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(54) **METHOD OF TREATING CORNEAL TRANSPLANT REJECTION**

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(58) **Field of Classification Search** None
See application file for complete search history.

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(57) **ABSTRACT**

Methods of preventing, reducing, or treating corneal transplant rejection to improve transplant survival in a subject in need thereof comprising administering an agent capable of blocking or inhibiting vascular endothelial growth factor (VEGF) are provided. The methods are useful for inhibiting or preventing corneal transplant rejection in a human subject who is the recipient of a transplanted cornea.

8 Claims, No Drawings

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METHOD OF TREATING CORNEAL TRANSPLANT REJECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 USC § 119(e) of U.S. Provisionals 60/473,734 filed 28 May 2003 and 60/492,865 filed 6 Aug. 2003, which applications are herein specifically incorporated by reference in their entirety.

BACKGROUND

1. Field of the Invention

The field of the invention is related to methods of using VEGF antagonists to reduce, prevent, or treat corneal transplant rejection, thus improving long-term transplant survival.

2. Description of Related Art

It has previously been reported that topical application of an anti-VEGF neutralizing antibody suppresses acute allograft rejection in a rat corneal transplant model (Yatoh et al. (1998) Transplantation 66(11):1519-24).

BRIEF SUMMARY OF THE INVENTION

The invention is based in part on the finding that administration of an agent capable of blocking or inhibiting vascular endothelial growth factor (VEGF) prevents corneas transplant rejection. The experiments, described below, conducted in an animal model of corneal transplantation show that long-term transplant survival is promoted by blocking VEGF-mediated activity.

In a first aspect, the invention features a method of improving transplant survival in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that transplant survival is improved.

In specific embodiments, the agent capable of blocking, inhibiting, or ameliorating VEGF-mediated activity is a VEGF antagonist. The VEGF antagonist may be a polypeptide, an antibody, a small molecule, or a nucleic acid. More specifically, the VEGF antagonist includes a VEGF trap selected from the group consisting of acetylated Flt-1(1-3)-Fc, Flt-1(1-3_{R→N})-Fc, Flt-1(1-3_{ΔB})-Fc, Flt-1(2-3_{ΔB})-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a). In a specific and preferred embodiment, the VEGF trap is VEGFR1R2-FcΔC1(a) (also termed VEGF trap_{R1R2}) having the nucleotide sequence set forth in SEQ ID NO: 1 and the amino acid sequence set forth in SEQ ID NO: 2. The invention encompasses the use of a VEGF trap that is at least 90%, 95%, 98%, or at least 99% homologous with the nucleotide sequence set forth in SEQ ID NO: 1 and/or the amino acid sequence set forth in SEQ ID NO:2.

In other embodiments, the agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity is a nucleic acid-based antagonist capable of interfering with the expression of VEGF. A specific example of this embodiment is one in which the nucleic acid-based antagonist is an aptamer, an siRNA, or an antisense molecule.

Administration of the agent may be by any method known in the art, including subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous, intranasal, oral, or topical routes of administration. Preferable, administration to the

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subject in need of the agent is topical administration to the eye or subconjunctival administration. Administration may occur prior to or following corneal transplantation, preferably following surgery. Administration may also include a second agent, such as an immunosuppressive agent.

The subject to be treated is preferably a human subject who has or will receive a corneal transplant.

In a second aspect, the invention features a method of preventing corneal transplant rejection in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that corneal transplant rejection is prevented.

In a third aspect, the invention features a method of reducing the incidence of corneal transplant rejection in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that the incidence of corneal transplant rejection is reduced.

In a fourth related aspect, the invention features a method of treating corneal transplant rejection in a subject in need thereof, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating vascular endothelial growth factor (VEGF)-mediated activity, such that corneal transplant rejection is treated.

In a fifth aspect, the invention features a pharmaceutical composition comprising a VEGF antagonist, for example the VEGF trap VEGFR1R2-FcΔC1(a), in a pharmaceutically acceptable carrier. Such pharmaceutical compositions may be liquid, gel, ointment, salve, slow release formulations or other formulations suitable for ophthalmic administration.

Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

General Description

Experiments were undertaken to evaluate occurrence and time course of hem- and lymphangiogenesis after normal-risk corneal transplantation and to test whether pharmaco-

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logic strategies inhibiting both processes improve long-term graft survival. As described in the experimental section below, normal-risk allogeneic (C57BL/6 to BALB/c) and syngeneic (BALB/c to BALB/c) corneal transplantations were performed and occurrence and time course of hem- and lymphangiogenesis after keratoplasty was observed using double immunofluorescence of corneal flatmounts (with CD31 as panendothelial and LYVE-1 as lymphatic vascular endothelial specific marker). A molecular trap designed to eliminate VEGF-A ("VEGF Trap_{R1R2}"; 12.5 mg/kg) was tested for its ability to inhibit both processes after keratoplasty and to promote long-term graft survival (intraperitoneal injections on the day of surgery and 3, 7, and 14 days later). The results show that no blood or lymph vessels were detectable immediately after normal-risk transplantation in either donor or host cornea, but hem- and lymphangiogenesis were clearly visible at day 3 after transplantation. Both vessel types reached donor tissue at one week after allo- and similarly after syngeneic grafting. Early postoperative trapping of VEGF-A significantly reduced both hem- and lymphangiogenesis and significantly improved long-term graft survival (78% versus 40%; $p < 0.05$). There is concurrent, VEGF-A-dependent hem- and lymphangiogenesis after normal-risk keratoplasty within the preoperatively avascular recipient bed. Inhibition of hem- and lymphangiogenesis (which mediate the efferent and afferent arms of an immune response) after normal-risk corneal transplantation improves long-term graft survival, establishing that early postoperative hem- and lymphangiogenesis are risk factors for graft rejection even in low-risk eyes.

Definitions

By the term "therapeutically effective dose" is meant a dose that produces the desired effect for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) *The Art, Science and Technology of Pharmaceutical Compounding*).

By the term "blocker", "inhibitor", or "antagonist" is meant a substance that retards or prevents a chemical or physiological reaction or response. Common blockers or inhibitors include but are not limited to antisense molecules, antibodies, antagonists and their derivatives. More specifically, an example of a VEGF blocker or inhibitor is a VEGF receptor-based antagonist including, for example, an anti-VEGF antibody, or a VEGF trap such as VEGFR1R2-FcΔC1(a) (SEQ ID NOs:1-2). For a complete description of VEGF-receptor based antagonists including VEGFR1R2-FcΔC1(a), see PCT publication WO/00/75319, the contents of which is incorporated in its entirety herein by reference.

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons, and may include chemical as well as peptide molecules.

VEGF Antagonists

In one aspect of the invention, VEGF-mediated activity is blocked or inhibited by the use of VEGF receptor-based blockers of VEGF-mediated activity. A non-limiting example of a VEGF receptor-based blocker includes, but is not limited to, VEGFR1R2-FcΔC1(a). Other suitable receptor-based blockers include acetylated Flt-1(1-3)-Fc, Flt-1(1-3_{R->N})-Fc, Flt-1(1-3_{ΔB})-Fc, Flt-1(2-3_{ΔB})-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a). For a complete description of these and other VEGF-receptor-based blockers, including pegylated receptor-based

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blockers, see PCT Publication No. WO/00/75319, the contents of which is incorporated in its entirety herein by reference.

In addition to the VEGF receptor-based blockers described in PCT Publication No. WO/00/75319, variants and derivatives of such VEGF receptor-based blockers are also contemplated by the invention. The sequence of the variants or derivatives may differ by a change which is one or more additions, insertions, deletions and/or substitutions of one or more nucleotides of the sequence set forth in SEQ ID NO:1. Changes to a nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code. Thus, nucleic acid according to the present invention may include a sequence different from the sequence shown in SEQ ID NO:1, yet encode a polypeptide with the same amino acid sequence as SEQ ID NO:2. On the other hand, the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in SEQ ID NO:2. Nucleic acid encoding a polypeptide which is an amino acid sequence variant or derivative of the sequence shown in SEQ ID NO:2 is further provided by the present invention. Nucleic acid encoding such a polypeptide may show at the nucleotide sequence and/or encoded amino acid level greater than about 90%, 95%, 98%, or 99% homology with the coding sequence shown in SEQ ID NO:1 and/or the amino acid sequence shown in SEQ ID NO:2. For amino acid "homology", this may be understood to be similarity (according to the established principles of amino acid similarity, e.g. as determined using the algorithm GAP (Genetics Computer Group, Madison, Wis.)) or identity. GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, the default parameters are used, with a gap creation penalty=12 and gap extension penalty=4.

Individual components of the VEGF-specific fusion proteins of the invention may be constructed by molecular biological methods known to the art with the instructions provided by the instant specification. These components are selected from a first cellular receptor protein, such as, for example, VEGFR1; a second cellular receptor protein, such as, for example, VEGFR2; a multimerizing component, such as an Fc.

Specific embodiments of the VEGF-specific fusion proteins useful in the methods of the invention comprise a multimerizing component which allows the fusion proteins to associate, e.g., as multimers, preferably dimers. Preferably, the multimerizing component comprises an immunoglobulin derived domain. Suitable multimerizing components are sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al. 1982 *Cell* 29:671-679); immunoglobulin gene sequences, and portions thereof.

The nucleic acid constructs encoding the fusion proteins useful in the methods of the invention are inserted into an expression vector by methods known to the art, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Host-vector systems for the production of proteins comprising an expression vector introduced into a host cell suitable for expression of the protein are known in the art. The suitable host cell may be a bacterial cell such as *E. coli*, a yeast cell, such as *Pichia pastoris*, an insect cell, such as *Spodoptera frugiperda*, or a mammalian cell, such as a COS, CHO, 293, BHK or NS0 cell.

Antisense Nucleic Acids

In one aspect of the invention, VEGF-mediated activity is blocked or inhibited by the use of VEGF antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to a gene or cDNA encoding VEGF or a portion thereof. As used herein, a VEGF "antisense" nucleic acid refers to a nucleic acid capable of hybridizing by virtue of some sequence complementarity to a portion of an RNA (preferably mRNA) encoding VEGF. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an mRNA encoding VEGF. Such antisense nucleic acids have utility as compounds that prevent VEGF expression, and can be used in the treatment or prevention of corneal transplant rejection. The antisense nucleic acids of the invention are double-stranded or single-stranded oligonucleotides, RNA or DNA or a modification or derivative thereof, and can be directly administered to a cell or produced intracellularly by transcription of exogenous, introduced sequences.

The VEGF antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 50 oligonucleotides. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded. In addition, the antisense molecules may be polymers that are nucleic acid mimics, such as PNA, morpholino oligos, and LNA. Other types of antisense molecules include short double-stranded RNAs, known as siRNAs, and short hairpin RNAs, and long dsRNA (>50 bp but usually ≥ 500 bp).

Short Interfering RNAs

In another embodiment, VEGF-mediated activity is blocked by blocking VEGF expression. One method for inhibiting VEGF expression is the use of short interfering RNA (siRNA) through RNA interference (RNAi) or post-transcriptional gene silencing (PTGS) (see, for example, Ketting et al. (2001) *Genes Develop.* 15:2654-2659). siRNA molecules can target homologous mRNA molecules for destruction by cleaving the mRNA molecule within the region spanned by the siRNA molecule. Accordingly, siRNAs capable of targeting and cleaving homologous VEGF mRNA are useful for treating, reducing or preventing corneal transplant rejection.

Inhibitory Ribozymes

In aspect of the invention, corneal transplant rejection may be treated or prevented in a subject suffering from such disease by decreasing the level of VEGF activity by using ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding VEGF, preventing translation of target gene mRNA and, therefore, expression of the gene product.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, e.g., U.S. Pat. No. 5,093,246. While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy mRNAs encoding VEGF, the use of hammerhead ribozymes

is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA has the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art. The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA). The Cech-type ribozymes have an eight base pair active site that hybridizes to a target RNA sequence where after cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences that are present in the gene encoding VEGF.

Generation of Antibodies to VEGF Proteins

In another aspect of the invention, the invention may be practiced with an anti-VEGF antibody or antibody fragment capable of binding and blocking VEGF activity. Anti-VEGF antibodies are disclosed, for example, in U.S. Pat. No. 6,121,230, herein specifically incorporated by reference. The term "antibody" as used herein refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant regions, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. Within each IgG class, there are different isotypes (eg. IgG₁, IgG₂, etc.). Typically, the antigen-binding region of an antibody will be the most critical in determining specificity and affinity of binding.

Antibodies exist as intact immunoglobulins, or as a number of well-characterized fragments produced by digestion with various peptidases. For example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)₂, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)₂ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)₂ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region. While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the terms antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv)(scFv) or those identified using phase display libraries (see, for example, McCafferty et al. (1990) *Nature* 348:552-554).

Methods for preparing antibodies are known to the art. See, for example, Kohler & Milstein (1975) *Nature* 256: 495-497; Harlow & Lane (1988) *Antibodies: a Laboratory Manual*, Cold Spring Harbor Lab., Cold Spring Harbor, N.Y.). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light

chain gene products generate a large pool of antibodies with different antigenic specificity. Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Pat. No. 4,946,778; U.S. Pat. No. 4,816,567) can be adapted to produce antibodies used in the fusion proteins and methods of the instant invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express human or humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens.

Antibody Screening and Selection

Screening and selection of preferred antibodies can be conducted by a variety of methods known to the art. Initial screening for the presence of monoclonal antibodies specific to a target antigen may be conducted through the use of ELISA-based methods, for example. A secondary screen is preferably conducted to identify and select a desired monoclonal antibody for use in construction of the multi-specific fusion proteins of the invention. Secondary screening may be conducted with any suitable method known to the art. One preferred method, termed "Biosensor Modification-Assisted Profiling" ("BiaMAP") is described in co-pending U.S. Ser. No. 60/423,017 filed 1 Nov. 2002, herein specifically incorporated by reference in its entirety. BiaMAP allows rapid identification of hybridoma clones producing monoclonal antibodies with desired characteristics. More specifically, monoclonal antibodies are sorted into distinct epitope-related groups based on evaluation of antibody: antigen interactions.

Treatment Population

A suitable subject for treatment by the method of the invention is a human who has received or will receive a corneal transplant. Corneal transplantation is the oldest, most successful and most commonly performed tissue transplantation, with nearly 40,000 transplantations a year alone in the US. When corneal grafts are placed into an avascular recipient bed (so-called normal-risk keratoplasty), 2-year graft survival rates approach 90% under cover of topical steroids, even without HLA-matching. This very successful outcome is attributed to corneal immune privilege, i.e. the phenomenon of suppressed corneal inflammation induced by an array of endogenous mechanisms downregulating alloimmune and inflammatory responses in the cornea and its bed. These mechanisms include the lack of both afferent lymphatic and efferent blood vessels in the normal-risk recipient cornea, lack of MHC II⁺ antigen presenting cells (APCs), FasL-expression on corneal epithelium and endothelium, and the anterior chamber associated immune privilege (ACAID) directed at graft antigens etc. (Streilein et al. (1999) *Transplant Proc.* 31:1472-1475).

In contrast, survival rates of cornea grafts placed into vascularized, not immune-privileged recipient beds (so called high-risk keratoplasty) drop significantly to below 50% (even with local and systemic immune suppression). Pre-existing corneal stromal blood vessels have been identified as strong risk factors for immune rejection after corneal transplantation, both in the clinical setting as well as in the well-defined mouse model of corneal transplantation (Sano et al. (1995) *Invest. Ophthalmol. Vis. Sci.* 36:2176-85). Recently, in addition to blood vessels, biomicroscopically undetectable lymphatic vessels have been found in association with blood vessels in vascularized high-risk human corneas (Cursiefen et al. (2003) *Cornea.* 22:273-81) and it is likely that corneal lymphatic vessels enable effective access of donor and host APCs and antigenic material to

regional lymph nodes where accelerated sensitisation to graft antigens occurs (Liu et al. (2002) *J. Exp. Med.* 195: 259-68) even in the normal-risk setting (with a preoperatively avascular recipient bed), where mild corneal hemangiogenesis develops after keratoplasty. Outgrowth of new blood vessels from the limbal arcade towards the graft can be observed within the first postoperative year in about 50% of patients undergoing normal-risk keratoplasty, and in 10% of patients these new blood vessels even reach the interface or invade donor tissue (Cursiefen et al. (2001) *Graefes Arch. clin. Exp. Ophthalmol.* 39:514-21) at corneal suture sites, and then proceed centrally.

Methods of Administration

The invention provides methods of treatment comprising administering to a subject an effective amount of an agent of the invention. In a preferred aspect, the agent is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, e.g., such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and can be used to administer an active agent of the invention, e.g., delivery systems suitable for topical administration, preferably topical administration directly to the eye, or subconjunctival administration, as well as other delivery systems such as those that utilize encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction are preferably topical or subconjunctival, but may be enteral or parenteral including but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The active agents may be administered by any convenient route, for example by absorption through epithelial (e.g. topical administration to the eye) or mucocutaneous linings (e.g., oral mucosa, intestinal mucosa, etc.) or infusion or bolus injection, and may be administered together with other biologically active agents. Administration can be systemic or local. Administration can be acute or chronic (e.g. daily, weekly, monthly, etc.) or in combination or alteration with other agents. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In another embodiment, the active agent can be delivered in a vesicle, in particular a liposome (see Langer (1990) *Science* 249:1527-1533). In yet another embodiment, the active agent can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer (1990) supra). In another embodiment, polymeric materials can be used (see Howard et al. (1989) *J. Neurosurg.* 71:105). In another embodiment where the active agent of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see, for example, U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:1864-1868), etc. Alternatively, a nucleic acid

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