* stability. In another preferred embodiment, cysteines are designed into variant or wild type TNF- α in order to incorporate (a) labeling sites for characterization and (b) incorporate PEGylation sites. For example, labels that may be used are well known in the art and include but are not limited to biotin, tag and fluorescent labels

(e.g. fluorescein). These labels may be used in various assays as are also well known in the art to achieve characterization. A variety of coupling chemistries may be used to achieve PEGylation, as is well known in the art. Examples include but are not limited to, the technologies of Shearwater and Enzon, which allow modification at primary amines, including but not limited to, lysine groups and the N-terminus See, Kinstler et al, Advanced Drug Deliveries Reviews, 54, 477-485 (2002) and M J Roberts et al, Advanced Drug Delivery Reviews, 54, 459-476 (2002), both hereby incorporated by reference.

[0102] In one preferred embodiment, the optimal chemical modification sites are 21, 23, 31 and 45, taken alone or in any combination. In an even more preferred embodiment, a TNF- α variant of the present invention includes the R31C mutation.

[0103] In a preferred embodiment, the variant TNF- α protein is purified or isolated after expression. Variant TNF- α proteins may be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample.

[0104] In another preferred embodiment, the TNF- α protein is administered via gene modified autologous or allogeneic cellular therapy, wherein the gene therapy comprises mesenchymal stem cells expressing a construct of the TNF- α protein, preferably a DN-TNF- α protein, more preferably XPRO1595.

[0105] Treatment Methods

[0106] The terms “treatment”, “treating”, and the like, as used herein include amelioration or elimination of a disease or condition once it has been established or alleviation of the characteristic symptoms of such disease or condition. A method as disclosed herein may also be used to, depending on the condition of the patient, prevent the onset of a disease or condition or of symptoms associated with a disease or condition, including reducing the severity of a disease or condition or symptoms associated therewith prior to affliction with said disease or condition. Such prevention or reduction prior to affliction refers to administration of the compound or composition as described herein to a patient that is not at the time of administration afflicted with the disease or condition. “Preventing” also encompasses preventing the recurrence or relapse-prevention of a disease or condition or of symptoms associated therewith, for instance after a period of improvement.

[0107] In one embodiment, a selective inhibitor of solTNF- α as described herein is administered peripherally to a patient in need thereof to reduce inflammation, improve cortical sparing and associated neurological outcome, mitigate injury induced by pathophysiological

changes in the hippocampus, and/or attenuate cognitive impairment, pain- and depressive-like behaviors.

[0108] In one embodiment, the treatment method includes administering to the patient suffering from traumatic brain injury a therapeutically effective amount of a selective inhibitor of solTNF- α , such as a DN-TNF- α protein and/or a nucleic acid encoding the DN-TNF- α protein, for example and without limitation, the biologic known as XPRO1595, whereby the patient is treated.

[0109] In some embodiments, the method may comprise subcutaneous injection of the selective inhibitor of solTNF- α for treatment of traumatic brain injury.

[0110] In another embodiment, the method may comprise intravenous administration of the selective inhibitor of solTNF- α for treatment of traumatic brain injury.

[0111] In an alternative embodiment the method may comprise topical administration of a selective inhibitor of solTNF- α as described herein. In this embodiment the DN-TNF- α may be formulated as a lotion or cream.

[0112] Other methods of administration are further described herein or may be appreciated by one having skill in the art.

Formulations

[0113] Depending upon the manner of introduction, the pharmaceutical composition may be formulated in a variety of ways. The concentration of the therapeutically active variant TNF- α protein in the formulation may vary from about 0.1 to 100 weight %. In another preferred embodiment, the concentration of the variant TNF- α protein is in the range of 0.003 to 1.0 molar, with dosages from 0.03, 0.05, 0.1, 0.2, and 0.3 millimoles per kilogram of body weight being preferred.

[0114] The pharmaceutical compositions for use in embodiments of the present invention comprise a variant TNF- α protein in a form suitable for administration to a patient. In the preferred embodiment, the pharmaceutical compositions are in a water-soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid,

methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

[0115] Pharmaceutical compositions are contemplated wherein a TNF- α variant of the present invention and one or more therapeutically active agents are formulated. Formulations of the present invention are prepared for storage by mixing TNF- α variant having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980, incorporated entirely by reference), in the form of lyophilized formulations or aqueous solutions. Lyophilization is well known in the art, see, e.g., U.S. Pat. No. 5,215,743, incorporated entirely by reference. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as histidine, phosphate, citrate, acetate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; sweeteners and other flavoring agents; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; additives; coloring agents; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS® or polyethylene glycol (PEG). In a preferred embodiment, the pharmaceutical composition that comprises the TNF- α variant of the present invention may be in a water-soluble form. The TNF- α variant may be present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid

addition salt” refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. “Pharmaceutically acceptable base addition salts” include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. The formulations to be used for *in vivo* administration are preferably sterile. This is readily accomplished by filtration through sterile filtration membranes or other methods.

Controlled Release

[0116] In addition, any of a number of delivery systems are known in the art and may be used to administer TNF- α variants in accordance with embodiments of the present invention. Examples include, but are not limited to, encapsulation in liposomes, microparticles, microspheres (e.g. PLA/PGA microspheres), and the like. Alternatively, an implant of a porous, non-porous, or gelatinous material, including membranes or fibers, may be used. Sustained release systems may comprise a polymeric material or matrix such as polyesters, hydrogels, poly(vinylalcohol), polylactides, copolymers of L-glutamic acid and ethyl-L-gutamate, ethylene-vinyl acetate, lactic acid-glycolic acid copolymers such as the LUPRON DEPOT®, and poly-D-(-)-3-hydroxybutyric acid. It is also possible to administer a nucleic acid encoding the TNF- α of the current invention, for example by retroviral infection, direct injection, or coating with lipids, cell surface receptors, or other transfection agents. In all cases, controlled release systems may be used to release the TNF- α at or close to the desired location of action.

[0117] The pharmaceutical compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers such as NaOAc; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and

other flavoring agents; coloring agents; and polyethylene glycol. Additives are well known in the art, and are used in a variety of formulations. In a further embodiment, the variant TNF- α proteins are added in a micellular formulation; see U.S. Pat. No. 5,833,948, incorporated entirely by reference. Alternatively, liposomes may be employed with the TNF- α proteins to effectively deliver the protein. Combinations of pharmaceutical compositions may be administered. Moreover, the TNF- α compositions of the present invention may be administered in combination with other therapeutics, either substantially simultaneously or co-administered, or serially, as the need may be.

[0118] Dosage forms for the topical or transdermal administration of a DN-TNF- α protein disclosed herein include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The DN-TNF- α protein may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required. Powders and sprays can contain, in addition to the DN-TNF- α protein, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Methods of Administration

[0119] The administration of the selective inhibitor of solTNF- α in accordance with embodiments of the present invention, preferably in the form of a sterile aqueous solution, is done peripherally, in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, the selective inhibitor of solTNF- α may be directly applied as a solution, salve, cream or spray. The selective inhibitor of solTNF- α may also be delivered by bacterial or fungal expression into the human system (e.g., WO 04046346 A2, hereby incorporated by reference).

[0120] Subcutaneous

[0121] Subcutaneous administration may be preferable in some circumstances because the patient may self-administer the pharmaceutical composition. Many protein therapeutics are not sufficiently potent to allow for formulation of a therapeutically effective dose in the maximum acceptable volume for subcutaneous administration. This problem may be addressed in part by the use of protein formulations comprising arginine-HCl, histidine, and polysorbate.

A selective inhibitor of solTNF- α may be more amenable to subcutaneous administration due to, for example, increased potency, improved serum half-life, or enhanced solubility.

[0122] Intravenous

[0123] As is known in the art, protein therapeutics are often delivered by IV infusion or bolus. The selective inhibitor of solTNF- α may also be delivered using such methods. For example, administration may be by intravenous infusion with 0.9% sodium chloride as an infusion vehicle.

[0124] Inhaled

[0125] Pulmonary delivery may be accomplished using an inhaler or nebulizer and a formulation comprising an aerosolizing agent. For example, inhalable technology, or a pulmonary delivery system may be used. The selective inhibitor of solTNF- α may be more amenable to intrapulmonary delivery. The selective inhibitor of solTNF- α may also be more amenable to intrapulmonary administration due to, for example, improved solubility or altered isoelectric point.

[0126] Oral Delivery

[0127] Furthermore, the selective inhibitor of solTNF- α may be more amenable to oral delivery due to, for example, improved stability at gastric pH and increased resistance to proteolysis.

[0128] Transdermal

[0129] Transdermal patches may have the added advantage of providing controlled delivery of the selective inhibitor of solTNF- α to the body. Dissolving or dispersing DN-TNF- α protein in the proper medium can make such dosage forms. Absorption enhancers can also be used to increase the flux of DN-TNF- α protein across the skin. Either providing a rate controlling membrane or dispersing DN-TNF- α protein in a polymer matrix or gel can control the rate of such flux.

[0130] Intraocular

[0131] Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being suitable for use in embodiments of this invention.

[0132] In a preferred embodiment, the selective inhibitor of solTNF- α is administered as a therapeutic agent, and can be formulated as outlined above. Similarly, variant TNF- α genes (including both the full-length sequence, partial sequences, or regulatory sequences of the variant TNF- α coding regions) may be administered in gene therapy applications, as is known in the art. These variant TNF- α genes can include antisense applications, either as gene therapy

(i.e. for incorporation into the genome) or as antisense compositions, as will be appreciated by those in the art.

[0133] Dosage

[0134] Dosage may be determined depending on the complication being treated and mechanism of delivery. Typically, an effective amount of the selective inhibitor of solTNF, sufficient for achieving a therapeutic or prophylactic effect, range from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Suitably, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 2000 mg per kilogram body weight per day. An exemplary treatment regime entails administration once every day or once a week or once a month. A DN-TNF protein may be administered on multiple occasions. Intervals between single dosages can be daily, weekly, monthly or yearly. Alternatively, A DN-TNF protein may be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the agent in the subject. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some subjects continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[0135] Toxicity

[0136] Suitably, an effective amount (e.g., dose) of a DN-TNF protein described herein will provide therapeutic benefit without causing substantial toxicity to the subject. Toxicity of the agent described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD50 (the dose lethal to 50% of the population) or the LD100 (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the agent described herein lies suitably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the

individual physician in view of the subject's condition. See, e.g., Fingl et al., In: The Pharmacological Basis of Therapeutics, Ch. 1 (1975).

Exemplary Features and Embodiments of the Invention

[0137] Certain preferred embodiments can be summarized as follows:

[0138] A method is disclosed for treating a subject suffering from traumatic brain injury, the method comprising: administering to the subject a therapeutically effective amount of a selective inhibitor of solTNF- α , whereby the subject is treated; for purposes herein, this shall constitute “the method”.

[0139] The selective inhibitor of solTNF- α may comprise a DN-TNF- α protein or a nucleic acid encoding the DN-TNF- α protein.

[0140] The DN-TNF- α protein may comprise XPRO1595. Thus, in an embodiment, the method may comprise administering XPRO1595 in a dose between 0.1 mg/kg and 10.0 mg/kg.

[0141] The DN-TNF- α protein can be administered: intravenously; subcutaneously; orally; via aerosol; via topical application; or via gene therapy. The gene therapy may comprise mesenchymal stem cells expressing a construct of the DN-TNF- α protein. The DN-TNF- α protein can be administered via gene modified autologous or allogeneic cellular therapy.

EXAMPLES

Example 1: Neutralization of solTNF- α Reduces Cortical and Hippocampal Inflammation

Following TBI in Mice

[0142] Brain inflammation following TBI is known to promote a plethora of undesirable pathologies including neuronal cell death, degeneration and aberrant neural plasticity. The pro-inflammatory cytokine solTNF- α plays a key role in promoting these pathologies by activating TNFR1, which not only enhances the inflammatory response, but also directly promotes these events. To determine whether Neutralization of solTNF- α reduces brain inflammation, mice received a TBI (CCI model), followed sixty-minutes later by the administration of XPRO1595 (10mg/kg S.C., twice weekly) or vehicle. After a two-week survival period, we assessed glial reactivity by immunohistochemically labelling cortical and hippocampal tissue with antibodies against GFAP (astrocytes) and IBA-1 (microglial/macrophage). Following semi-quantitation of protein immunoreactivity using NIH ImageJ software, only minimal GFAP and IBA-1 reactivity was observed in the uninjured hemisphere, independent of treatment (See for example, Figures 3A, B, G & H: contralateral

hippocampus not shown). In comparison, GFAP and IBA-1 are all upregulated in the cortical peri-lesional region and hippocampus in the vehicle-treated injured mice (See for example, Figures 3C, E, I, K, M-P). Systemic treatment with XPRO1595 significantly reduced astrocytic and microglial reactivity in the cortical peri-lesional region and hippocampus (See for example, Figures 3D, F, J, L, M-P), indicating that administration of XPRO1595 prevents brain inflammation following TBI.

[0143] Referring to the drawings, Figures 3(A-B) show inflammatory response in the uninjured cortex two-weeks following TBI, wherein negligible GFAP protein (astrocytes) is expressed, independent of treatment.

[0144] Figure 3C shows inflammatory response in the injured cortex two-weeks following TBI, wherein injury upregulates GFAP protein (astrogliosis).

[0145] Figure 3D shows inflammatory response in the injured cortex two-weeks following TBI, wherein injury upregulates GFAP protein (astrogliosis), but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0146] Figure 3E shows inflammatory response in the injured hippocampus two-weeks following TBI, wherein injury upregulates GFAP protein (astrogliosis).

[0147] Figure 3F shows inflammatory response in the injured hippocampus two-weeks following TBI, wherein injury upregulates GFAP protein (astrogliosis), but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0148] Figures 3(G-H) show inflammatory response in the uninjured cortex two-weeks following TBI, wherein negligible IBA-1 protein (microglial activity) is expressed.

[0149] Figure 3I shows inflammatory response in the injured cortex two-weeks following TBI, wherein injury upregulates IBA-1 (microglial reactivity).

[0150] Figure 3J shows inflammatory response in the injured cortex two-weeks following TBI, wherein injury upregulates IBA-1 (microglial reactivity), but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0151] Figure 3K shows inflammatory response in the injured hippocampus two-weeks following TBI, wherein injury upregulates IBA-1 (microglial reactivity).

[0152] Figure 3L shows inflammatory response in the injured hippocampus two-weeks following TBI, wherein injury upregulates IBA-1 (microglial reactivity), but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0153] Figure 3M shows glial reactivity (peri-lesional GFAP) in the ipsilateral and contralateral hemisphere following TBI.

[0154] Figure 3N shows glial reactivity (hippocampal GFAP) in the ipsilateral and contralateral hemisphere following TBI.

[0155] Figure 3O shows glial reactivity (peri-lesional IBA-1) in the ipsilateral and contralateral hemisphere following TBI.

[0156] Figure 3P shows glial reactivity (hippocampal IBA-1) in the ipsilateral and contralateral hemisphere following TBI.

Example 2: Neutralization of solTNF- α Improves Cortical Sparing and Associated Neurological Outcome Following TBI in Mice

[0157] To assess whether neutralization of solTNF- α can prevent neuronal cell death and thus improve tissue viability, solTNF- α was selectively inhibited by systematically administering XPRO1595 beginning sixty-minutes following TBI for a period of two-weeks. Coronal serial brain sections forty microns (40 μ m) thick were then cut and stained with the DNA dye DAPI to show the location of intact cortical tissue. Two weeks following TBI it was observed that XPRO1595 therapy prevented tissue loss at the cortical injury site, compared to vehicle-treated injured mice, resulting in a significant increase in the amount of spared cortical tissue (See for example, Figures 4A-C). Since the injury site overlies the motor cortex, the animals' motor function was also assessed using the accelerating rotarod. Prior to injury, the mice can maintain their ability to walk on the accelerating rod for up to 220-240 seconds (Figure 4D), although TBI renders the mice less able to walk on the accelerating rod, resulting in them falling off sooner. In comparison, the XPRO1595-treated injured group do not exhibit a motor deficit following the injury and are significantly better able to walk on the accelerating rotarod than the vehicle-treated injured group. Collectively, this data suggests that treating the mice systemically with XPRO1595 improves the cortical injury site pathophysiology leading to improved motor skills.

[0158] To confirm that XPRO1595 is acting directly on the brain to regulate motor function, XPRO1595 or vehicle was infused into the lateral ventricle of the brain immediately following a TBI. Seventeen days following the injury, the motor performance of the mice was assessed on the accelerating rotarod. XPRO1595-treated injured mice had significantly improved motor performance, compared to vehicle-treated injured mice (Figure 4E), suggesting that XPRO1595 is having a direct effect on the brain to regulate motor performance.

[0159] With reference to the drawings, Figures 4(A-B) show images of serial sections through an injury site from individual mice, wherein it is observed that controlled cortical injury (CCI) at a velocity of 3.0m/s and a depth of 0.5mm results in a small peri-lesional cavity.

[0160] Figure 4A shows the amount of cortical tissue loss after TBI.

[0161] Figure 4B shows systemic XPRO1595 treatment reduces the amount of cortical tissue loss relative to Vehicle treatment.

[0162] Figure 4C shows a plot summarizing spared cortical tissue according to that of Figures 4A and 4B.

[0163] Figure 4D shows a plot illustrating time to fall (seconds) pre- and post- TBI injury; wherein when assessed on the accelerating rotarod, the injured mice systemically treated with XPRO1595 take longer to fall off the rotating rod, and therefore have better motor-function than Vehicle-treated injured mice.

[0164] Figure 4E shows XPRO1595-treated injured mice also performed better than Vehicle-treated injured mice, after XPRO1595 or Vehicle was infused into the lateral ventricles of mice immediately following TBI. This result confirms the brain-specific effect of TBI.

Example 3: Neutralization of $\text{solTNF-}\alpha$ Prevents Hippocampal Dendritic Degeneration and Altered Plasticity

[0165] Since hippocampal inflammation, specifically TNF/TNFR1 activity, promotes pathophysiological changes in the hippocampus, it was investigated whether XPRO1595 treatment can prevent injury-induced by these pathophysiological changes. Thy-1 YFP mice display auto-fluorescent labelling of hippocampal neurons (Figure 5A), which in the CA1 regions of naïve mice have extensive dendritic spines (Figure 5B), although the number of spines is drastically reduced in the injured brain three-days after injury. This reduction corresponds with reduced PSD-95 expression seventy-two hours following TBI as identified using Western blotting (Figure 5C), but can be rescued by systemically treating the mice sixty minutes following injury with XPRO1595, and leads to significant improvements in the long-term potentiation of neurons in the CA1 region (Figure 5D: mPSC rise and decay times seventy-two hours after injury; electrophysiology preliminary data n=1 animal per group). Within the DG, doublecortin-immunoreactive immature neurons in the naïve mouse appear long and curvy without beading (Figure 5E), while vehicle-treated injured mice displayed extensive dendritic degeneration (i.e. beading) two weeks following injury. The number of dendritic beads were markedly reduced by treating mice systemically with XPRO1595 treatment (Figures 5E and 5F). Therefore, the use of XPRO1595 is shown to be associated with improved hippocampal-related neurological outcomes following TBI.

[0166] With reference to the drawings, Figure 5A shows Thy1-YFP mouse hippocampus showing dentate gyrus (DG), CA1, CA2 and CA3 regions.

[0167] Figure 5B shows neurons from CA1 region in naïve mice show extensive dendritic spines, whereas TBI promotes spine regression.

[0168] Figure 5C shows spine regression due to TBI reduced post-synaptic protein expression (PSD-95), which was rescued by XPRO1595 treatment.

[0169] Figure 5D shows XPRO1595 rescued LTP in hippocampal slices.

[0170] Figure 5E shows doublecortin immunoreactivity reveals long, thin and straight primary dendrites, within the dentate gyrus, which degenerate (promotion of beading) in vehicle-treated injured mice, but wherein systemic treatment with XPRO1595 attenuates this upregulation.

[0171] Figure 5F shows XPRO1595 treatment significantly reduced the amount of hippocampal dendritic beading, compared to vehicle-treated injured mice.

Example 4: Neutralization of $\text{solTNF-}\alpha$ Improves Hippocampal-Related Neurological Outcomes (attenuation of cognitive impairment, pain- and depressive-like behaviors) Following TBI in Mice

[0172] One of the most well-characterized neurological symptoms associated with hippocampal injury is cognitive impairment related to learning and memory. A common behavioral test to quantitate learning and memory deficits in rodents following TBI is the Morris water maze (MWM). Here the MWM was used to assess the level of cognitive impairment seven-days post-TBI. It was observed that systemic XPRO1595 treatment significantly improved the ability of injured mice to learn the task (Figures 6(A-B)), and showed a trend to memory improvement three-days following completion of the training phase (Figure 6C). This data indicates that XPRO1595 is a significant modulator of cognitive ability, which is likely due to reduced inflammation resulting in less aberrant hippocampal pathophysiology.

[0173] As well as cognitive impairment, depression is frequently associated with aberrant hippocampal pathology following TBI, therefore the development of depressive-like symptoms in injured mice was assessed. For this the sucrose preference test was utilized, where mice are given free access to both water and a 2% sucrose solution overnight, and the volume of sucrose drunk is assessed as a percentage of total liquid drunk. As expected, prior to injury the naïve uninjured untreated mice showed ~ 90% preference for the sucrose solution (Figure 6E), but this was significantly reduced in vehicle-treated mice acutely following injury, suggesting the CCI brain injury model promotes depressive-like symptoms. Conversely, treating injured mice systemically with XPRO1595 prevented a reduction in their preference

for the 2% sucrose solution, suggesting that XPRO1595 may affect the onset of depressive-like symptoms, and may be a useful clinical tool.

[0174] Since depression and pain are frequently comorbid following TBI, and chronic pain has links to hippocampal inflammation (TNF/TNFR1 activation), the mechanical von Frey assay was used to assess hindpaw hypersensitivity, as a marker of pain in mice. This is an especially useful test because individuals with injury-induced chronic pain are also at increased risk of developing dermal mechanical and thermal hypersensitivity. Both sham and injured adult mice were systemically treated with either XPRO1595 or vehicle twice a week for two-weeks starting sixty-minutes following TBI, and underwent hindpaw von Frey testing prior to injury and on post-injury days 3, 7 and 14. It was observed that all sham injured animals, independent of treatment, experienced a small increase in hypersensitivity three-days following injury (indicated by reduced mechanical threshold: Figure 6F), but this was transient and quickly returned to baseline levels. In comparison, vehicle-treated injured mice were significantly more hypersensitive on day three (almost a 3-fold increase compared to vehicle-treated sham mice), and this remained until the end of testing on day fourteen, suggesting these mice were in more pain, which lasted longer than sham-operated mice. Conversely, XPRO1595-treated injured mice experienced less hypersensitivity than vehicle-treated injured mice (1.6-fold increase compared to XPRO1595-treated sham mice), and this quickly improved such that they were no longer experiencing significantly more hypersensitivity at the end of testing. This indicates that using XPRO1595 therapy can reduce levels of pain associated with brain injury.

[0175] Here, it is shown that XPRO1595 therapy improves hippocampal functions following TBI in mice.

[0176] With reference to the drawings, Figure 6A shows a plot indicating latency (seconds) to find platform in both Vehicle- and XPRO1595-treated mice post-TBI (day 7 thru day 11). XPRO1595 treatment improves learning.

[0177] Figure 6B shows a plot indicating time in platform quadrant (seconds) in both Vehicle- and XPRO1595-treated mice post-TBI (day 7 thru day 11). XPRO1595 treatment improves learning.

[0178] Figure 6C shows a plot indicating cognitive abilities in the Morris water maze test (time in platform quadrant (seconds)) in both Vehicle- and XPRO1595-treated mice post-TBI (day 11 and day 14). XPRO1595 treatment has a tendency to improve learning.

[0179] Figure 6D shows a plot indicating cognitive abilities in the Morris water maze probe trial (number of quadrants entered (integer)) in both Vehicle- and XPRO1595-treated mice post-TBI (day 11 and day 14).

[0180] Figure 6E shows a plot indicating depressive-like behavior (% sucrose preference) in Vehicle- and XPRO1595-treated mice post-TBI (baseline injury, and days 3, 7, and 14). XPRO1595 treatment prevent the onset of depressive-like behavior following TBI.

[0181] Figure 6F shows a plot indicating hindpaw mechanical hypersensitivity threshold) in sham- and TBI-, Vehicle- and XPRO1595-treated mice post-TBI (baseline injury, and days 3, 7, and 14). XPRO1595 treatment prevents the progression of hindpaw hypersensitivity following TBI.

Example 5: Neutralization of α TNF Modulates the Brain's Perception of Pain

[0182] Since TNF can regulate peripheral nociceptor activation, as well as central hippocampal control of pain perception, it was investigated whether systemic XPRO1595 therapy is primarily acting centrally or peripherally to reduce injury-induced pain. For this, XPRO1595 or vehicle was infused directly into the lateral ventricles of the brain following TBI, and neuropathic pain testing was performed similar to that performed following systemic administration. It was observed that vehicle-treated injured mice had significantly greater mechanical hypersensitivity (Figure 7A), compared to baseline testing. Interestingly, infusing XPRO1595 into the lateral ventricles following an injury prevented this effect, suggesting a significant component of the increased dermal hypersensitivity following TBI could be due to pain perception within the brain.

[0183] It was next investigated whether XPRO1595 treatment can regulate neuronal activation in the somatosensory cortex, where the perception of pain is processed. In mice systemically treated with either XPRO1595 or vehicle, the somatosensory cortex was immunohistochemically examined to observe c-Fos expression, as a marker of neural activity in that region. Brain injury is known to cause a loss of input to the somatosensory cortex, resulting in hyper-excitability of neurons (homeostatic activity regulation), which increases the number of labelled c-Fos-positive neurons. Consistent with this, an extensive number of c-Fos-positive cells were observed in layers II and III of the ipsilateral and contralateral somatosensory cortex of vehicle-treated injured mice (indicated by black arrows in Figure 7B). In contrast, no labelled cells were detected in the somatosensory cortex of XPRO1595-treated mice. As a positive control for the immunohistochemistry technique, c-Fos was constitutively expressed in stem cells of the hippocampal DG in all tissue examined (images not shown).

[0184] The ability of XPRO1595 infused directly into the brain to improve pain outcomes (Figure 7A), and the reduction in c-Fos activity in the somatosensory cortex of XPro1595-treated injured mice (Figure 7B), suggests XPRO1595 may regulate pain perception by preserving hippocampal-cortical connectivity, potentially by preventing both dendritic degeneration and synaptic plasticity.

Conclusion

[0185] Following a short sixty-minute delay after TBI, XPro1595 was administered systemically and reduced inflammation (glial reactivity) was observed in cortical and subcortical brain regions, along with improved cortical tissue sparing (equates to improved neuronal survival) and reduced hippocampal dendritic degeneration and plasticity. This improved pathophysiology lead to significant improvements in motor function, learning and memory, depressive-like behavior, and pain perception. .

INDUSTRIAL APPLICABILITY

[0186] The invention finds utility in the treatment of traumatic brain injury and is therefore applicable to the medical field.

CLAIMS

1. A method for treating a subject suffering from traumatic brain injury, comprising:
administering to the subject a therapeutically effective amount of a selective inhibitor
of solTNF- α , whereby the subject is treated.
2. The method of claim 1, wherein the selective inhibitor of solTNF- α comprises a DN-
TNF- α protein and/or a nucleic acid encoding the DN-TNF- α protein.
3. The method of claim 2, wherein the DN-TNF- α protein comprises XPRO1595.
4. The method of claim 3, wherein the method comprises administering XPRO1595 in a
dose between 0.1 mg/kg and 10.0 mg/kg.
5. The method of claim 2, wherein the DN-TNF- α protein is administered:
intravenously; subcutaneously; orally; via aerosol; via topical application; or via gene
therapy.
6. The method of claim 5, wherein the gene therapy comprises mesenchymal stem cells
expressing a construct of the DN-TNF- α protein.
7. The method of claim 2, wherein the DN-TNF- α protein is administered via gene
modified autologous or allogeneic cellular therapy.
8. A composition comprising selective inhibitor of solTNF- α for use in a method of
treating a subject suffering from traumatic brain injury, the method comprising: administering
to the subject a therapeutically effective amount of the selective inhibitor of solTNF- α ,
whereby the subject is treated.
9. The composition of claim 8, wherein the selective inhibitor of solTNF- α comprises a
DN-TNF- α protein and/or a nucleic acid encoding the DN-TNF- α protein.
10. The composition of claim 9, wherein the DN-TNF- α protein comprises XPRO1595.

11. The composition of claim 10, wherein the method comprises administering XPRO1595 in a dose between 0.1 mg/kg and 10.0 mg/kg.
12. The composition of claim 9, wherein the DN-TNF- α protein is administered: intravenously; subcutaneously; orally; via aerosol; via topical application; or via gene therapy.
13. The composition of claim 12, wherein the gene therapy comprises mesenchymal stem cells expressing a construct of the DN-TNF- α protein.
14. The composition of claim 12, wherein the DN-TNF- α protein is administered via gene modified autologous or allogeneic cellular therapy.

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1   atgcaccacc accaccacca cgtacgctcc tcctcccgca ctccgtccga caaaccggta
61  gctcacgtag tagctaaccg gcaggctgaa ggtcagctgc agtggctgaa ccgccgcgct
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361 tgggtacgaac cgatctacct ggggtggtga ttccagctgg aaaaagggtga ccgcctgtcc
421 gctgaaatca accgcccgga ctacctggac ttcgctgaat ccggtcaggt atacttcggt
481 atcatcgctc tgtga
```

(SEQ ID NO:1)

FIG.1A

```
1   MHHHHHHVRS SSRTPSDKPV AHVVANPQAE GQLQWLNRRRA NALLANGVEL RDNQLVVPSE
61  GLYLIYSQVL FKGQGCPSSTH VLLTHTISRI AVSYQTKVNL LSAIKSPCQR ETPEGAEAKP
121 WYEPIYLGGV FQLEKGDRLS AEINRPDYLD FAESGQVYFG IIAL
```

(SEQ ID NO:2)

FIG.1B

Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro Gln Ala Glu
Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp
Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln
Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys
Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro
Trp Tyr Glu Pro Ile Thr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile
Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu

(SEQ ID NO:3)

FIG.1C

Wild-type TNF amino acid	Wild-type TNF amino acid number	Mutants
Q	21	R
N	30	D
R	31	I, D, E
R	32	D, E, S
A	33	E
A	35	S
K	65	D, T, M, W, I, Q, S, N, V, E
G	66	Q, K
Q	67	D, W, Y, R, K, S
A	111	R, E
K	112	D, E
Y	115	Q, K, E, N, R, F, H, M, L, I, W, D, T, S
D	140	R, K
D	143	E, N, Q, S, R, K
F	144	N
A	145	R, D, K, N, H, T, Q, E, Y, M, S, F
E	146	N, K, R, S
S	147	R

FIG. 2

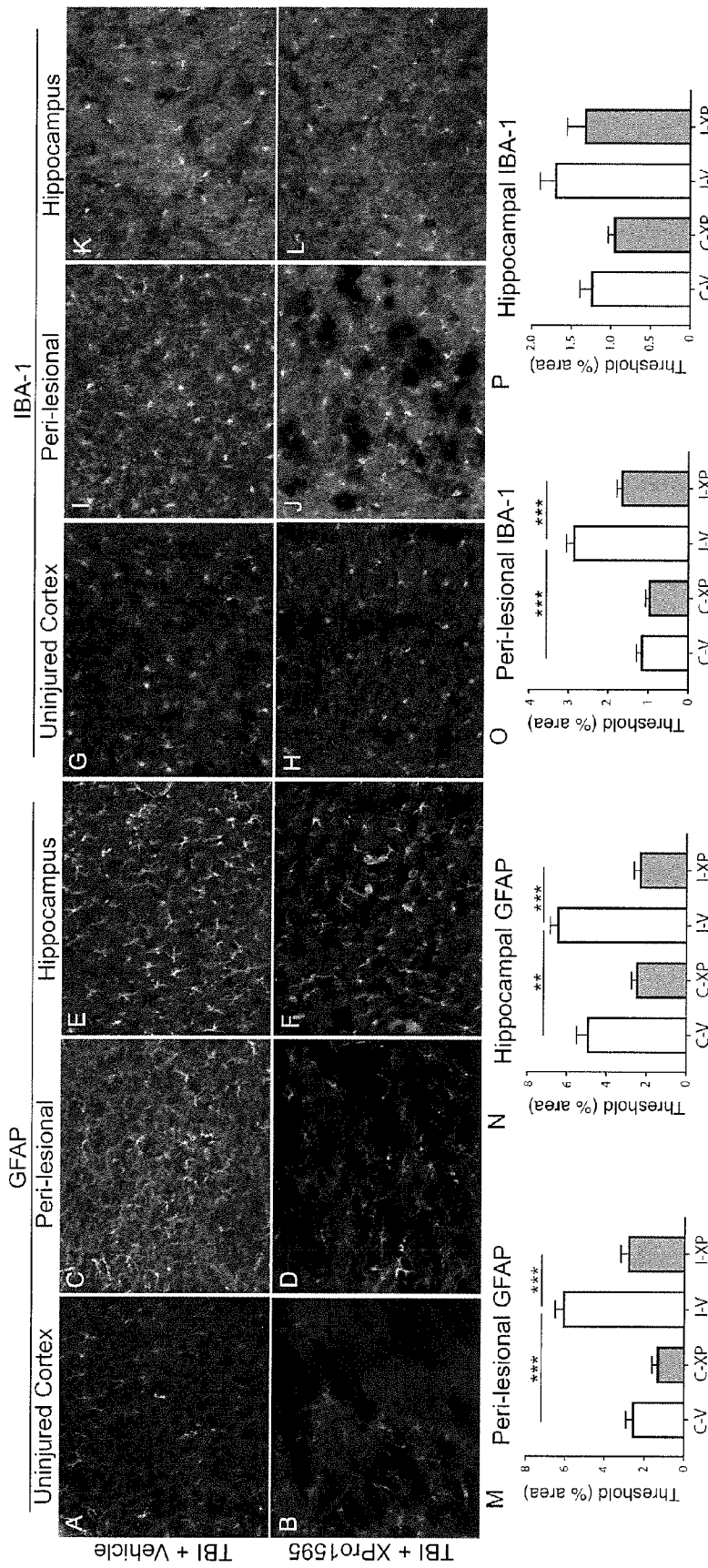


Fig. 3. Inflammatory response in the cortex and hippocampus two weeks following TBI. Negligible GFAP (astrocytes) and IBA-1 (microglia/macrophages) protein is expressed in the uninjured contralateral cortex (A,B,G&H), and hippocampus (images not shown), independent of treatment. Injury upregulates gliosis in both the cortex and hippocampus (C,E,I&K), although systemic treatment with XPro1595 (10 mg/kg) 60 minutes following injury attenuates this upregulation (D, F, J & L). Graphs show semiquantitation of glial reactivity in the ipsilateral and contralateral hemisphere following TBI (M-P). C-V = contralateral hemisphere + vehicle; C-XP = contralateral hemisphere + XPro1595; I-V = ipsilateral hemisphere + vehicle; I-XP = ipsilateral hemisphere + XPro1595; n=9, XPro1595 treated n=8; O&P all groups n=9. *** = $p < 0.001$, ** = $p < 0.01$.

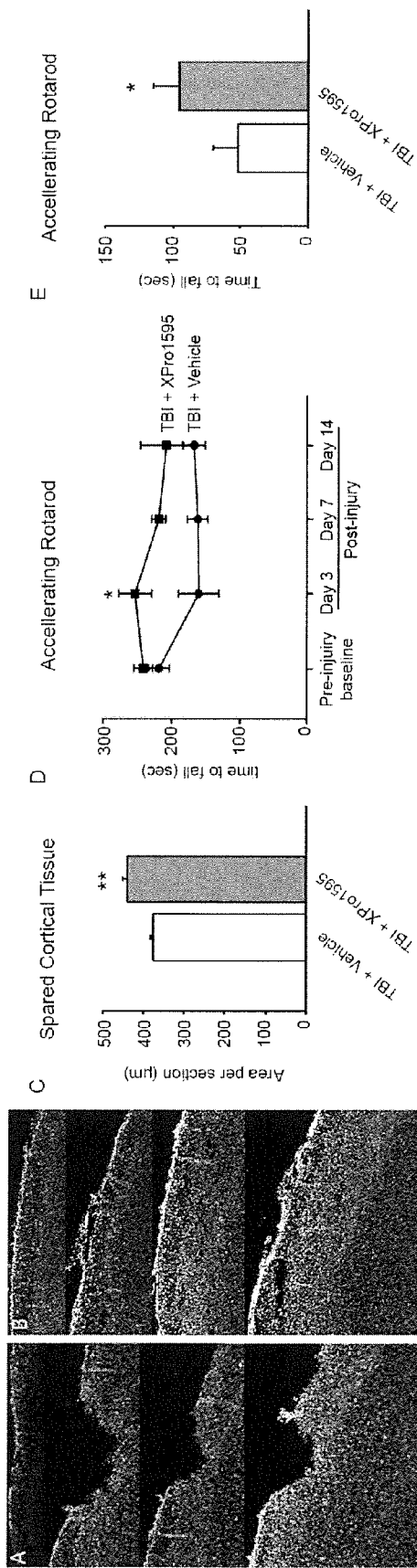


Fig 4. Effect of XPro1595 therapy on cortical injury neuropathology following TBI in mice. Controlled cortical injury (CCI) at a velocity of 3.0m/s and a depth of 0.5mm results in a small peri-lesional cavity (A&C; images shown are serial sections through the injury site from individual mice). Systemic XPro1595 treatment reduces the amount of cortical tissue loss (B&C). When assessed on the accelerating rotarod, the injured mice systemically treated with XPro1595 take longer to fall off the rotating rod, and therefore have better motor function than vehicle-treated injured mice (D). To confirm this is a brain-specific effect, XPro1595 or vehicle was infused into the lateral ventricles of mice immediately following TBI, and showed XPro1595-treated mice also performed better than vehicle-treated injured mice (E). * = $p < 0.05$ and ** = $p < 0.01$.

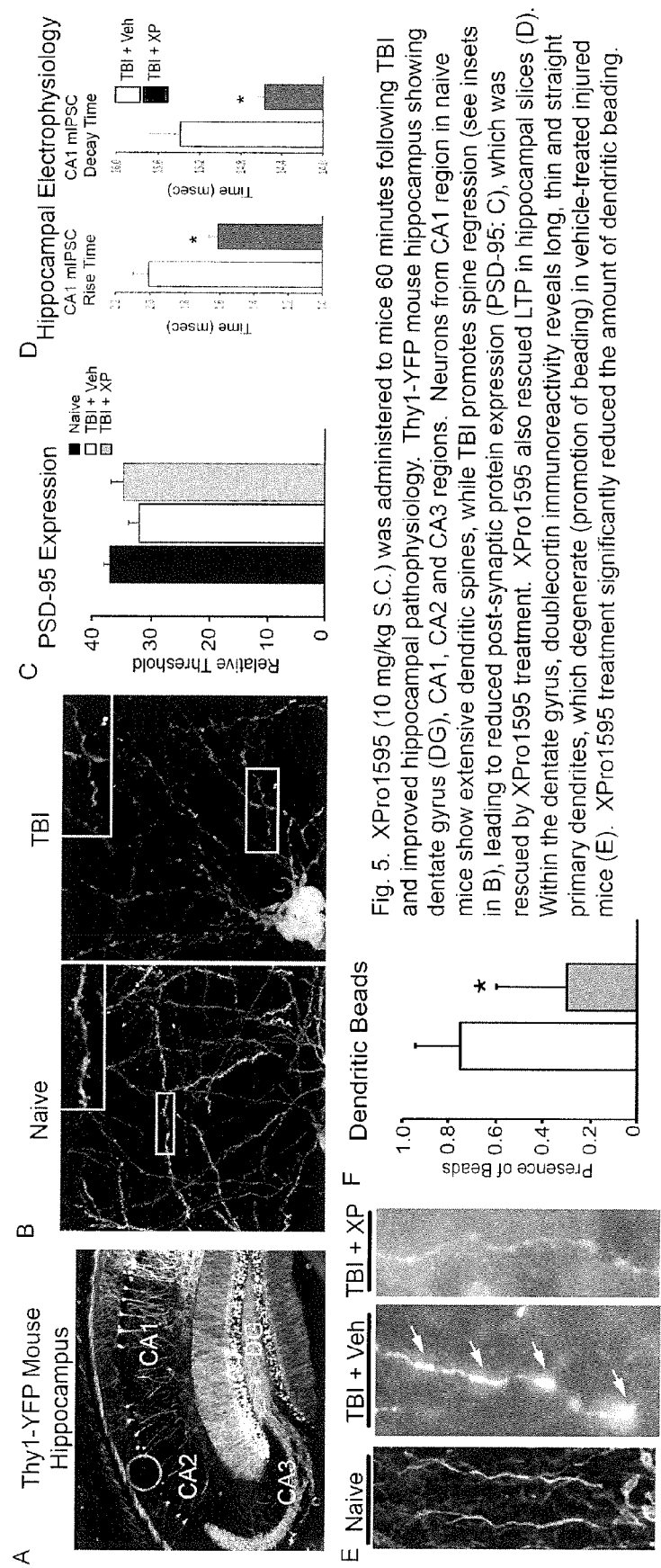


Fig. 5. XPro1595 (10 mg/kg S.C.) was administered to mice 60 minutes following TBI and improved hippocampal pathophysiology. Thy1-YFP mouse hippocampus showing dentate gyrus (DG), CA1, CA2 and CA3 regions. Neurons from CA1 region in naive mice show extensive dendritic spines, while TBI promotes spine regression (see insets in B), leading to reduced post-synaptic protein expression (PSD-95: C), which was rescued by XPro1595 treatment. XPro1595 also rescued LTP in hippocampal slices (D). Within the dentate gyrus, doublecortin immunoreactivity reveals long, thin and straight primary dendrites, which degenerate (promotion of beading) in vehicle-treated injured mice (E). XPro1595 treatment significantly reduced the amount of dendritic beading.

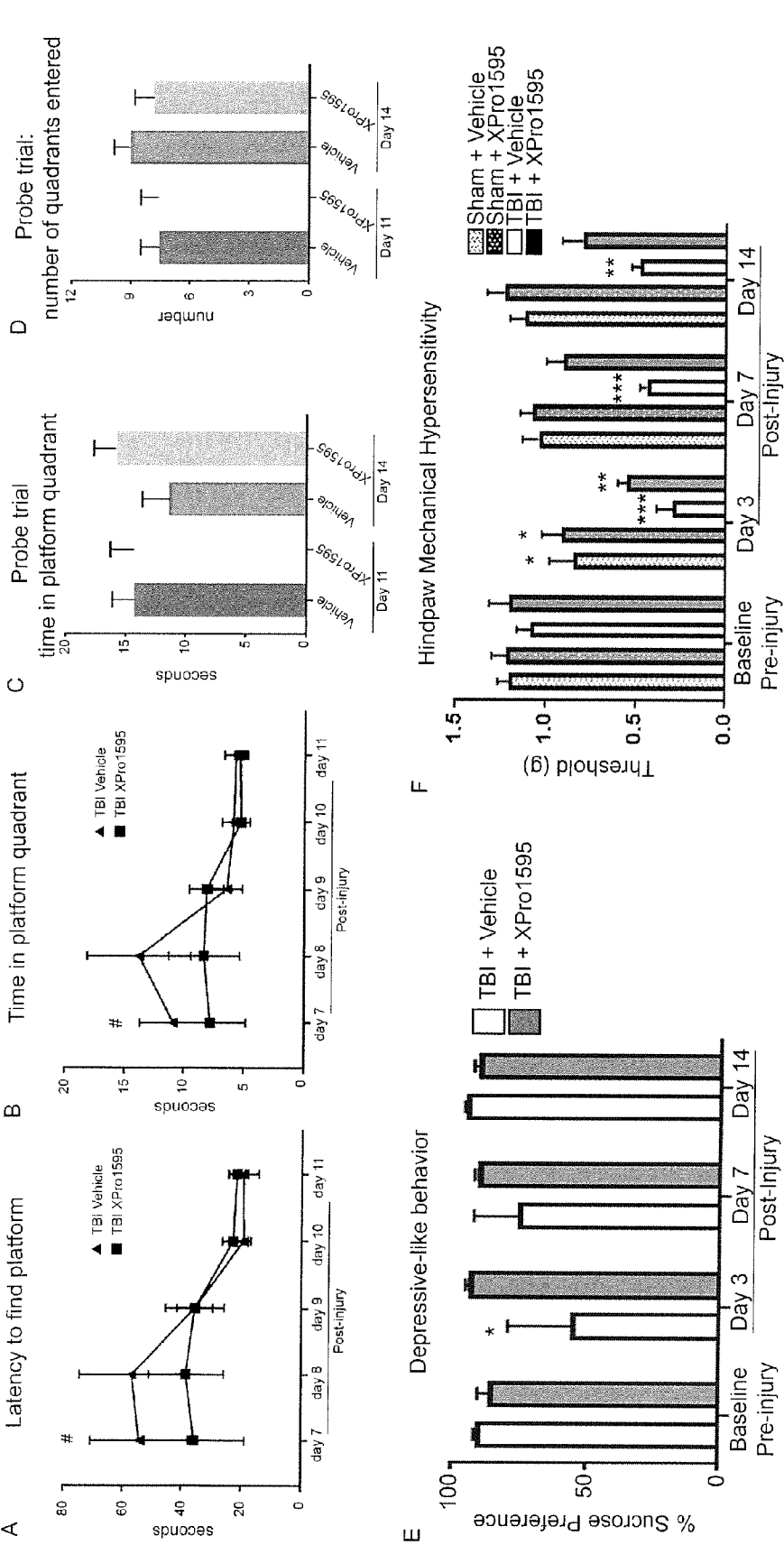


Fig. 6. Systemic XPro1595 therapy improves hippocampal functions following TBI in mice. Cognitive impairment was assessed by subjecting the mice in the Morris Water Maze (A-D) beginning 7 days post injury. On day 1 the vehicle-treated injured mice, performed worse than both groups of sham-injured mice, as well as XPro1595-treated injured mice at both time to find platform, and time in platform quadrant (A&B). No differences were observed between XPro1595-treated injured mice and both vehicle- and XPro1595-treated sham groups. There was a tendency for XPro1595-treated injured mice to perform better on remembering the task 3 days later, compared to vehicle-treated injured mice (C). (Sham + veh n=8; sham + XP n=7, TBI + veh n=10; TBI + sham n=9). Since the hippocampus is also associated with the development of depression, we used the sucrose preference test to assess depressive-like behavior in the mice following TBI. Injured vehicle-treated mice displayed a reduction in desire to drink the sucrose water within 3 days of the injury (E: sham injury data not shown), suggestive of depressive-like behavior, however this effect was abolished following systemic XPro1595 therapy. (Sham + veh n=2; sham + XP n=2; TBI + veh n=4; TBI + XP n=5). Often depression and chronic pain are observed together, and pain has been associated with both TBI and hippocampal inflammation, therefore we assessed hindpaw hypersensitivity using the mechanical von Frey assay, as an indicator of pain. Vehicle-treated injured mice displayed a significant and long-lasting increase in dermal hypersensitivity (F). Comparatively, systemic XPro1595 therapy alleviated this pain (Sham + veh n=13; sham + XP n=14; TBI + veh n=9; TBI + XP n=9). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to same group and treatment at baseline, $p < 0.05$ compared to other treatment at same time-point.

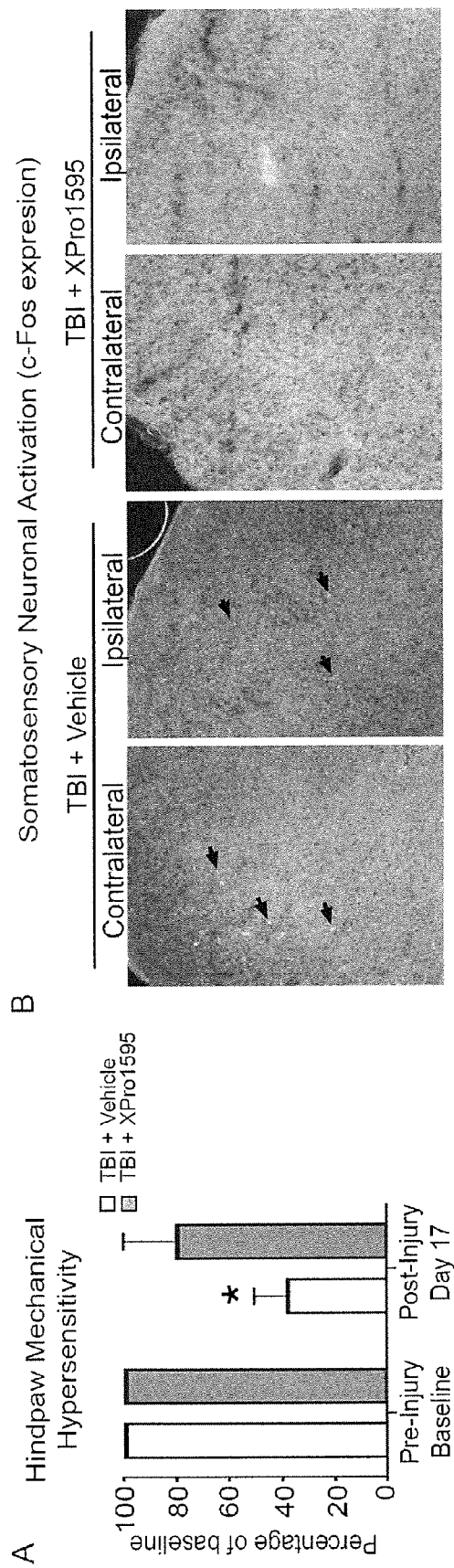


Fig. 7. XPro1595 therapy modulates brain-regulation of pain. XPro1595 or vehicle were infused directly into the lateral ventricles of the brain following TBI, and hindpaw mechanical hypersensitivity was assessed. At post-injury day 17 the vehicle-infused injured mice had increased hypersensitivity (reduced threshold score), compared to baseline testing, however the XPro1595-infusion group was not significantly different to baseline (A). In other mice XPro1595 or vehicle was systemically administered and neuronal activation was observed in the somatosensory cortex by labelling c-Fos protein using fluorescent IHC two weeks following TBI (B). c-Fos IHC constitutively labelled hippocampal stem cells as expected (images not shown), confirming immunoreactivity in all tissues tested, but only labelled neurons in the vehicle-treated injured cortex, and not in the XPro1595-treated injured cortex. c-Fos-positive neurons indicated by black arrows. * = $p < 0.05$.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/59981

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 14/525; A61K 38/19; A61P 25/00 (2020.01)

CPC - C07K 14/525; A61K 38/191; A61K 9/0085; A61P 25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/0239951 A1 (Xencor, Inc.) 27 August 2015 (27.08.2015). Especially para [0006], [0008], [0136].	1-5, 8-12
A	US 2015/0079086 A1 (TACT IP LLC) 19 March 2015 (19.03.2015). Especially para [0019], [0041], [0042], [0046], [0064].	1-3, 5, 8-10, 12

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 January 2020

Date of mailing of the international search report

30 MAR 2020

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/59981

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-----Go to Extra Sheet for continuation-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 8-12, limited to DN-TNF-alpha is a protein and is administered intravenously

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 19/59981

Continuation of Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-14, drawn to a method of treating a subject suffering from traumatic brain injury.

The method of treating will be searched to the extent that the solTNF-alpha comprises a DN-TNF-alpha protein (claims 2, 9) administered intravenously (claims 5, 12). It is believed that claims 1-5, 8-12 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass DN-TNF-alpha administered intravenously. Additional forms of DN-TNF-alpha and means of administration will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional forms of DN-TNF-alpha and means of administration. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: a nucleic acid encoding the DN-TNF-alpha protein (claims 2, 9), administered via gene therapy (claims 5, 12) (claims 1, 2, 5-9, 12-14).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Among the inventions listed as Groups I+ are the forms of DN-TNF-alpha protein or nucleic acid encoding said protein) and the specific means of administering a solTNF-alpha (e.g., administered as a protein or as a nucleic acid via gene therapy). Each invention requires a specific form of DN-TNF-alpha and a specific method of administering not required by any other inventions.

Common Technical Feature:

Group I+ inventions share claim 1 and 8.

However, said common technical features do not represent a contribution over the prior art, and is disclosed by US 2015/0239951 A1 to Xencor, Inc. (hereinafter "Xencor").

As to claim 1, Xencor discloses a method for treating a subject suffering from traumatic brain injury, comprising: administering to the subject a therapeutically effective amount of a selective inhibitor of solTNF-alpha whereby the subject is treated (para [0006]; "certain dominant negative TNF-alpha proteins (DN-TNF) have effects on the CNS and brain when administered peripherally. In an embodiment the effects are mediated via direct contact of the DN-TNF with the brain and/or CNS as the DN-TNF agents cross the blood brain barrier. Accordingly, provided herein is a method of treating a patient with a neurological disease comprising administering to the patient a therapeutically effective amount of a dominant negative TNF-alpha inhibitor, wherein said dominant negative TNF-alpha inhibitor is administered peripherally"; para [0008]; "In another preferred embodiment, the dominant negative TNF-alpha polypeptide is XPro1595").

As to claim 8, Xencor discloses a composition comprising selective inhibitor of solTNF-alpha for use in a method of treating a subject suffering from traumatic brain injury, the method comprising: administering to the subject a therapeutically effective amount of the selective inhibitor of solTNF-alpha, whereby the subject is treated (para [0006]; "certain dominant negative TNF-alpha proteins (DN-TNF) have effects on the CNS and brain when administered peripherally. In an embodiment the effects are mediated via direct contact of the DN-TNF with the brain and/or CNS as the DN-TNF agents cross the blood brain barrier. Accordingly, provided herein is a method of treating a patient with a neurological disease comprising administering to the patient a therapeutically effective amount of a dominant negative TNF-alpha inhibitor, wherein said dominant negative TNF-alpha inhibitor is administered peripherally"; para [0008]; "In another preferred embodiment, the dominant negative TNF-alpha polypeptide is XPro1595").

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Group I+ inventions lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.