Apolipoprotein E/Growth Factor Complexes and Methods of Use

Related Application Information

This application claims the benefit of United States Provisional Application No. 60/060,533 filed September, 30, 1997, which is incorporated by reference herein in its entirety.

Statement of Federal Support

This invention was made with Government support under grant number R01 AG12532-01 from the National Institutes of Health. The government has certain rights in this invention.

Field of the Invention

The present invention relates to compositions comprising complexes containing apolipoprotein E and nerve growth factor, neurotrophin 4, or γ-interferon and methods of administering the same.

Background of the Invention

Apolipoprotein E (protein: apoE; allele: APOE) is the principal apolipoprotein in the brain (for review, *see* Mahley, (1988) *Science* 240, 622) and cerebrospinal fluid (CSF) (Pitas *et al.*, (1987) *J. Biol. Chem.* 262, 14352). Several observations have implicated a role for apoE in the injured nervous system. Expression of apoE mRNA by astrocytes in the hippocampus increases following

entorhinal cortex lesion (Poirier *et al.*, (1991) *Mol. Brain Res.* 11, 97). Oligodendrocytes and macrophages increase expression of apoE following optic and sciatic nerve injury, respectively (optic: Stoll *et al.*, (1989) *GLIA* 2, 170; sciatic: Skene and Shooter, (1983) *Proc. Nat. Acad. Sci. USA* 80, 4169; Stoll and Mueller, (1986) *Neurosci. Lett.* 72, 233), and apoE protein accumulates to 5% of total extracellular protein following peripheral nervous system (PNS) injury (Skene and Shooter, (1983) *Proc. Nat. Acad. Sci. USA* 80, 4169). APOE is a susceptibility gene for familial and late-onset Alzheimer's disease (AD: Strittmatter *et al.*, (1993) *Proc. Nat. Acad. Sci. USA* 90, 1977; for review see Strittmatter and Roses, (1995) *Proc. Nat. Acad. Sci. USA* 92, 4725). The gene dose of APOE4, one of the three major alleles of APOE in humans, is correlated with increased risk and decreased average age of onset of AD. These observations suggest a role for apoE in the injured or diseased nervous system.

Three major isoforms of apoE in humans -- apoE2, apoE3 and apoE4 -- are distinguished by cysteine-arginine substitutions at positions 112 and 158. The most common isoform, apoE3, is secreted as a 299 amino acid protein with a single cysteine at position 112 and an arginine at position 158: apoE2 contains a cysteine at position 158 and apoE4 contains an arginine at position 112. ApoE contains two distinct structural and functional domains, a hydrophobic domain and a hydrophilic receptor binding domain (Weisgraber, (1994) *Adv. Prot. Chem.* 45, 249). The crystal structure of the hydrophilic domain of apoE is homologous to the family of four-helix bundle growth factors, including ciliary neurotrophic factor, although the sequences of these proteins diverge greatly. CNTF; reviewed by Bazan, (1991) *Neuron* 7, 197; Mott and Campbell, (1995) *Curr. Opin. Struc. Biol.* 5, 114; apoE crystal structure by Wilson *et al.*, (1991) *Science* 252, 1817; CNTF crystal structure by McDonald *et al.*, (1995) *EMBO J.* 14, 2689.

Nerve growth factor (NGF) belongs to a family of neurotrophins that includes brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), neurotrophin 4/5 (NT4/5), and neurotrophin 6 (NT6). All of these proteins bind to the low affinity NGF receptor p75 and a specific member of the trk family of tyrosine kinase receptors. For NGF, this is the trkA receptor. Binding of the 26

kDa homodimer of NGF to trkA induces an intracellular signaling cascade that begins with autophosphorylation of trkA. Ultimately, activation of trkA ends with changes in gene expression that effect neuronal survival, outgrowth, excitability, and differentiation.

It is well-established that NGF promotes survival and neurite outgrowth of sympathetic and neural-crest derived sensory cells during development (for review, *see* Snider and Johnson, (1989) *Annual Neurology* **26**, 489). This is evidenced by the fact that mice that are homozygote mutants for either NGF or trkA have severe loss of cells in the superior cervical ganglion (SCG; >90%) and in dorsal root ganglia (DRG, 70-80%) (Crowley *et al.*, (1994) *Cell* **78**, 1001; Smeyne *et al.*, (1994) *Nature* **368**, 246) and die within 3 weeks of birth. The ability of NGF to promote neurite outgrowth is one of the most dramatic activities of NGF. Indeed, neurite outgrowth from explanted embryonic chick DRGs is the standard bioassay to test for NGF activity. Dissociated cultures of DRGs and SCGs also respond to NGF with increased neuritic growth. The PC12 cell line, derived from rat adrenal medulla, has provided an excellent system in which to study the neurite outgrowth and survival effects of NGF. If PC12 cells are cultured in medium supplemented with fetal bovine and horse serum, they proliferate and appear as small, round phase-bright cells. Upon addition of NGF to the culture medium, these cells stop dividing and differentiate into a sympathetic-like neuronal phenotype with multiple neuritic processes and large cell bodies. NGF is also required for survival of PC12 cells in a serum-free environment. Because of the ease of this paradigm, much of the work elucidating the mechanism of NGF action has used PC12 cells.

As DRG neurons mature, the time course and sensitivity of their dependence on NGF for survival changes. To illustrate this, 24 hours following transection of the sciatic nerve in a newborn rat there is loss of 45% of the cells in the corresponding DRG. Yip *et al.*, (1984) *J. Neurosci.* **4**, 2986. Systemic administration of NGF reduces cell loss to 18%. *Id.* In contrast, in the adult rat, transection without regeneration results in a 22% loss of axotomized cells in the DRG at 3 weeks post axotomy. Application of NGF directly to the proximal stump,

prevents all of the axotomy induced cell death. Rich *et al.*, (1987) *J. Neuro. Cytol.* **16**, 569.

Exogenous NGF can promote the survival and regeneration of transected sciatic nerves. Multiple studies have confirmed that silastic tube implants filled with NGF enhance the rate and degree of functional sensory recovery. Rich *et al.*, (1987) *J. Neuro. Cytol.* **16**, 261; Rich *et al.*, (1989) *J. Neuro. Cytol.* **18**, 569; Derby *et al.*, (1993) *Exp. Neurol.* **119**, 176.

Neurotrophin 4 (NT4) is another member of the neurotrophin family. NT4 has been reported to promote survival of corticospinal motor neurons from neonatal rats (Junger and Varon, (1997) *Brain Res.* **762**, 56), increase survival of cultured rat septal neurons under normal and stress conditions (Nonner et al, (1996) *J. Neurosci.* **16**, 6665), promote survival and morphological and biochemical differentiation of embryonic rat striatal neurons in culture (Ventimiglia *et al.* (1995), *Eur. J. Neurosci.* **7**, 213), and enhance survival of cultured rat vestibular ganglion neurons and protect these cells against neurotoxic agents (Zheng *et al.*, (1995) *J. Neurobiol.* **28**, 330).

Interferons (IFN) are proteins secreted by eukaryotic cells after virus infections, which in turn protect against virus infections. Three classes of interferons are known at present: they are referred to as IFNα, IFNβ and IFNγ, which differ in both their structure and biological effects. All of the IFNγ identified thus far are glycosylated, although glycosylation does not appear to influence biological activity. Keller *et al.*, (1983) *J. Biol. Chem.* **258**, 8010. Gamma-interferon is regarded as a lymphokine since it is produced by lymphoctyes after either specific or non-specific stimulation by antigens. Gamma-interferon is well-known as an anti-viral and anti-tumor agent. *See* U.S. Patent No. 5,602,010 to Hauptmann *et al.* Other investigators have found that IFNγ increases intravascular C1 inhibitor concentrations in patients exhibiting or at risk for C1 inhibitor deficiencies. U.S. Patent No. 5,271,931 to Lotz *et al.* C1 inhibitor is a serine protease inhibitor that is involved in the regulation of several proteolytic systems including the complement, contact, coagulation, and fibrinolytic systems. Davis *et al.* (1988) *Ann. Rev. Immunol.* **6**, 595. Other activities of IFNγ include promotion

of polynuclear giant cell formation and activation of macrophages (Weinberg *et al.*, (1984) *Proc. Natl. Acad. Sci. USA* 81, 4554), inhibition of bone resorption (U.S. Patent No. 4,921,697 to Peterlik *et al.*), and prevention of type I diabetes mellitus (U.S. Patent No. 5,624,895 to Sobel).

Thus, NGF, NT4 and IFNγ possess biologically significant activities. Accordingly, there is a need in the art for strategies of potentiating the activity of these factors. In particular, it is an object of this invention to provide means of enhancing the activity of the neurotrophic growth factors, NGF and NT4, to slow the progression of neurodegenerative diseases, to protect against neural degeneration after injury, and to facilitate nerve regeneration.

Summary of the Invention

Disclosed herein is the novel discovery that apoE binds to and potentiates the biological action of the neurotrophic factor NGF. By "potentiate" it is meant that apoE enhances at least one biological action of NGF, by increasing the maximal response achieved and/or increasing the potency of the growth factor (*i.e.*, shifting the dose-response curve to the left). Alternately, the term "potentiate" indicates that apoE stabilizes the NGF molecule and decreases its degradation. Further disclosed is the finding that apoE advantageously binds to NT4 and IFNγ.

As a first aspect, the present invention provides a composition comprising a complex of apoE and NGF. Also disclosed are compositions comprising a complex of apoE and NT4. Further disclosed are compositions comprising a complex of apoE and IFNγ. The apoE component can be apoE2, apoE3, or apoE4, and can be in the native lipid-bound or a delipidated state. Furthermore, the complex can be formed by either covalent or noncovalent interactions between the apoE and NGF, NT4 or IFNγ molecules.

As a second aspect, the present invention provides a method of enhancing the survival of neural cells, comprising administering to the neural cells a survival-enhancing amount of a composition comprising a complex of apoE and NGF or NT4.

As a third aspect, the present invention provides a method of administering a composition comprising a complex of apoE and NGF or NT4 in a therapeutically-effective amount to a subject in need thereof.

As a fourth aspect, the present invention provides a method of protecting cells against virus infection, comprising administering to the cells an anti-virus effective amount of a composition comprising a complex of apoE and IFNγ. Further provided is a method of administering IFNγ in an anti-viral effective amount to a subject in need thereof.

As a further aspect, the present invention provides a method of administering IFNγ in an anti-tumor effective amount to a subject in need thereof.

These and other aspects of the invention are set forth in more detail in the description of the invention below.

Brief Description of the Drawings

Figure 1 demonstrates that apoE3 potentiates the survival promoting activity of NGF in PC12 cells. The data are presented as the mean percent survival ± standard deviation as compared to the control treatment of 1000 ng/ml NGF and no apoE. Using the Student's *t* test for the difference between the means, $p<0.001$ at 100 and 1000 ng/ml NGF. Open bars: substrate-bound apoE3 (50 ng/ml) preincubated with 0, 100 and 1000 ng/ml NGF. Black bars: soluble NGF alone added at 0, 100 and 1000 ng/ml.

Figure 2 demonstrates that apoE3, but not apoE4, enhances neurite outgrowth from PC12 cells in response to NGF. Neurite outgrowth was measured after 3 days in culture with serum-free medium containing 100 ng/ml NGF and 1 μg/ml apoE3, apoE4 or bovine serum albumin (BSA) as a control protein. Phase images of cells were digitally collected on random fields using IMAGE 1™ analysis software (Universal Imaging Corp.; West Chester, PA). Neurite length is displayed as percent of outgrowth of the control treatment (NGF+BSA) ± SEM. Using a

paired Student's *t*-test, $p<0.005$ for apoE3 vs. BSA and $p<0.01$ for apoE3 vs. apoE4.

Figure 3 demonstrates that apoE knockout (KO) mice have a reduced number of unmyelinated fibers as compared with wildtype (WT) mice. The total area covered by high-powered electron micrographs from 3 wildtype and 3 knockout animals was approximately 18,000 μm^2 for each group (17,900 for wildtype and 18,200 for knockout). The total number of myelinated and unmyelinated axons in this area were counted. It was found that knockout nerves had 50% fewer unmyelinated fibers, but an equal number of myelinated fibers, as compared with nerves from wildtype animals.

Figure 4 demonstrates that apoE knockout mice have a delayed response to noxious thermal stimuli. For this experiment, the withdrawal latency in seconds for mice to remove their hind feet from a 55°C water bath was measured. The data presented are the means ± SEM for 3 mice in each group and 3 measurements/per hind foot for each mouse. Knockout mice have over a 50% increase in their response latency as compared with wildtype mice. The p value for the difference between the two means is $p<0.001$ using a paired Student's *t* test.

Detailed Description of the Invention

A. Neurotrophic Factors – NGF and NT4.

The nervous system responds to acute injury and to chronic neurodegenerative diseases by the coordinated expression of many proteins, including growth and survival factors, cell surface receptors, and secreted extracellular proteins. Many of these proteins, most notably the neurotrophic factors, play a role in recovery from injury and protection from neurodegenerative disease. Within the complex milieu of the traumatized nervous system it is extremely unlikely that any one growth factor functions alone. Cooperative interactions of growth factors with other proteins are likely to play important roles in the stability, localization or presentation of growth factors. Optimal functioning

of growth and survival factors released at sites of injury is critical for recovery from neural trauma. In both the peripheral and central nervous systems the expression of apoE is increased in acute and chronic stress.

Disclosed herein is the discovery that apoE binds to the neurotrophic factors NGF and NT4. Further disclosed is the discovery that apoE potentiates the biological activity of NGF. As described above, by "potentiate" it is meant that apoE enhances at least one biological action of NGF, by increasing the maximal response achieved and/or increasing the potency of the growth factor (*i.e.*, shifting the dose-response curve to the left). Alternately, the term "potentiate" indicates that apoE stabilizes the NGF molecule and decreases its degradation.

While not wishing to be held to any particular theory of the invention, the finding that apoE binds to NGF and NT4 suggests that apoE may act as an accessory protein for these growth factors, regulating their metabolism or biological activities. One mechanism may entail the localization of the growth factors to extracellular matrix by tethering them through an apoE molecule. It has previously been shown that apoE binds to the extracellular matrix protein laminin, and thereby increases neuron adhesion, and alters growth cone spreading (Huang *et al.*, (1995) *Exp. Neurology* **136**, 251). ApoE may additionally alter the biological activity of the bound growth factor by other mechanisms, such as inhibiting its proteolytic inactivation, or by altering its ability to interact with its cell-surface receptor. Interactions between apoE and NGF and/or NT4 may be important in modulating the role of these growth factors in neural regeneration responses, both in acute and in chronic disorders of the nervous system.

Disclosed herein are compositions containing apoE:NGF and apoE:NT4 complexes. The claimed complexes can be formed by simply mixing apoE and the neurotrophic factor(s) (typically aqueous solutions) together, or by any other suitable method known in the art. The compositions can contain both complexes of apoE with NGF and complexes of apoE with NT4. Also encompassed by the present invention are compositions containing apoE:NGF and/or apoE:NT4 complexes and/or complexes of apoE with other neurotrophic factors, such as ciliary neurotrophic factor.

Those skilled in the art will appreciate that compositions containing the claimed apoE complexes produced in this manner will generally contain apoE, NGF and/or NT4 monomers, homodimers and multimers. In general, as low as 35%, 25%, 20%, 15%, or lower, or as high as 40%, 50%, 70%, 85%, 90%, 95%, 99% or higher of the NGF or NT4 molecules in the composition will be complexed with apoE. The terms "apoE:NGF" and "apoE:NT4", as used herein, encompass apoE:NGF and apoE:NT4 complexes containing monomers, dimers, trimers and larger multimers of NGF or NT4, respectively. Alternately, apoE monomers, homodimers, homotrimers, *etc.* may associate with NGF or NT4 monomers, homodimers, homotrimers, *etc.* Thus, the present invention encompasses complexes between one or more apoE molecules with one or more NGF or NT4 molecules.

While not wishing to held to any particular theory of the invention, the active form of NGF is a homodimer, and it is believed that the NGF homodimer complexes with an apoE monomer. Heterodimeric apoE:NGF and heterotrimeric apoE:$(NGF)_2$ complexes are preferred. Likewise, heterodimers of apoE and NT4 are preferred. Complexes between one or more apoE molecules with one or more NT4 molecules are also aspects of the present invention. Those skilled in the art will appreciate that apoE:NGF and apoE:NT4 complexes may also be more loosely associated with additional molecules.

Alternatively, apoE may first be bound to a substrate, such as a polymeric surface (*i.e.*, tissue culture plate, test tube), prior to being exposed to and forming a complex with NGF or NT4. Such apoE-bound substrates are useful for collecting NGF or NT4 from a solution. As a further alternative, the apoE:NGF or apoE:NT4 complex can be bound to a substrate after complex formation. Such substrates are useful for culturing cells *in vitro*.

The strength of the binding interaction between apoE and NGF or NT4 is high; with a dissociation constant of the apoE complexes of at least 10^{-7}, preferably at least 10^{-8}, more preferably at least 10^{-9}. Complexes can be formed by covalent or noncovalent interactions, with covalent complexes of apoE and NGF or NT4 being preferred. Typically, apoE:NGF and apoE:NT4 complexes are stable in a solution

containing at least 1% SDS. Also preferred are complexes formed between apoE3 and NGF or NT4.

The apoE component of the claimed complexes can be apoE2, apoE3, apoE4 or a combination thereof. Preferably, the complexes contain apoE3. ApoE variants and fragments that bind to the apoE receptor and/or complex with and potentiate the biological actions of NGF and/or NT4 are also encompassed by the present invention. ApoE can be from any species of origin, preferably of mammalian origin, more preferably human origin. The apoE molecules can be in the native lipid-bound state or a delipidated state, with delipidated being preferred.

ApoE can be purified from natural sources (*i.e.*, blood, serum or peritoneal fluid). United States Patent No. 5,672,685 describes the isolation of native apoE from peritoneal fluid, the disclosure of which is incorporated herein in its entirety by reference. The majority of apoE from sera is associated with lipoprotein particles. Purification of apoE from sera requires delipidation with organic solvents or detergents, which causes significant protein denaturation. Lipoprotein isolation by ultracentrifugation, with subsequent lyophilization and delipidation of lipoproteins, and chromatographic isolation of apoE, is described in Rall *et al.*, (1986) *E. Methods Enzymol.* **128**, 273. An alternative method for isolation of apoE from a mixture of apolipoproteins utilizes gel electrophoresis. Purification of apoE isoforms may be accomplished using isoelectric focusing techniques (Rall *et al.*, (1986) *E. Methods Enzymol.* **128**, 273).

ApoE may also be separated from contaminating proteins using heparin-sepharose chromatography, which utilizes the heparin-binding property of apoE. Rall *et al.*, (1986) *E. Methods Enzymol.* **128**, 273. ApoE may be isolated and/or purified, optionally to homogeneity, by conventional techniques such as affinity chromatography, size-exclusion chromatography, gas chromatography, HPLC, and combinations thereof. Separation of the non-cysteine containing E4 isoform of apoE from contaminating cysteine-containing proteins may be accomplished using thiopropyl chromatography on thiopropyl Sepharose (Weisgraber *et al.* (1983), *J. Biol. Chem.* **258**, 2508).

Recombinant apoE can be produced using methods known in the art, and human recombinant apoE is commercially available. However, recombinant protein is not in the native glycosylated form and is subject to denaturation and oxidation during purification.

The NGF component of the complex can also be from a native or recombinant source, and can be produced by any means known in the art. Native: U.S. Patent Nos. 5,210,185 and 5,057,223 to Della Valle *et al.*; U.S. Patent No. 4,407,744 to Young. Recombinant: U.S. Patent No. 5,272,063 to Chan *et al.*; U.S. Patent No. 5,288,622 to Gray *et al.*; U.S. Patent No. 5,082,774 to Heinrich. Moreover, the NGF component of the claimed apoE:NGF complexes can be from any species of origin, preferably of mammalian origin, more preferably of human origin. The term "NGF" encompasses NGF variants, analogs and derivatives. For example, U.S. Patent No. 5,349,055 to Persson *et al.* discloses NGF analogs with significantly reduced binding to the low affinity p75 receptor and essentially no alteration in binding to the trk receptor. Further disclosed by this reference are NGF analogs having increased stability. Also known in the art are chimeric NGF molecules in which regions of the NGF peptide are replaced by the corresponding residues of brain derived growth factor (BDGF). Ibanez *et al.*, (1991) *EMBO J.* **10**, 2105. Finally, the term "NGF" also includes NGF fragments that bind to NGF receptors and/or elicit the neurotrophic actions of NGF. For example, U.S. Patent No. 5,134,121 to Mobley *et al.* discloses NGF peptides and analogs thereof that induce NGF-associated biological response.

Likewise, the NT4 component of the disclosed apoE:NT4 complexes can be from a native or recombinant source, and can be produced by any means known in the art. Recombinant: U.S. Patent No. 5,364,769 to Rosenthal. Moreover, the NT4 component of the claimed apoE:NT4 complexes can be from any species of origin, preferably of mammalian origin, more preferably of human origin. The term "NT4" also encompasses NGF variants, analogs and derivatives, such as those disclosed in U.S. Patent No. 5,364,769 to Rosenthal and U.S. Patent No. 5,349,055 to Persson. The term "NT4" also includes NT4 fragments that bind to NGF receptors and/or elicit the neurotrophic actions of NT4.

Methods of enhancing the survival of neural cells by administration of an apoE:NGF and/or apoE:NT4 complex are also an aspect of the present invention. The term "enhancing the survival" of neural cells is intended to be construed broadly, and includes neurotrophic and neuro-regenerative actions of the claimed compositions. Alternatively, the expression "enhancing the survival" of neural cells refers to the action of the claimed compositions in protecting neural cells from injury and/or improving recovery from neural injury. By "enhancing the survival" it is meant that the inventive complexes provide some improvement to neural cell survival, as defined above. The improvement in neural cell survival can be 5%, 10%, 25%, 50%, 75%, 100% or more. "Neural cells" includes the cells and tissues of the central nervous system and the peripheral nervous system, both *in vitro* and *in vivo*. Compositions comprising apoE:NGF and/or apoE:NT4 complexes for use in the claimed methods are as described in more detail hereinabove.

The claimed apoE complexes can be administered to neural cells *in vitro*. Typically, *in vitro* administration will simply require adding a solution (*i.e.*, aqueous) containing the apoE:NGF and/or apoE:NT4 complexes to the culture medium. Alternatively, the apoE and NGF or NT4 components can be added individually, either concurrently or sequentially, to the culture medium. As a further alternative, the apoE molecule or the apoE:growth factor complex can be bound to a substrate (*i.e.*, a tissue culture dish or petri dish), preferably prior to culturing cells on the substrate. It is well-known in the art that apoE binds to polymeric or coated (*i.e.*, with extracellular matrix proteins such as laminin) surfaces of tissue culture plates.

Complexes of apoE and NGF or NT4, which potentiate the activity of these neurotrophic factors, are useful *in vitro* for culturing neural cells, such as immortalized PC12 cells (*see, e.g.*, U.S. Patent No. 5,349,055 to Persson *et al.*) and primary cultures of neurons (*see, e.g.*, Varon, (1997) *Brain Res.* 762, 56; Barrett and Barrett, (1996) *J. Neurosci.* 16, 6665; Abiru *et al.*, (1996) *Brain Res. Dev. Brain Res.* 91, 260; Snider and Johnson, (1989) *Annual Neurology* 26, 489; Yip *et al.*, (1984) *J. Neurosci.* 4, 2986).

As provided by U.S. Patent No. 5,134,121 to Mobley, NGF additionally finds use as a component of culture medium to enhance the survival of neurons. Also disclosed is the use of NGF to provide a therapeutic treatment for Alzheimer's disease, Huntington's disease, and other neurodegenerative disorders. As established by U.S. Patent No. 5,604,202 to Kessler *et al.*, NGF also finds use in methods of treating drug-induced neuropathy. Thus, the presently claimed NGF complexes possessing enhanced neurotrophic activity find similar uses as previously known NGF compositions.

As established by U.S. Patent No. 5,364,769 to Rosenthal, NT4 finds use as a component of culture media to enhance the survival or induce the outgrowth of nerve cells *in vitro*. Also disclosed is use of NT4 in the therapy of neurodegenerative diseases. Thus, the presently claimed NT4 complexes possessing enhanced neurotrophic activity find similar uses as previously known NT4 compositions.

The claimed apoE:NGF and apoE:NT4 complexes also find use in tissue culture, because the complexed growth factors are protected against proteolytic degradation.

Finally, the claimed complexes find use in methods of quantifying or purifying NGF or NT4 receptors or other proteins that bind to these neurotrophic factors. ApoE is readily bound to surfaces, such as test tubes and microtiter plates. Thus, NGF or NT4 can be tethered to surfaces for use in sandwich assays or affinity purification techniques, which are well-known to those skilled in the art.

The claimed apoE:NGF or apoE:NT4 complexes can also be administered to a subject *in vivo*. Methods of administration and pharmaceutical formulations of the claimed compositions are described in more detail hereinbelow. The methods of the invention are useful for treating a subject afflicted with a neurodegenerative disease and subjects who have experienced injury or trauma to neural tissue. Such subjects include but are not limited to those afflicted with Alzheimer's Disease, Parkinson's Disease, peripheral nerve injury, peripheral neuropathy (in particular, diabetes-induced peripheral neuropathy), amyotrophic lateral sclerosis, head injury, and

stroke. The present invention is particularly useful in treating Alzheimer's Disease and diabetes-induced peripheral neuropathy.

Subjects suitable for carrying out the present invention are, in general, mammals and avians, including but not limited to humans, monkeys, equines, caprines, bovines, ovines, porcines, dogs, cats, rabbits, rats, hamsters, mice, quail, chickens and turkeys. Human subjects are presently preferred for *in vivo* administration. Similarly, cultured neural tissues/cells for use with the inventive methods include mammalian and avian tissues and cells, including but not limited to neural tissues and cells derived from humans, monkeys, equines, caprines, bovines, ovines, porcines, dogs, cats, rabbits, rats, hamsters, mice, quail, chickens and turkeys.

The disclosed compositions may be contained in a physiologically acceptable carrier (preferably sterile), which is a carrier that is not unduly detrimental to cells and includes pharmaceutically acceptable carriers.

In the manufacture of a medicament according to the present invention, hereinafter referred to as a pharmaceutical formulation, the inventive compositions are typically admixed with a pharmaceutically acceptable carrier. For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid, such as sterile, pyrogen-free water or sterile pyrogen-free phosphate-buffered saline solution. Alternately, one may incorporate or encapsulate the claimed complexes in a suitable polymer matrix, liposome or membrane, thus providing a sustained-release delivery device suitable for implantation near the site to be treated locally.

For the preparation of these compositions for administration to a subject, use can be made of pharmaceutical carriers adapted for conventional forms of administration, for example, injection solutions, tablets, capsules, dragees, syrups, solutions, suspension and the like. As an injection medium, it is preferred to use water which contains the additives usual in the case of injection solutions, such as stabilizing agents, salts or saline, and/or buffers. The active agent or its pharmaceutical formulation may be contained within a nutritional medium, *e.g.*, in nutritional

supplements. Oral formulations may be slow release preparations or enteric coated preparations to facilitate delivery of the peptide to the small intestine.

When administering the claimed apoE complexes to a subject in need thereof, any suitable route of administration may be employed, including administration by parenteral injection (*e.g.*, subcutaneous, intramuscular, or intradermal), or by oral, rectal, topical, nasal, ophthalmic, intrathecal, and intracerebral administration.

The apoE:NGF and apoE:NT4 complexes are included in an amount effective to accomplish the intended treatment. In general, the claimed apoE complexes are present in an amount effective to enhance survival of neural cells. ApoE:NGF and apoE:NT4 complexes may be administered concurrently or in combination with other agents. In particular, apoE:NGF and/or apoE:NT4 complexes can be administered with other neurotrophic factors, such as CNTF.

The precise amount of an apoE:NGF or apoE:NT4 complex to be administered is determined in a routine manner, and will vary depending on the age and species of subject, the desired effect, the apoE isoform, and the route of administration. Preferred dosages may be determined by simply administering a composition containing a known amount of apoE:NGF or apoE:NT4 complexes *in vitro* or *in vivo* to a subject, and monitoring the cells, tissue or subject for the desired effect, as would be known by one skilled in the art.

There are no particular upper or lower limits to the dosage of apoE:NGF or apoE:NT4 complexes to be administered according to the present invention. For *in vivo* administration, dosages can be as low as 10, 3, 1, 0.5 or 0.1 μg/kg body weight, or less. *In vivo* dosages can be as high as 10, 30, 50 or 100 μg/kg body weight, or more. In general, the *in vivo* dosage of apoE:NGF or apoE:NT4 complexes administered will be sufficient to result in peak plasma concentration of the complex of from about $1x10^{-1}$, 1×10^{0} or 1×10^{1} picomole per Liter to about 1×10^{2}, 1×10^{3} or even 1×10^{4} picomole per Liter or more.

Similarly, there are no particular lower or upper limits to the dosages of the claimed complexes to be administered *in vitro*. For *in vitro* administration dosages can be as low as 10, 5, 1, 0.5, or 0.1 ng/ml of medium, or less. *In vitro* dosages can be as high as 10, 50, 100, 500, 1000, or 1500 ng/ml of medium, or more.

B. Gamma-Interferon.

Another aspect of the present invention is apoE:IFNγ complexes. In preferred embodiments, the biological activity of IFNγ is potentiated (as defined above with respect to NGF and NT4) by complexing with apoE. ApoE:IFNγ complexes can be formed as described above for apoE:NGF and apoE:NT4 complexes.

Those skilled in the art will appreciate that compositions containing the claimed apoE:IFNγ complexes will generally contain apoE and IFNγ monomers, homodimers and multimers as well. In general, as low as 35%, 25%, 20%, 15%, or lower, or as high as 40%, 50%, 70%, 85%, 90%, 95%, 99% or higher of the IFNγ molecules in the composition are complexed with apoE. The term "apoE:IFNγ" as used herein encompasses apoE:IFNγ complexes containing monomers, dimers, trimers and larger multimers of IFNγ. Alternately, apoE monomer, homodimers, homotrimers, *etc.* may associate with IFNγ monomers, homodimers, homotrimers, *etc.* Thus, the present invention encompasses complexes between one or more apoE molecules with one or more IFNγ molecules. While not wishing to be held to any particular theory of the invention, the active form of IFNγ is a homodimer, and it is believed that the IFNγ homodimer complexes with an apoE monomer. Such heterotrimeric complexes of apoE:IFNγ are preferred. Those skilled in the art will appreciate that apoE:IFNγ complexes may also be more loosely associated with additional molecules.

Alternatively, apoE may first be bound to a substrate prior to being exposed to and forming a complex with IFNγ. As a further alternative, the apoE:IFNγ complex can be bound to a substrate after complex formation.

The strength of the binding interaction between apoE and IFNγ is as described above for apoE:NGF and apoE:NT4 complexes.

The apoE component of the disclosed complexes is as described above in connection with apoE:NGF and apoE:NT4 complexes. The IFNγ component of the complex can be from a native or recombinant source, and can be produced by any

means known in the art. Native: U.S. patent No. 5,518,899 to Kurimoto; U.S. Patent No. 4,723,000 to Georgiades *et al.*; U.S. Patent No. 5,132,110 to Fleischmann *et al.* Recombinant: U.S. Patent No. 5,602,010 to Hauptmann *et al.*; U.S. Patent No. 4,970,161 to Kakutani *et al.*; U.S. Patent No. 4,889,803 to Revel *et al.* Moreover, the IFNγ component of the claimed apoE:IFNγ complexes can be from any species of origin, preferably of mammalian origin, more preferably of human origin. The term "IFNγ" also encompasses IFNγ variants, analogs and derivatives, where the variants, analogs and derivatives retain IFNγ biological activity. Exemplary are IFNγ variants, analogs and derivatives disclosed in U.S. Patent No. 4,845,196 to Cowling; PCT application No. 83/04053; U.S. Patent Nos. 4,898,931 and 4,758,656 to Itoh *et al.*; Franke *et al.*, (1982) *DNA* 1, 223; King *et al.*, (1983) *J. Gen. Virol.* 64, 1815. The term "IFNγ" also includes fragments of the IFNγ molecule that bind to IFNγ receptors and/or elicit the biological actions of IFNγ.

A further aspect of the present invention is methods of administering compositions containing apoE:IFNγ complexes to cells in a biologically effective amount. Compositions containing apoE:IFNγ complexes find use in methods of protecting cells against virus infection, such methods comprise administering to the cells an anti-virus effective amount of a composition comprising a complex of apolipoprotein E and IFNγ. *See, e.g.*, Wheelock (1965) *Science* 149, 310. By "protecting" cells against viral infection, it is meant that virus infection rates are reduced or eliminated or that an already-existing infection is reduced or eliminated by the disclosed methods. Such methods can be used to protect cultured cells *in vitro* or a subject *in vivo*.

Another aspect of the invention is methods of administering an anti-tumor effective amount of a composition containing apoE:IFNγ complexes. An "anti-tumor effective amount" of the claimed complexes is a dosage that is effective in reducing the incidence of tumor formation or in decreasing the size, growth or metastasis of a tumor. Likewise, the present invention can be employed to administer the inventive apoE:IFNγ complexes to treat a subject afflicted with a

tumor (*e.g.*, by decreasing the size, growth or metastasis of a tumor) or to reduce the incidence of tumor formation in a subject at risk of developing tumors.

The claimed apoE complexes can be administered to cells *in vitro*. Typically, *in vitro* administration will simply require adding a solution (*i.e.*, aqueous) containing the apoE:IFNγ complexes to the culture medium. Alternatively, the apoE and IFNγ components can be added individually, either concurrently or sequentially, to the culture medium. As a further alternative, the apoE molecule or the apoE complex can be bound to a substrate (*i.e.*, a tissue culture dish or petri dish), preferably prior to culturing cells on the substrate. It is well-known in the art that apoE binds to polymeric or coated (*i.e.*, with extracellular matrix proteins such as laminin) surfaces of tissue culture plates.

The disclosed apoE:IFNγ complexes can also be administered to a subject *in vivo*. Suitable subjects, methods of administration, pharmaceutical formulations of the compositions containing apoE:IFNγ, and suitable dosages thereof are as described above in connection with apoE:NGF and apoE:NT4 complexes. The disclosed ApoE:IFNγ complexes can be administered alone or in combination with other therapeutic agents.

Compositions containing apoE:IFNγ complexes can be administered to a subject *in vivo* to provide anti-viral and anti-tumor treatments to a subject in need thereof. The inventive complexes can also be administered to treat bone degradation and resorption, for example in the elderly, post-menopausal women, and women afflicted with or at risk for developing osteoporosis. Finally, IFNγ can be administered to facilitate blood coagulation, by stimulating blood C1 inhibitor levels, and to treat type I diabetes mellitus.

Complexes of apoE and IFNγ, which potentiate the activity of the IFNγ, are useful *in vitro* for culturing cells that are responsive to IFNγ, including but not limited to bone cells (*see* U.S. Patent No. 4,921,697 to Peterlik *et al.*).

As established by U.S. Patent No. 6,268,169 to Brandely *et al.*, IFNγ is useful in methods of treating ovarian cancer. Also known is use of IFNγ to treat type I diabetes mellitus. U.S. Patent No. 5,624,895 to Sobel. In addition, it has

been established that IFNγ is useful in methods of treating blood trauma (U.S. Patent No. 5,271,931 to Lotz *et al.*) and treating bone degradation (U.S. Patent No. 4,921,697 to Peterlik *et al.*). Thus, the presently claimed IFNγ complexes possessing enhanced biological activity find similar uses as previously known IFNγ compositions.

The claimed apoE:IFNγ complexes also find use in tissue culture, because the complexed IFNγ molecule is protected against proteolytic degradation.

Finally, the apoE:IFNγ complexes find use in methods of quantifying or purifying IFNγ receptors or other proteins that bind to IFNγ. ApoE is readily bound to plastic surfaces, such as test tubes and microtiter plates. Thus, IFNγ can be tethered to plastic surfaces for use in sandwich assays or affinity purification techniques, which are well-known to those skilled in the art.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof.

Example 1

Gel-Shift Assays

Delipidated apoE was purified from individuals homozygous for apoE3 or apoE4, as previously described (Rall *et al.*, (1986) *Methods Enzymol.* 128, 273). ApoE3 or apoE4 were incubated with growth factors in Tris-buffered saline (TBS) for up to 4 hours at 37°C. Incubations were terminated by adding 4X SDS-Laemmli buffer without reducing agents. Proteins were electrophoretically separated by SDS-PAGE, and transferred to PVDF membrane (Immobilon P, Millipore, Bedford, MA). The membranes were blocked in Blotto (5% dried milk in TBS, pH 7.6, with 0.05% Tween [Surfact Amps-20, Pierce, Rockford, IL]) for one hour, then incubated in primary antibody for one hour. The anti-apoE antibody was a polyclonal goat anti-human apoE (Calbiochem, San Diego, CA) diluted 1:2000 in Blotto. Membranes were washed in Blotto three times, ten minutes each wash, then incubated with the secondary antibody for one hour. All incubations and washes were done at 25°C.

For detecting the anti-apoE antibody, the secondary antibody was a porcine anti-goat IgG conjugated to horseradish peroxidase (HRP; Boehringer Mannheim Biochemicals, Indianapolis, IN) diluted 1:3000 in Blotto. The enzyme-conjugated antibodies were visualized by addition of ECL chemiluminescent substrate (Amersham, Arlington Heights, IL) and exposure to Hyperfilm (Amersham) as previously described (Strittmatter *et al.*, (1993) *Proc. Nat. Acad. Sci. USA* 90, 1977).

Example 2

ApoE3 forms an SDS-Stable Complex with NGF, IFNγ and NT4

Gel shift assays, as described in Example 1, were used to screen for growth factors that bind apoE. Fifty ng of apoE3 or apoE4 and 50 ng of growth factor were incubated together for up to four hours. The growth factors evaluated were: recombinant human ciliary neurotrophic factor (CNTF), IFNγ, neurotrophin-3 (NT3), neurotrophin-4 (NT4), NGF, the fibroblast growth factor bFGF, and LIF. CNTF, IFNγ and LIF are members of the four-helix bundle family of growth factors. Sources of growth factors are as follows: CNTF -- generously provided by Regeneron Pharmaceuticals (Tarrytown, NY); IFNγ -- Human, recombinant #40044, Collaborative Biomedical Products (Bedford, MA); NT3 -- Regeneron Pharmaceuticals; NT4 -- human, recombinant #G1511, Promega (Madison, WI); NGF -- mouse, natural #40005, Collaborative Biomedical Products (Bedford, MA); bFGF -- Collaborative Biomedical Products (Bedford, MA); and LIF -- R&D Systems (Minneapolis, MN).

Samples were boiled in non-reducing SDS sample buffer, separated by SDS-PAGE, transferred to PVDF paper and probed with anti-apoE and peroxidase conjugated secondary antibody. Immunoreactivity was detected using the Amersham chemiluminescent detection reagent.

ApoE3 forms a SDS-stable complex, in the absence of reducing agents, with CNTF, IFNγ, NGF and NT4. Complexes were not observed between ApoE3 and IL-6, LIF, NT3, bFGF or BDNF. ApoE4 does not form a SDS-stable complex with any of these growth factors. The gel mobility of each apoE3/growth factor complex is consistent with a bimolecular complex between apoE3 and the growth factor (the active forms of NGF and IFNγ are dimers, so the apoE3/NGF complex is likely a trimer).

In further studies it was found that the apoE3 complexes with NGF and CNTF are detected within 30 minutes of incubation and reach equilibrium by 3-6 hours. The SDS-stable apoE3/CNTF complex reducible with β-mercaptoethanol, suggesting disulfide bond formation. Rigorous proof that one of these bands is a

true molecular complex has been obtained by showing that the CNTF/apoE complex is immunoreactive with both CNTF and apoE antibodies.

Example 3

ApoE Promotes NGF Activity in PC12 Cells

PC12 cells require NGF to survive in serum-free medium. Experiments were carried out to assess PC12 cell survival in the presence of serum-free medium containing NGF, apoE3 or NGF+apoE3.

PC12 cells were plated in 96-well dishes that had been pre-coated with poly-l-lysine and then treated with a solution containing serial dilutions of NGF and apoE3 that had been preincubated together. It is known that apoE3 will adsorb to tissue culture dishes under these conditions. For the preincubation, serial dilutions of NGF were added to a constant amount of apoE3 (50 ng/ml), incubated for 3 hours at 37°C, and then added to each well. These proteins were allowed to bind to the substrate for an additional 3 hours at 37°C. The final treatments were 50 ng/ml apoE3 + 0, 100 and 1000 ng/ml NGF. All the wells were washed three times with PBS to remove any soluble NGF and apoE. Another set of wells received soluble NGF at the same concentrations that were adsorbed to the wells (0, 100 and 1000 ng/ml).

The PC12 cells were incubated for 72 hours in serum-free medium alone or with substrate-bound apoE3+NGF, or soluble NGF as described above. At 72 hours, cell viability was assessed using the Promega CellTiter Aqueous MTS Assay as described in **Example 4**. Each condition was done in triplicate and non-specific background was subtracted.

The substrate-bound complex of apoE3 and NGF promotes the survival of serum-deprived PC12 cells to a greater degree than does substrate-bound NGF or soluble NGF alone (**Figure 1**). In fact, 50 ng/ml of apoE3 mixed with 100 ng/ml NGF and bound to the substrate enhanced survival of PC12 cells by more than 2-fold as compared with 100 ng/ml soluble NGF alone ($p < 0.001$). The differences at 1000 ng/ml NGF were also significant at the $p < 0.001$ level. ApoE alone has no

survival promoting activity toward PC12 cells (even at 1 μg/ml), regardless of whether the apoE is absorbed to the substrate or included in the culture medium.

The best interpretation of the survival promoting activity of the apoE/NGF complex is a facilitation of NGF activity. Thus, apoE potentiates the activity of NGF in the maintenance of NGF-responsive peripheral nerve cells.

Example 4

MTS Survival Assay

Viability of PC12 cells was assayed using the Promega viability assay (CellTiter 96 Aqueous, Promega, Madison, WI). *See* Ip and Yancopoulos, (1992) *Progress in Growth Factor Res.* 4, 1; Riddle *et al.*, (1995) *Nature* 378, 189. This is a colometric assay in which the tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-trazolium (MTS) is reduced by viable cells into a soluble formazan product. The absorbance of the formazan is measured directly from the 96-well plates. The quantity of formazan product as measured by absorbance at 490nm is proportional to the number of viable cells. For performing this assay, 100 μl of media were removed from each well before adding 20 μl of MTS solution to each well. Plates were returned to the incubator and absorbance read by a Dynatech MR5000 microplate reader (Dynatech Laboratories) after a four-hour incubation. The data were collected and calculated as the percent difference between each treatment group and the matched control (no apoE/no NGF condition) for each experiment. The value for the no apoE/no NGF condition was set at 100%. The percent differences for the separate experiments were then averaged.

Example 5

ApoE Potentiates NGF Activity on Neurite Outgrowth

Another well-described activity of NGF is the ability of NGF to promote neurite outgrowth. Studies were carried out in PC12 cells to evaluate whether apoE would promote NGF activity in this regard. PC12 cells were first primed for neurite outgrowth by growing cells in 500 ng/ml NGF and serum for 7 days. At

this time, all cells possessed neurites greater than 1 cell body in diameter. These neurites were mechanically removed as the cells were replated in 96-well plates. This NGF priming allows for rapid growth of neurites in the experimental condition.

After 3 days in culture in serum-free medium with 100 ng/ml NGF and 1 μg/ml apoE3, apoE4 or BSA (as a control), phase images of cells were digitally collected. Measurements of neurites were made on random fields using IMAGE 1™ analysis software (Universal Imaging Corp.; West Chester, PA). ApoE3, but not apoE4, potentiates neurite outgrowth from primed PC12 cells (**Figure 2**). The average neurite outgrowth of cells grown in apoE3 plus NGF was 1.5-fold greater than in cells grown in BSA or apoE4.

Example 6

ApoE "Knockout" Mice Exhibit Peripheral Neuropathy

In three separate experiments, we analyzed the ultrastructure of the sciatic nerves from apoE knockout mice and control mice. ApoE knockout mice were generated using gene targeting in embryonic stem cells. Piedrahita *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89, 4471. Sciatic nerves from 12-week-old wildtype and knockout mice were carefully dissected, fixed by immersion in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide. Samples were rinsed, then dehydrated, in an ethanol gradient with final rinses in propylene oxide. The nerves were embedded in epoxy resin which was cured at 60°C for 24 hours. Eighty nanometer thin sections were cut on a diamond knife and picked up on formvar covered slot grids which made it possible to view the entire cross-section. Photomicrographs were made on a Phillips 410 electron microscope.

In all three experiments, a striking loss of unmyelinated fibers was observed, particularly around the perimeter of the nerve. Although the number of myelinated fibers between knockout and wildtype mice was essentially equal, the number of unmyelinated fibers is reduced by 50% in the apoE knockout mice (**Figure 3**). Furthermore, the morphology of the remaining unmyelinated fibers is highly abnormal. While the unmyelinated fibers in the wildtype are circular and are

clearly separated from each other by Schwann cell cytoplasm, the knockout fibers are irregularly shaped and are surround by very little Schwann cell cytoplasm. There is also a loss of the sharp distinction between the axons and Schwann cell cytoplasm in the knockout.

These abnormalities in apoE knockout mice are consistent with a role for apoE in potentiating NGF activity *in vivo*. The unmyelinated fibers within the sciatic nerve express trkA NGF receptors and are responsive to NGF. These fibers are lost from animals treated with anti-NGF antibodies and, following nerve transection, these neurons can be rescued by NGF. These results are also relevant to diabetic neuropathy, in which NGF-sensitive neurons are compromised.

Example 7

Neurite Outgrowth and Schwann Cell Migration from Neonatal Rat DRGs are Impaired on NGF Treated Distal Stumps from ApoE Knockout Mice

Neurite outgrowth and Schwann cell migration from neonatal rat dorsal root ganglia (DRGs) were measured on NGF-treated distal stumps from apoE knockout and wildtype mice. For these experiments, neonatal rat DRGs were explanted onto cryostat sections of distal stump from either apoE knockout or wildtype animals. Five days following transection, distal stumps were removed, fresh frozen in O.C.T. compound and cut into 20 μm thick longitudinal sections. Sections were incubated in 100 μl of 100 ng/ml NGF for 3 hours at 37°C. Nerves were then washed to remove unbound NGF and neonatal DRG explants were placed on the nerves and cultured in serum-free medium. Images of neurite outgrowth and Schwann cell migration were obtained after 72 hours using the vital dye carboxyfluorescein and fluorescence microscopy.

Both neurite outgrowth and Schwann cell migration are impaired on NGF-treated distal stumps of transected sciatic nerve prepared from apoE knockout mice. It has previously been established that NGF promotes both neurite outgrowth (see Example 3) and Schwann cell migration. Thus, these studies provide further evidence of the role of apoE in enhancing NGF action *in vivo*.

Example 8

ApoE Knockout Mice have a Delayed Response to Noxious Thermal Stimuli

Unmyelinated fibers are the fibers that relay pain and temperature sensitivity. Thus, the finding that apoE knockout animals have a substantial decrease in unmylenated fibers (Example 6) suggests that these animals might have a reduced response to noxious thermal stimuli. To test this, the withdrawal latency for wildtype and apoE knockout mice from a hot waterbath was measured.

Withdrawal latency was determined for wildtype and knockout mice to remove their hind feet from a 55°C water bath. As shown in Figure 4, apoE knockout mice exhibit over a 50% increase in their response latency ($p < 0.001$). A similar effect was observed with a tail withdrawal paradigm (data not shown).

These data further support a role for apoE in promoting the health and survival of unmyelinated fibers through interaction with NGF.

Example 9

Summary of Primary Findings

Supporting the Hypothesis that ApoE Potentiates NGF Activity

The data presented in the Examples above strongly suggest that apoE potentiates NGF activity. This evidence is summarized below:

- ApoE3, but not ApoE4, forms an SDS-stable complex with NGF
- Substrate-bound complexes of apoE3 and NGF potentiate the survival-promoting activity of NGF
- ApoE3, but not apoE4, potentiates the neurite-outgrowth activity of NGF
- NGF-dependent neurite outgrowth and Schwann cell migration are impaired on substrates from apoE knockout animals
- ApoE knockout animals have a 50% loss of unmyelinated, NGF-sensitive, sensory fibers
- ApoE knockout mice have a delayed response to noxious thermal stimuli

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

That Which is Claimed is:

1. A composition comprising a complex of apolipoprotein E and nerve growth factor.

2. A composition according to claim 1, wherein said composition further comprises a complex of apolipoprotein E and neurotrophin 4.

3. A composition according to claim 1, wherein said apolipoprotein E is a delipidated apolipoprotein E.

4. A composition according to claim 1, wherein said complex is a covalent complex.

5. A composition according to claim 1, wherein said complex is a noncovalent complex.

6. A composition according to claim 1, wherein said complex is bound to a substrate.

7. A composition according to claim 1, wherein said apolipoprotein E is apolipoprotein E2.

8. A composition according to claim 1, wherein said apolipoprotein E is apolipoprotein E3.

9. A composition according to claim 1, wherein said apolipoprotein E is apolipoprotein E4.

10. A composition according to claim 1, wherein said apolipoprotein E is human apolipoprotein E.

11. A composition according to claim 1, wherein said nerve growth factor is a human nerve growth factor.

12. A composition according to claim 1, wherein said composition is in a pharmacologically acceptable carrier.

13. A method of enhancing the survival of neural cells, comprising administering to the neural cells a survival-enhancing amount of a composition comprising a complex of apolipoprotein E and nerve growth factor.

14. A method according to claim 13, wherein the composition further comprises a complex of apolipoprotein E and neurotrophin 4.

15. A method according to claim 13, wherein the apolipoprotein E is a delipidated apolipoprotein E.

16. A method according to claim 13, wherein the complex is a covalent complex.

17. A method according to claim 13, wherein the complex is a noncovalent complex.

18. A method according to claim 13, wherein the apolipoprotein E is apolipoprotein E2.

19. A method according to claim 13, wherein the apolipoprotein E is apolipoprotein E3.

20. A method according to claim 13, wherein the apolipoprotein E is apolipoprotein E4.

21. A method according to claim 13, wherein the apolipoprotein E is human apolipoprotein E.

22. A method according to claim 13, wherein the nerve growth factor is a human nerve growth factor.

23. A method according to claim 13, wherein the administration is carried out *in vitro*.

24. A method according to claim 23, wherein the complex is bound to a substrate.

25. A method according to claim 24, wherein the apolipoprotein E is bound to the substrate before forming the complex with the nerve growth factor.

26. A method according to claim 13, wherein the administration is carried out *in vivo*.

27. A method according to claim 26, wherein the neural cells are human neural cells.

28. A method according to claim 27, wherein the neural cells are in a human subject afflicted with a neurodegenerative disease.

29. A method according to claim 27, wherein the neural cells are in a human subject who has experienced injury or trauma to neural tissue.

30. A method according to claim 27, wherein the neural cells are in a human subject afflicted with a condition selected from the group consisting of Alzheimer's Disease, Parkinson's disease, peripheral nerve injury, peripheral neuropathy, amyotrophic lateral sclerosis, head injury, and stroke.

31. A method of administering a composition comprising a complex of apolipoprotein E and nerve growth factor in a therapeutically-effective amount to a subject in need thereof.

32. A method according to claim 31, wherein the complex is a covalent complex.

33. A method according to claim 31, wherein the complex is a noncovalent complex.

34. A method according to claim 31, wherein the apolipoprotein E is apolipoprotein E2.

35. A method according to claim 31, wherein the apolipoprotein E is apolipoprotein E3.

36. A method according to claim 31, wherein the apolipoprotein E is apolipoprotein E4.

37. A method according to claim 31, wherein the apolipoprotein E is human apolipoprotein E.

38. A method according to claim 31, wherein the nerve growth factor is human nerve growth factor.

39. A method according to claim 31, wherein the subject is a human subject.

40. A method according to claim 39, wherein the human subject is afflicted with a neurodegenerative disease.

41. A method according to claim 39, wherein the human subject has experienced injury or trauma to neural tissue.

42. A method according to claim 39, wherein the human subject is afflicted with a condition selected from the group consisting of Alzheimer's Disease, Parkinson's disease, peripheral nerve injury, peripheral neuropathy, amyotrophic lateral sclerosis, head injury, and stroke.

43, A composition comprising a complex of apolipoprotein E and neurotrophin 4.

44. A composition according to claim 43, wherein said composition is in a pharmaceutically acceptable carrier.

45. A method of enhancing the survival of neural cells, comprising administering to said neural cells a survival-enhancing amount of a composition comprising a complex of apolipoprotein E and neurotrophin 4.

46. A method of administering a composition comprising a complex of apolipoprotein E and neurotrophin-4 in a therapeutically-effective amount to a subject in need thereof.

47. A composition comprising a complex of apolipoprotein E and γ-interferon.

48. A composition according to claim 47, wherein said composition is in a pharmacologically acceptable carrier.

49. A method of protecting cells against virus infection, comprising administering to the cells an anti-virus effective amount of a composition comprising a complex of apolipoprotein E and γ-interferon.

50. A method of administering an anti-viral effective amount of a composition comprising a complex of apolipoprotein E and γ-interferon to a subject in need thereof.

51. A method according to claim 50, wherein the subject is afflicted with a virus infection.

52. A method according to claim 50, wherein the subject is at risk of developing a virus infection.

53. A method of administering an anti-tumor effective amount of a composition comprising a complex of apolipoprotein E and γ-interferon to a subject in need thereof.

54. A method according to claim 53, wherein the subject is afflicted with a tumor.

55. A method according to claim 54, wherein the tumor is malignant.

56. A method according to claim 53, wherein the subject is at risk of developing a tumor.

57. A method of administering a composition comprising a complex of apolipoprotein E and γ-interferon in a therapeutically-effective amount to a subject in need thereof.