Title: COMPOSITION AND METHOD FOR USING A CYTOKINE TO TREAT RESTENOSIS

Abstract: The present invention provides devices, including stents and vascular catheters, comprising interleukin-10, for the treatment of restenosis and intimal hyperplasia associated with acute vascular injury. Also provided are methods for treating restenosis and preventing vascular obstruction.
COMPOSITION AND METHOD FOR USING A CYTOKINE TO TREAT RESTENOSIS

CROSS-REFERENCE TO RELATED APPLICATION

The present application is a continuation-in-part of U.S. Provisional Application No. 60/622,482 as filed on October 26, 2004, the disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally for devices and methods for treating restenosis and vascular hyperplasia. More specifically, the invention relates to methods and devices which include interleukin-10 for treating restenosis and vascular hyperplasia.

BACKGROUND OF THE INVENTION

Intimal hyperplasia is a universal response of the arterial wall to mechanical injury, and it is a major cause of restenosis after percutaneous coronary interventions. The multifactorial pathophysiology of restenosis remains as yet not entirely defined. Emerging experimental and clinical data indicate that inflammation is of major importance to the restenotic process. Inflammatory cells are activated promptly after vascular injury and recruited to the site of injury (Hancock W, 1994, Welt FGP 2000, Wilensky R 1995, Serrano C, 1997, Assoian RK, 1987, Moreno, PR, 1996, Schillinger M, 2003, Fakuda et al, 2004, colombo A, 2004]. These cells are capable of releasing mediators such as TNF and other pro-inflammatory cytokines that facilitate VSMC migration and proliferation on one hand and inhibition of endothelial cell proliferation and re-endothelialization on the other (Bendek MP, 1994, Libby, P, 1992, Danenberg, 2002, G. Peri G, 1990, Krasinski, 2001, Kishore, 2002). In human studies, the severity of postangioplasty luminal loss has been found to correlate with activation of circulating leukocytes, (Pietersma A, 1995) and restenosis in patients undergoing directional atherectomy has been determined to be correlated with the percentage of macrophages in retrieved tissue at the time of angioplasty (Moreno, PR, 1996). Recent reports also suggest that VSMC proliferation may not be the key event in lesion formation and that inflammatory cells may play a more significant role [Welt FGP 2000, Wilensky R
Monocytes have been hypothesized to serve as markers, initiators, and promoters of arterial occlusive disease. In particular, activated monocytes may contribute to neointimal thickening (Moreno PR, 1996, ) through the production of pro-inflammatory cytokines like TNF and IL-6 and chemotactic factors which activates other effector cells like VSMC and EC to produce additional monocyte chemoattractants like MCP-1 facilitating accumulation, infiltration and adhesion of monocytes to these cells. Furthermore, monocytes have been implicated to contribute in neointima formation by generating reactive oxygen species (Assoian RK, 1987) binding to a broad repertoire of ligands (Languino LR, 1995) and/or by matrix metalloprotease production capable of degrading matrix constituents and consequently facilitating VSMC migration (Sukhova GK, 1998). Several experimental studies in different animal models suggest that intimal inflammation is also a determinant of in-stent neointimal growth (Bayes-Genis A, 2002, Danenberg HD, 2002, C. Rogers C, 1995, Rogers C, 1996) Furthermore, a number of studies demonstrate that the blockade of inflammation and cell adhesion molecules important for neutrophil recruitment attenuates neointimal growth M.K. Barron, 1997, C. Rogers, 1998, M. Usui, 2002, Mori E, 2002, Lumsden AB, 1997) Danenberg et al. (Danenberg, 2002b) recently demonstrated that the systemic inaction and depletion of monocytes and macrophages by liposomal clodronate in animal models reduced neointimal hyperplasia and restenosis.

In view of the above, there exists a need in the art for methods and compositions which can reduce inflammation and hyperplasia in response to vascular injury.

**SUMMARY OF THE INVENTION**

The present invention is based, at least in part, in on the discovery that interleukin-10 (also referred to herein as "IL-10") can prevent restenosis and/or vascular hyperplasia.

Accordingly, in one embodiment, the invention included a device suitable for implantation in a patient, the device comprising a body having open ends, a sidewall and a polymer on at least a part of a surface of the sidewall, wherein the polymer comprises a therapeutically sufficient amount of mammalian interleukin-10; or a portion or variant thereof. In a preferred embodiment, the device includes a stent, catheter, graft, pump, needle, or a suture, and may optionally comprising a graft material. In another preferred embodiment, the polymeric material is a biostable, biocompatible, and non-thrombogenic material. In a further preferred embodiment, the polymeric material is a swellable hydrogel
polymer and/or a hydrophobic polymer, including, but not limited to, a polymer selected from the group consisting of hysilicones, polysiloxanes, substituted polysiloxanes, polyurethanes, thermoplastic elastomers, ethylene vinyl acetate copolymers, polyolefin elastomers, polyamide elastomers, EPDM rubbers, fluorosilicones, polyethylene glycol (PEG), polysaccharides, phospholipids, polycarboxylic acids, cellulosic polymers, gelatin, polyvinylpyrrolidone, maleic anhydride polymers, polyamides, polyvinyl alcohols, polyacrylic acid, and polyethylene oxides and combinations thereof. In one embodiment, the polymer is adhered to the stent. In a preferred embodiment, the interleukin-10 or portion or variant thereof and said polymer in a coating upon said device is capable of slow time release of interleukin-10 or portion or variant thereof.

In another embodiment, the device comprises an expandable portion, e.g., a balloon-expandable stent, wherein said expandable portion is a stent positioned about said balloon. In one embodiment, the stent and balloon both include said polymer coating incorporating said interleukin-10 or a portion or variant thereof. In another embodiment, the expandable portion is a dilatation balloon expandable to pressures in the range of about 1 to 20 atmospheres.

In a preferred embodiment, the interleukin-10 comprises the amino acid sequence of SEQ ID NO:2, or a portion or variant thereof. In a further preferred embodiment, the interleukin-10 comprises a polypeptide which is at least about 60% identical to the amino acid sequence of SEQ ID NO:2, or a polypeptide which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:2.

In another embodiment, the device further comprises at least one agent selected from the group consisting of: antithrombotics, anticoagulants, antibiotics, antiplatelet agents, thrombolitics, antiproliferatives, steroidal and nonsteroidal antiinflammatories, agents that inhibit hyperplasia, agents that inhibit restenosis, smooth muscle cell inhibitors, growth factors, growth factor inhibitors, cell adhesion inhibitors, chemotherapeutic agents, and combinations thereof. In a further embodiment, the antithrombotic is selected from the group consisting of heparin, PPACK, enoxaprin, aspirin, hirudin, and combinations thereof. In another embodiment, the antiproliferative is selected from the group consisting of monoclonal antibodies capable of blocking smooth muscle cell proliferation, heparin, angiopeptin, enoxaprin, and combinations thereof. In still another embodiment, the chemotherapeutic agent is taxol.
In another embodiment, the invention includes a vascular catheter for delivering mammalian interleukin-10 or a portion or variant thereof into tissue or occlusive formation at a desired location, comprising a catheter constructed for insertion into a vascular lumen having a catheter shaft and an expandable portion mounted on said catheter shaft, and at least a part of the exterior surface of the expandable portion comprises interleukin-10 or a portion or variant thereof. In a preferred embodiment, the desired location is the vascular lumen. In another preferred embodiment, the expandable portion is expandable in response to controlled inflation pressure to fill the cross-section of the vascular lumen and engage the tissue or occlusive formation of said vascular lumen.

In a preferred embodiment, the interleukin-10 or portion or variant thereof is contained in a polymer coating. In one embodiment, the polymer is a swellable hydrogel polymer and/or a hydrophobic polymer, including, but not limited to, a polymer is selected from the group consisting of: hydrosilicones, polysiloxanes, substituted polysiloxanes, polyurethanes, thermoplastic elastomers, ethylene vinyl acetate copolymers, polyolefin elastomers, polyamide elastomers, EPDM rubbers, fluorosilicones, polyethylene glycol (PEG), polysaccharides, phospholipids, and combinations thereof. In one embodiment, the polymer is adhered to the catheter.

In one embodiment, the catheter is a dilatation catheter sized, constructed and arranged for insertion in a stenosed vascular lumen, and said expandable portion is an inflatable dilatation balloon adapted for brief inflation at pressures in the range for effecting widening of said stenosed vascular lumen, said elevated compressive pressure being in a range effective to simultaneously cause, with said compression of said hydrogel an administration of said interleukin-10 or a portion or variant thereof, widening of said vascular lumen. In a preferred embodiment, the hydrogel polymer is selected from the group consisting of polycarboxylic acids, cellulotic polymers, gelatin, polyvinylpyrrolidone, maleic anhydride polymers, polyamides, polyvinyl alcohols, polyethylene oxides, polyacrylic acid and combinations thereof.

In a preferred embodiment, the interleukin-10 comprises the amino acid sequence of SEQ ID NO:2, or a portion or variant thereof. In a further preferred embodiment, the interleukin-10 comprises a polypeptide which is at least about 60% identical to the amino acid sequence of SEQ ID NO:2, or a polypeptide which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:2.
In another embodiment, the vascular catheter further comprises at least one agent selected from the group consisting of: antithrombotics, anticoagulants, antibiotics, antiplatelet agents, thrombolytics, antiproliferatives, steroidal and nonsteroidal antiinflammatories, agents that inhibit hyperplasia, agents that inhibit restenosis, smooth muscle cell inhibitors, growth factors, growth factor inhibitors, cell adhesion inhibitors, chemotherapeutic agents, and combinations thereof. In a further embodiment, the antithrombotic is selected from the group consisting of heparin, PPACK, enoxaprin, aspirin, hirudin, and combinations thereof. In another embodiment, the antiproliferative is selected from the group consisting of monoclonal antibodies capable of blocking smooth muscle cell proliferation, heparin, angiopeptin, enoxaprin, and combinations thereof. In still another embodiment, the chemotherapeutic agent is taxol.

In another embodiment, the expandable portion is adapted for application of heat to said polymer material to control the rate of administration. In a further embodiment, the catheter further comprises a member, extending over said expandable portion to inhibit release of said interleukin-10 or a portion or variant thereof into body fluids during placement of said catheter. In a preferred embodiment, the expandable portion is a retractable sheath. In another embodiment, the expandable portion is constructed to withstand sufficient inflation pressure that application of said pressure to rapidly administer said interleukin-10 or portion or variant thereof simultaneously facilitates delivery of said interleukin-10 or portion or variant thereof into and through cracks in plaque formed by balloon angioplasty to reach smooth muscle tissue. In a preferred embodiment, said pressure is in the pressure range of about 1 to 20 atmospheres. In another preferred embodiment, polymer is effective to release about 20% or more of said interleukin-10 or a portion or variant thereof during inflation in said pressure range.

In another embodiment, said expandable portion is constructed to withstand sufficient inflation pressure that application of said pressure to rapidly administer said interleukin-10 or a portion or variant thereof simultaneously facilitates delivery of said interleukin-10 or a portion or variant thereof into tissue or occlusive formation that lies deeper than the surface portion of the tissue or occlusive formation directly engaged by said expandable portion.

In another embodiment, the response characteristic of said hydrogel to said compressive pressure is effective to deliver said dose of said interleukin-10 or a portion or variant thereof over a duration of about 10 minutes or less. In a further embodiment, said hydrogel polymer coating is about 10 to 50 microns thick in the swelled, uncompressed state.
In another embodiment, said expandable portion includes a stent, placeable in said body lumen by expansion thereof. In another embodiment, the hydrogel releases about 50% of the interleukin-10 or a portion or variant thereof upon compression. In yet another embodiment, the interleukin-10 or a portion or variant thereof is precipitated into the hydrogel. In still another embodiment, said catheter is constructed as a perfusion catheter having an expandable balloon.

In another embodiment, wherein said expandable portion is constructed to withstand sufficient inflation pressure that application of said pressure to rapidly administer said interleukin-10 or portion or variant thereof simultaneously facilitates delivery of said interleukin-10 or portion or variant thereof into tissue or occlusive formation that lies deeper than the surface portion of the tissue or occlusive formation directly engaged by said expandable portion. In another embodiment, said expandable portion includes a stent, placeable in said body lumen by expansion thereof. In a further embodiment, the vascular catheter includes a binding of said interleukin-10 or a portion or variant thereof and hydrogel polymer in a coating upon said stent for slow time release of interleukin-10 or portion or variant thereof remaining in said hydrogel polymer on said stent after said compression of said hydrogel polymer by said expansion. In a further embodiment, said hydrogel polymer coating on said stent is a polyacrylic acid including an ammonium anion and said binding is an electrostatic binding. In a further embodiment, said stent is a balloon-expandable stent and said expandable portion is a stent positioned about said balloon. In still a further embodiment, said stent and balloon both include said swellable hydrogel coating incorporating said interleukin-10 or a portion or variant thereof.

In another embodiment, the invention provides a vascular balloon catheter for rapidly delivering a dose of mammalian interleukin-10 or portion or variant thereof into tissue or occlusive formation at a desired location of the wall of a vascular lumen, comprising: a catheter constructed for insertion into a vascular lumen having a catheter shaft and an expandable dilatation balloon mounted on said catheter shaft, wherein at least a portion of the exterior surface of the expandable balloon comprises said dose of said interleukin-10 or portion or variant thereof. In one embodiment, the interleukin-10 or portion thereof is contained in a polymer coating. In a preferred embodiment, the polymer is selected from the group consisting of: a swellable hydrogel polymer and a hydrophobic polymer. In another preferred embodiment, the polymer is further selected from the group consisting of: hysilicones, polysiloxanes, substituted polysiloxanes, polyurethanes, thermoplastic
elastomers, ethylene vinyl acetate copolymers, polyolefin elastomers, polyamide elastomers, EPDM rubbers, fluorosilicones, polyethylene glycol (PEG), polysaccharides, phospholipids, and combinations thereof. In another preferred embodiment, said hydrogel polymer is selected from the group consisting of polycarboxylic acids, cellulosic polymers, gelatin, polyvinylpyrrolidone, maleic anhydride polymers, polyamides, polyvinyl alcohols, polyethylene oxides, and combinations thereof. In one embodiment, the polymer is adhered to the catheter. In still another preferred embodiment, said hydrogel polymer is polyacrylic acid.

In one embodiment, said expandable balloon is expandable by an expansion controller to press against said tissue or occlusive formation at a compressive pressure in the range of about 1 to 20 atmospheres. In a further embodiment, said compressive pressure is elevated above the inflation pressure needed to fill the cross-section of the vascular lumen and engage the tissue or occlusive formation of said vascular lumen.

In a further embodiment, said catheter further comprises a sheath member, extendable over said balloon to inhibit release of said interleukin-10 or portion or variant thereof into body fluids during placement of said catheter.

In a preferred embodiment, said interleukin-10 comprises the amino acid sequence of SEQ ID NO:2, or a portion or variant thereof. In a further preferred embodiment, said portion or variant thereof comprises a polypeptide selected from the group consisting of: a polypeptide which is at least about 60% identical to the amino acid sequence of SEQ ID NO:2; and a polypeptide which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:2.

In another embodiment, the catheter further comprises at least one agent selected from the group consisting of: antithrombotics, anticoagulants, antibiotics, antiplatelet agents, thrombolytics, antiproliferatives, steroidal and nonsteroidal antiinflammatories, agents that inhibit hyperplasia, agents that inhibit restenosis including restenosis preventatives, smooth muscle cell inhibitors, growth factors, growth factor inhibitors, cell adhesion inhibitors, chemotherapeutic agents, and combinations thereof. In a preferred embodiment, the antithrombotic is selected from the group consisting of heparin, PPACK, enoxaparin, aspirin, hirudin, and combinations thereof. In another preferred embodiment, the antiproliferative is selected from the group consisting of monoclonal antibodies capable of blocking smooth muscle cell proliferation, heparin, angiotensin, enoxaprin, and combinations thereof. In still another preferred embodiment, the chemotherapeutic agents is taxol.
In one embodiment, the hydrogel releases about 50% of the interleukin-10 or portion or variant thereof upon compression. In another embodiment, the hydrogel includes up to about 20-30 mg of aqueous solution of interleukin-10 or portion or variant thereof. In yet another embodiment, the interleukin-10 or portion or variant thereof is precipitated into the hydrogel.

In another embodiment, the invention provides a bioprosthetic graft material comprising interleukin-10 or a portion or variant thereof, the graft material being capable of eluting the interleukin-10 or portion or variant thereof from the graft material.

In another embodiment, the device, vascular catheter, vascular balloon catheter or bioprosthetic graft material of the invention includes said interleukin-10 or portion or variant thereof which is capable of slow time release of interleukin-10 or portion or variant thereof.

In another embodiment, the invention provides an apparatus adapted to administer a biologically active agent to a patient comprising the device, vascular catheter, vascular balloon catheter or bioprosthetic graft material of the invention.

In another embodiment, the invention provides a method of treating or preventing vascular occlusion or obstruction comprising contacting the vascular lumen with interleukin-10 or portion or variant thereof. In one embodiment, the method comprises contacting the vascular lumen with interleukin-10 or portion or variant thereof with the device, vascular catheter, or vascular balloon catheter of the invention.

In a preferred embodiment, the method is performed following revascularization by a method selected from the group consisting of angioplasty, stenting, and bypass grafting.

In another embodiment, the invention provides a method of preventing blood vessel obstruction after blood vessel grafting comprising isolating a blood vessel from a subject; contacting the blood vessel ex vivo with a compound selected from the group consisting of interleukin-10 protein or a portion or variant thereof and interleukin-10 nucleic acid or a portion or variant thereof; and returning the blood vessel into the subject. In a preferred embodiment, the blood vessel is returned to the subject by grafting at a heterogeneous location (e.g., the heart). In another preferred embodiment, the blood vessel is saphenous vein.

In a preferred embodiment, the interleukin-10 protein comprises the amino acid sequence of SEQ ID NO:2, or a portion or variant thereof. In a further preferred embodiment, said portion or variant thereof comprises a polypeptide selected from the group consisting of: a polypeptide which is at least about 60% identical to the amino acid sequence.
of SEQ ID NO:2; and a polypeptide which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:2. In another preferred embodiment, the interleukin-10 comprises the nucleic acid sequence of SEQ ID NO:1, or a portion or variant thereof. In a further preferred embodiment, said portion or variant thereof comprises a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence which is at least about 60% identical to the amino acid sequence of SEQ ID NO:1; and a nucleic acid sequence which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:1.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the ability of IL-10 to downregulate inflammatory markers after treatment with LPS.

Figure 2 depicts the ability of IL-10 to downregulate IP-10 in response to LPS.

Figure 3 depicts the ability of IL-10 to downregulate TNF and IP-10 expression in response to actinomycin D.

Figure 4 depicts the ability of IL-10 to downregulate TNF in response to LPS.

Figure 5 depicts the ability of IL-10 to downregulate endogenous and exogenous TNF.

Figure 6 depicts the influence of IL-10 treatment on STAT3 and SOCS3 expression.

Figure 7 depicts the influence of IL-10 treatment on ERK1/2, JNK, and p38 signaling.

Figure 8 depicts the influence of IL-10 on TNF expression.

Figure 9 depicts the influence of IL-10 on IP-10 expression in the presence of inflammatory agents.

Figure 10 depicts a graph of IL-10 treatment of cells.

Figure 11 depicts the ability of IL-10 to suppress BrdU incorporation by cells.

Figure 12 depicts the fractions of cells in G0/G1 phase versus S phase in cells treated with IL-10.

Figure 13 depicts Cyclin A expression in cells treated with IL-10.

Figure 14 depicts cellular adhesion in cells treated with IL-10.

Figure 15 depicts arterial reendothelialization in mouse arteries treated with and without IL-10.
Figure 16 depicts ET-staining and HE staining in mouse arteries treated with and without IL-10.

Figure 17 depicts graphs of medial area and intimal area in mouse arteries treated with and without IL-10.

Figure 18 depicts immunostaining for inflammatory cells in sections of arterial tissue from mice treated with and without IL-10.

Figure 19 depicts staining for TNF expression in sections of arterial tissue from mice treated with and without IL-10.

Figure 20 depicts the RNase protection probes used in distinguishing transfected and endogenous TNF mRNA.

**DETAILED DESCRIPTION OF THE INVENTION**

IL-10 was originally described as an inhibitor of cytokine synthesis exhibiting a broad spectrum of suppressive activity (Trinchieri G, 1994; Mosmann TR, 1991) Though initial studies examined suppression of T-cell derived cytokines, subsequent work has demonstrated the mononuclear phagocytes to be a major target (Fiorentino DF, 1991; Moore KW, 1993, D’Andrea A, 1993). IL-10 is produced by a diverse array of cell types including but not limited to B cells, T cells, monocytes, macrophages and neutrophils (Moore KW, 1993). More importantly, IL-10 has been identified as an important regulator of homeostasis with respect to the inflammatory status of the whole organism. It has emerged as a macrophage deactivator competent to suppress the expression of inflammatory mediators as well as the macrophages’ ability to support accessory functions to adaptive immunity (Moore KW, 1993). Mice in which the IL-10 gene has been deleted by gene targeting exhibit unregulated inflammatory activity exemplified by enhanced TNF accumulation and which is associated with a variety of pathogenic outcomes (Berg DJ, 1995; Rennick D, 1995). The anti-inflammatory potential of IL-10 in vivo has also been demonstrated in preventing endotoxemia (Gerard C, 1993; Marchant A, 1994) and suppressing the development of intestinal inflammation (Kühn R, 1993). Information regarding the anti-inflammatory effects of IL-10 to the neointimal thickening and restenosis following arterial injury is limited and has only begun to emerge lately. A recent study demonstrated that IL-10 inhibits intimal hyperplasia after angioplasty or stent implantation in hypercholesterolemic rabbits (Feldman LJ, 2000). Another study demonstrated that overexpression of IL-10 by T cells inhibits advanced atherosclerotic lesions in LDL receptor-null mice fed an atherogenic diet (Pinderski LJ, 2002).
intramuscular gene transfer of IL-10 cDNA was shown to reduce atherosclerosis in apolipoprotein E-knockout mice (Namiki M, 2004). In Apolipoprotein E and IL-10 double knock out mice (E-/- x IL-10-/-) IL-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins (Caligiuri G, 2003). None of these studies looked either at the specific inflammatory gene repression in response to IL-10 or the mechanisms of such inhibition thus necessitating further in vivo and in vitro mechanistic studies. With regard to potential of IL-10 to contribute to cell type specific expression in vivo, IL-10 receptor and IL-10 sensitivity are more restricted and include predominantly (though not exclusively) hematopoietic cells (Weber-Nordt RM, 1994; Ho AS, 1994).

Isolated Nucleic Acid Molecules Used in the Devices and Methods of the Invention

Preferred embodiments of the invention include human interleukin-10 or portions or variants thereof. Other embodiments of the invention may also include interleukin-10 derived from other organisms, including, but not limited to, mouse interleukin-10.

The cDNA sequence of the isolated human interleukin-10 (also referred to herein as "interleukin 10", "IL10", and/or "IL-10") cDNA and the amino acid sequence of the human IL-10 polypeptide are shown in SEQ ID NOs: 1 and 2, respectively, and in GenBank Accession Nos. NM_000572 and NP_000563, respectively. The cDNA sequence of the isolated mouse IL-10 cDNA and the amino acid sequence of the mouse IL-10 polypeptide are shown in SEQ ID NOs: 3 and 4, respectively, and in GenBank Accession Nos. NM_010548 and NP_034678, respectively. When aligned using a global algorithm (Myers and Miller, CABIOS (1989) 4:11-17), the human and mouse IL-10 polypeptide sequences are 73% identical.

The devices and methods of the invention include the use of isolated nucleic acid molecules that encode IL-10 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify IL-10-encoding nucleic acid molecules (e.g., IL-10 mRNA) and fragments for use as PCR primers for the amplification or mutation of IL-10 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.
A nucleic acid molecule used in the methods of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or 3, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1 or 3 as a hybridization probe, IL-10 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual. 2nd ed. ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1 or 3 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1 or 3.

A nucleic acid used in the methods of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. Furthermore, oligonucleotides corresponding to IL-10 nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, the isolated nucleic acid molecules used in the methods of the invention comprise the nucleotide sequence shown in SEQ ID NO:1 or 3, a complement of the nucleotide sequence shown in SEQ ID NO:1 or 3, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1 or 3 thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1 or 3, or a portion of any of this nucleotide sequence.

Moreover, the nucleic acid molecules used in the methods of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1 or 3, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of an IL-10 protein, e.g., a biologically active portion of an IL-10 protein. The probe/ primer typically
comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1 or 3 or an anti-sense sequence of SEQ ID NO:1 or 3, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or 3. In one embodiment, a nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1 or 3.

As used herein, the term “hybridizes under stringent conditions” is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in Molecular Cloning: A Laboratory Manual, Sambrook et al., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X or 6X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A further preferred, non-limiting example of stringent hybridization conditions includes hybridization at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X or 6X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, e.g., at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1X SSPE is 0.15 M NaCl,
10 mM Na$_2$HPO$_4$, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1X SSC is 0.15 M NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature ($T_m$) of the hybrid, where $T_m$ is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(°C)=2(\# \text{ of A+T bases})+4(\# \text{ of G+C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(°C)=81.5+16.6(\log_{10}[\text{Na}^+])+0.41(\% \text{G+C})-(600/N)$, where N is the number of bases in the hybrid, and [Na$^+$] is the concentration of sodium ions in the hybridization buffer ([Na$^+$] for 1X SSC=0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (e.g., BSA or salmon or herring sperm carrier DNA), detergents (e.g., SDS), chelating agents (e.g., EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5 M NaH$_2$PO$_4$, 7% SDS at about 65°C, followed by one or more washes at 0.02 M NaH$_2$PO$_4$, 1 % SDS at 65°C, see e.g., Church and Gilbert (1984) Proc. Natl. Acad. Sci. USA 81:1991-1995 (or alternatively 0.2X SSC, 1% SDS).

In preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which express an IL-10 nucleic acid or protein (e.g., exogenous IL-10 nucleic acid or protein), such as by measuring a level of an IL-10-encoding nucleic acid in a sample of cells from a subject e.g., detecting IL-10 mRNA levels or determining whether a genomic IL-10 gene has been mutated or deleted.

The methods of the invention further encompass the use of nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or 3 due to degeneracy of the genetic code and thus encode the same IL-10 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1 or 3. In another embodiment, an isolated nucleic acid molecule included in the methods of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2 or 4.
The methods of the invention further include the use of allelic variants of human IL-10, e.g., functional and non-functional allelic variants. Functional allelic variants are naturally occurring amino acid sequence variants of the human IL-10 protein that maintain an IL-10 activity. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or 4, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally occurring amino acid sequence variants of the human IL-10 protein that do not have an IL-10 activity. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2 or 4, or a substitution, insertion or deletion in critical residues or critical regions of the protein.

The methods of the present invention may further use non-human orthologues of the human IL-10 protein. Orthologues of the human IL-10 protein are proteins that are isolated from non-human organisms and possess the same IL-10 activity.

The methods of the present invention further include the use of nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1 or 3, or a portion thereof, in which a mutation has been introduced. The mutation may lead to amino acid substitutions at “non-essential” amino acid residues or at “essential” amino acid residues. A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of IL-10 (e.g., the sequence of SEQ ID NO:2 or 4) without altering the biological activity, whereas an “essential” amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the IL-10 proteins used in the present invention and other members of the interleukin family are not likely to be amenable to alteration.

Mutations can be introduced into SEQ ID NO:1 or 3 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine,
valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an IL-10 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an IL-10 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for IL-10 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or 3, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using an assay described herein.

Isolated IL-10 Proteins and Anti-IL-10 Antibodies Used in the Devices and Methods of the Invention

The methods of the invention include the use of isolated IL-10 proteins, as well as biologically active portions and variants thereof. In one embodiment, native IL-10 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In a preferred embodiment, IL-10 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an IL-10 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

As used herein, a “biologically active portion” of an IL-10 protein includes a fragment of an IL-10 protein having an IL-10 activity. Biologically active portions of an IL-10 protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the IL-10 protein, e.g., the amino acid sequence shown in SEQ ID NO:2 or 4, which include fewer amino acids than the full length IL-10 proteins, and exhibit at least one activity of an IL-10 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the IL-10 protein. A biologically active portion of an IL-10 protein can be a polypeptide which is, for example, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175 or more amino acids in length. Biologically active portions of an IL-10 protein can be used in the methods and devices of the present invention.

In a preferred embodiment, the IL-10 protein used in the methods and devices of the invention has an amino acid sequence shown in SEQ ID NO:2 or 4. In other embodiments, the IL-10 protein is substantially identical to SEQ ID NO:2 or 4, and retains the functional activity of the protein of SEQ ID NO:2 or 4, yet differs in amino acid sequence due to natural
allelic variation or mutagenesis, as described in detail above. Accordingly, in another embodiment, the IL-10 protein used in the methods of the invention is a protein which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more identical to SEQ ID NO:2 or 4.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the IL-10 amino acid sequence of SEQ ID NO:2 or 4 having 178 amino acid residues, at least 53, preferably at least 71, more preferably at least 89, even more preferably at least 107, and even more preferably at least 125, 142, 160 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available online through the Genetics Computer Group), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available online through the Genetics Computer Group).
Group), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of Meyers, E. and Miller, W. (Comput. Appl. Biosci. 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The methods and devices of the invention may also use IL-10 chimeric or fusion proteins. As used herein, an IL-10 "chimeric protein" or "fusion protein" comprises an IL-10 polypeptide operatively linked to a non-IL-10 polypeptide. A "IL-10 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an IL-10 molecule, whereas a "non-IL-10 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the IL-10 protein, e.g., a protein which is different from the IL-10 protein and which is derived from the same or a different organism. Within an IL-10 fusion protein the IL-10 polypeptide can correspond to all or a portion of an IL-10 protein. In a preferred embodiment, an IL-10 fusion protein comprises at least one biologically active portion of an IL-10 protein. In another preferred embodiment, an IL-10 fusion protein comprises at least two biologically active portions of an IL-10 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the IL-10 polypeptide and the non-IL-10 polypeptide are fused in-frame to each other. The non-IL-10 polypeptide can be fused to the N-terminus or C-terminus of the IL-10 polypeptide.

For example, in one embodiment, the fusion protein is a GST-IL-10 fusion protein in which the IL-10 sequences are fused to the C-terminus of the GST sequences. In other embodiments, the fusion protein comprises IL-10 fused to a myc tag or a polyhistidine tag. Such fusion proteins can facilitate the purification of recombinant IL-10.

In another embodiment, this fusion protein is an IL-10 protein containing a signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of IL-10 can be increased through use of a signal sequence. In one embodiment, the signal sequence is the endogenous IL-10 signal sequence (e.g., amino acid residues 1-18 of SEQ ID NO:2 or 4). In another embodiment, the signal sequence is a heterologous signal sequence. Such signal sequences are known in the art.
The IL-10 fusion proteins used in the methods of the invention can be incorporated into pharmaceutical compositions and administered to a subject in vivo, e.g., using the devices of the invention.

Preferably, an IL-10 chimeric or fusion protein used in the methods of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al., John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). An IL-10-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the IL-10 protein.

The present invention also pertains to the use of variants of the IL-10 proteins. Variants of the IL-10 proteins can be generated by mutagenesis, e.g., discrete point mutation or truncation of an IL-10 protein. An agonist of the IL-10 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of an IL-10 protein. An antagonist of an IL-10 protein can inhibit one or more of the activities of the naturally occurring form of the IL-10 protein by, for example, competitively modulating an IL-10-mediated activity of an IL-10 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the IL-10 protein.

In one embodiment, variants of an IL-10 protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of an IL-10 protein for IL-10 protein agonist activity. In one embodiment, a variegated library of IL-10 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a
variegated gene library. A variegated library of IL-10 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential IL-10 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of IL-10 sequences therein. There are a variety of methods which can be used to produce libraries of potential IL-10 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential IL-10 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S. A. (1983) Tetrahedron 39:3; Itakura et al. (1984) Annu. Rev. Biochem. 53:323; Itakura et al. (1984) Science 198:1056; Ike et al. (1983) Nucleic Acids Res. 11:477).

In addition, libraries of fragments of an IL-10 protein coding sequence can be used to generate a variegated population of IL-10 fragments for screening and subsequent selection of variants of an IL-10 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an IL-10 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the IL-10 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of IL-10 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble
mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify IL-10 variants (Arkin and Youvan (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815; Delagrave et al. (1993) Prot. Eng. 6(3):327-331).

Recombinant Expression Vectors and Host Cells Used in the Methods of the Invention

The methods of the invention (e.g., the screening assays described herein) include the use of vectors, preferably expression vectors, containing a nucleic acid encoding an IL-10 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors to be used in the methods of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when
the vector is introduced into the host cell). The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) Methods Enzymol. 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., IL-10 proteins, mutant forms of IL-10 proteins, fusion proteins, and the like).

The recombinant expression vectors to be used in the methods of the invention can be designed for expression of IL-10 proteins in prokaryotic or eukaryotic cells. For example, IL-10 proteins can be expressed in bacterial cells such as E. coli, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel (1990) supra. Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in E. coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D. B. and Johnson, K. S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.)
which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in IL-10 activity assays (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for IL-10 proteins. In a preferred embodiment, an IL-10 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathobiology of the subject recipient is then examined after sufficient time has passed (e.g., six weeks).

In another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987) EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J. et al., Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissuespecific regulatory elements are used to express the nucleic acid).

Another aspect of the invention pertains to the use of host cells into which an IL-10 nucleic acid molecule of the invention is introduced, e.g., an IL-10 nucleic acid molecule within a recombinant expression vector or an IL-10 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, an IL-10 protein can be expressed in bacterial cells such as E. coli, insect cells, yeast or mammalian cells.
(such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms “transformation” and “transfection” are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

A host cell used in the methods of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) an IL-10 protein. Accordingly, the invention further provides methods for producing an IL-10 protein using the host cells of the invention. In one embodiment, the method comprises cultivating the host cell of the invention (into which a recombinant expression vector encoding an IL-10 protein has been introduced) in a suitable medium such that an IL-10 protein is produced. In another embodiment, the method further comprises isolating an IL-10 protein from the medium or the host cell.

Devices

The present invention embodies devices which can deliver IL-10 to the vascular lumen and/or wall. Such devices include, but are not limited to, stents, vascular catheters (including vascular balloon catheters), and bioprosthetic graft materials. Such devices are well-known in the art, and are described, for example, in U.S. Patent No. 6,620,194, U.S. Patent No. 5,588,962, U.S. Patent No. 6,358,556, U.S. Patent No. 5,304,121, U.S. Patent No. 6,702,850, and U.S. Patent No. 5,674,192, all of which are herein incorporated by reference.

As discussed, it will usually be preferred to deliver IL-10 to a subject (including biologically active fragments or variants thereof) using a device in accord with the invention. If desired, the device may also include one or more of the agents disclosed herein such as the restenosis preventatives discussed herein. However in other embodiments, it will be useful to deliver the IL-10, fragment or variant directly to the subject (e.g., via injection or infusion).
instead of (or in addition to) administration of the cytokine by the device. In such embodiments, the IL-10, fragment or variant is administered as the sole active agent or it can be administered with one or more other active agents such as the restenosis preventatives. In embodiments in which the IL-10, fragment or variant is administered directly to a subject (either with or without one or more of the other agents) such delivery can take the form of a pharmaceutical composition in mixture with conventional excipient, i.e. pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral or intranasal application which do not deleteriously react with the active compounds and are not deleterious to the recipient thereof. Suitable pharmaceutically acceptable carriers are known in the field and include but are not limited to water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethyl-cellulose, polyvinylpyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously react with the active compounds.

Such compositions that include the IL-10 as well as biologically active fragments or variants thereof may be prepared for use in parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; intranasally, particularly in the form of powders, nasal drops, or aerosols; vaginally; topically e.g. in the form of a cream; rectally e.g. as a suppository; etc.

The compositions that include the IL-10 as well as biologically active fragments or variants thereof may be conveniently administered in unit dosage form and may be prepared by any of the methods well known in the pharmaceutical arts, e.g., as described in Remington's Pharmaceutical Sciences (Mack Pub. Co., Easton, Pa., 1980). Formulations for parenteral administration may contain as common excipients such as sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. In particular, biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be useful excipients to control release.
Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxymethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration.

The concentration of one or more treatment compounds in a therapeutic composition will vary depending upon a number of factors, including the dosage of the IL-10, fragment or variant to be administered, the chemical characteristics (e.g., hydrophobicity) of the composition employed, and the intended mode and route of administration. In general terms, one or more than one of the IL-10 (including biologically active fragments or variants thereof) may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v of a compound for parenteral administration.

It will be appreciated that the actual preferred amounts of active compounds used in a given therapy will vary according to e.g. the specific compound being utilized, the particular composition formulated, the mode of administration and characteristics of the subject, e.g. the species, sex, weight, general health and age of the subject. Optimal administration rates for a given protocol of administration can be readily ascertained by those skilled in the art using conventional dosage determination tests conducted with regard to the foregoing guidelines. Suitable dose ranges may include from about 1 microgram/kg to about 100 mg/kg of body weight per day. Administration routes according to the invention will be for a time needed to achieve a desired therapeutic effect such as less than about two or three months, preferably between about a few weeks up to a month.

It will be apparent that practice of the invention can achieve many advantages including preventing, treating or alleviating symptoms associated with cardiac bypass surgery including development of potentially deadly occlusions. Such occlusions can be in a new vascular graft or in an existing graft or vessel. Thus in one invention embodiment, a device
as disclosed herein is used to administer IL-10 to a vascular graft in an amount needed to treat the occlusion. Examples of suitable grafts include saphenous vein grafts (SVG) used for bypass and related surgeries. Such grafts often feature obstructions related to thrombosis, neointimal hyperplasia or both. See generally Motwani, J.G. and Topol, E.J. (1998) in Cardiology 97: 916; and Cameron, A. et al. (1996) in N. Engl. J. Med. 334: 216.

It will also be apparent that practice of the invention is suitable for preventing, treating or alleviating the symptoms associated with occlusions or restenosis of a wide range of bodily vessels including peripheral arteries, coronaries, and grafts.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the sequence listing and the figures, are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: LOCAL ADENOVIRAL IL-10 GENE DELIVERY INHIBITS NEOINTIMAL GROWTH IN A MOUSE MODEL OF ARTERIAL INJURY

The association of inflammation with atherosclerosis and restenosis is now fairly well established. Mononuclear phagocytes are likely participants in the host response to vascular injury, via the secretion of cytokines and chemokines. In this context, TNF produced largely by activated monocytes/macrophages is known to be negatively associated with restenosis and atherosclerosis TNF expression has been shown to be elevated in human restenotic athrectomized lesions and at the sites of arterial injury in animals. Others and we have previously shown that TNF represses re-endothelialization, inhibits endothelial cell (EC) proliferation and is a strong mitogen for vascular smooth muscle cell (VSMC) proliferation. Thus regulation of TNF production via deactivation of inflammatory cells within revascularized arteries would be a desirable approach for restenosis prevention.

The data presented below and in the figures indicates that systemic injections of recombinant murine IL-10 not only diminishes inflammatory cell accumulation at the sites of arterial injury which correlates strongly with diminished TNF expression, but also has a
functional benefit on endothelial recovery as indicated by accelerated re-endothelialization and significantly reduced medial thickening. Studies were performed using systemic recombinant IL-10. There are several potential problems in pursuing systemic IL-10 therapy. First, global deactivation of mononuclear phagocytes may mask local effects of IL-10 in the arterial microenvironment. Second, the half-life of IL-10 protein is itself short, and would require daily IL-10 injections with prohibitory costs associated with it. Adenoviral IL-10 cDNA constructs are available and described elsewhere (Ostlund, LJ, 1999; Chen D, 2003).

**Mouse Carotid Injury Model and adenoviral-IL-10 transduction**

Previous methods of carotid injury in the mouse (Lindner, 1993) have resulted in highly variable lesion formation (Kalichman, 1998) limiting the utility of this model. The method we have developed (Goukassian D, 2003; Ishikawa, 2003; see data) results in consistent lesion formation 2 weeks after injury, similar to the pattern seen in the well-characterized rat model. This method employs a .014" wire that has been modified to induce the denuding and barotraumatic injury, which is common to angioplasty in humans and previously described animal models. The result is consistent de-endothelialization as has been described in previous studies from our labs (Spyridopoulos, 1997; Krasinski, 2001, Goukassian D, 2003). We are able to evaluate endothelial barrier function, using Evans Blue dye in this model and therefore have a similar means of evaluating ReEndo in this model (along with immunostaining for EC). Carotid artery denudation surgery will be performed using a dissecting microscope. Four-six week old C57/Bl6 mice (50 per trial 25 /IL-10 group and 25/ad-bGal group) are used and at least 4 such trials are performed in order to achieve statistical significance at all time points studied. Mice are anesthetized with an intraperitoneal injection of ketamine (45mg/kg body weight). The bifurcation of the left external carotid artery is surgically exposed via a midline incision on the ventral aspect of the neck. Size 6-0 surgical silk sutures are placed around the common carotid, internal and external carotid arteries in order to temporarily restrict the flow of blood to the area of surgical manipulation. The artery is injured using a .014" diameter flexible angioplasty wire (ACS, Temecula, CA) modified to create a barotraumatic/stretch injury, which is introduced into the external carotid artery and advanced to the common carotid artery. The wire is advanced and withdrawn three times in order to ensure a reliable effect. Immediately after injury, 50 μL (2×10⁶ pfu/mL) Ad- IL-10 and or Ad-bGal (as control of transduction efficiency) is be infused and incubated for 30 min as described previously (Goukassian D, 2003, Tulis, DA, 2001, Agata J, 2003). The wire is removed from the artery, the external carotid artery permanently ligated and the temporary
ligatures are released in order to allow blood flow to be restored through the internal carotid artery. The connective tissue of the sub-cutis is then closed with interrupted 6-0 absorbable sutures and skin closed with interrupted 6-0 silk sutures. Antibiotics (0.1 ml Di-trim, SC) are given immediately post-operatively via subcutaneous injection. Surgical procedure can be completed typically in 15 min following the initial incision. All animal procedures are performed in accordance with Caritas St. Elizabeth’s Institutional Animal Care and Use Committee. Thus there are 25 mice/Ad-Il-10 and Ad-bgal groups in each trial. The following parameters are examined in each group of mice:

a) Re-endothelialization.

Following injury, carotid arteries are harvested on days 3, 7 and 14 post-injury (5 mice/time point/group). Before animal sacrifice, arteries are perfused with 0.5% Evans Blue dye. Excised arteries are then cut open to expose endothelial layer and will be subjected to microscopy to evaluate the re-endothelialized area. These procedures are routine in our laboratories and have been published before (Asahara, 1995; Krasinski, 1997; 2001; Spyridopoulos, 1998; Goukassian, 2003; Iwakura, 2003).

b) Neointimal thickening

Neointimal thickening is quantified by measuring media to intima ratios in the injured arteries by histomorphometric analysis. These standard procedures have been described previously in previous manuscripts from our laboratory (Krasinski, 1997; Goukassian, 2003; Iwakura, 2003).

c) Immunostaining.

For immunostaining of tissue sections for inflammatory cells, TNF and IL-10, endogenous peroxidase activity is blocked and incubation with specific primary antibodies is performed at 37°C for 1h or at 4°C for overnight. Primary immune complexes are detected with species matched biotinylated secondary antibodies and streptavidin peroxidase (Goukassian, 2003; Krasinski, 2001; Asahara, 1995). Sections adjacent to those used for immunohistochemistry are stained with hematoxylin/eosin. The specimen is mounted with glycerol gelatin aqueous mounting media and examined on an Olympus Vanox-T microscope (Olympus American, Inc., Melville, NY). Pictures are recorded on Kodak Gold Plus films.
(Eastman Kodak Co, Rochester, NY). BrdU incorporation is evaluated after incubating tissue sections in 2M HCl for 10 min at 37°C to denature DNA. Immunostaining results will be confirmed independently by laser confocal microscopy.

d) Ex vivo binding assays.

Our in vitro data suggest that IL-10 inhibits TNF induced binding of monocytes to endothelial cell monolayer. This phenomenon is confirmed in ex vivo binding of monocytes to mouse carotid arteries. Mice are euthanized by pentobarbital overdose, and injured and control carotid arteries are excised and used for ex vivo monocyte binding assays. THP-1 in HBSS buffer is fluorescently labeled with Dil, preincubated with or without different dose of IL-10 (10, 50, 100 ng/ml) for 30 min, then seeded on carotid arteries (endothelial side up), with or without LPS (10 ng/ml). After 30 min incubation, carotid arteries are washed with HBSS and adherent THP-1 be counted by fluorescence microscopy.

Results

As shown in the figures, systemic IL-10 delivery results in the reduction of arterial lesion, reduced inflammatory cell accumulation and TNF expression as well as enhanced endothelial recovery.

EXAMPLE 2: INHIBITION OF INFLAMMATORY CELL RECRUITMENT AND INTIMAL HYPERPLASIA AND ENHANCEMENT OF ENDOTHELIAL RECOVERY FOLLOWING ENDOTHELIAL DENUDATION

Two distinct, gene specific molecular mechanisms for IL-10 Suppression of pro-inflammatory cytokine expression are involved in the inhibition of inflammatory cell recruitment and intimal hyperplasia. As discussed, inflammation plays an essential role in vascular injury and repair. Mononuclear phagocytes participate in these processes, in part via adhesive interactions and secretion of pro-inflammatory cytokines/chemokines. These functions are associated with new gene expression, which reflects the inflammatory cell response to stimuli encountered in the arterial microenvironment. The anti-inflammatory cytokine IL-10 suppresses such responses by repressing inflammatory cytokine expression. IL-10 knockout mice are prone to atherosclerosis and exhibit a severe inflammatory phenotype. As shown, systemic IL-10 treatment of mice following carotid artery denudation
blunted the inflammatory cell infiltration, Pro-inflammatory cytokine, including TNF-alpha, expression and intimal hyperplasia while significantly accelerating re-endothelialization. At molecular level, in human monocytic cell line THP1, IL-10 suppressed LPS-induced mRNA expression of a number of inflammatory cytokines via at least two distinct, gene specific mechanisms: post-transcriptional mRNA destabilization (e.g. TNFα) and reduced gene transcription (e.g. chemokine IP-10). Detailed studies using TNF mRNA sequences linked to reporter gene revealed that A+U rich elements (ARE) in the 3’ untranslated region (UTR) are necessary for IL-10-mediated TNF mRNA destabilization. IL-10 mediated TNF mRNA instability depends on the ability of IL-10 to inhibit the activation of p38 MAP kinase in stat3 independent but SOC3 dependent pathway. Transcriptional inhibition of IP-10 gene by IL-10, however was indirect and depends upon the inhibition of LPS-induced expression of interferon β. Furthermore, IL-10 co-treatment abrogated TNF-induced EC dysfunctions and significantly diminished TNF-induced adhesion of monocytes to EC, in vitro. Accordingly, IL-10 function and signaling are important components for control of inflammatory responses, and are important in modulating vascular repair and other accelerated arteriopathies, including transplant vasculopathy and vein graft hyperplasia.

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modifications and improvements within the spirit and scope of the invention as set forth in the following claims.
What is claimed:

1. A device suitable for implantation in a patient, the device comprising a body having open ends, a sidewall and a polymer on at least a part of a surface of the sidewall, wherein the polymer comprises a therapeutically sufficient amount of mammalian interleukin-10; or a portion or variant thereof.

2. The device of claim 1, wherein the device is a stent, catheter, graft, pump, needle, or a suture.

3. The device of any one of claims 1-2, optionally comprising a graft material.

4. The device of any one of claims 1-3, wherein the polymeric material is a biostable, biocompatible, and non-thrombogenic material.

5. The device of any one of claims 1-4, wherein the device is a stent comprising interleukin-10 or a portion or variant thereof.

6. The device of any one of claims 1-5, wherein the polymer is selected from the group consisting of: a swellable hydrogel polymer and a hydrophobic polymer.

7. The device of any one of claims 1-6, wherein the polymer is selected from the group consisting of: hydrosilicones, polysiloxanes, substituted polysiloxanes, polyurethanes, thermoplastic elastomers, ethylene vinyl acetate copolymers, polyolefin elastomers, polyamide elastomers, EPDM rubbers, fluorosilicones, polyethylene glycol (PEG), polysaccharides, phospholipids, and combinations thereof.

8. The device of any one of claims 1-7, wherein the polymer is adhered to the stent.

9. The device of any one of claims 1-8, wherein said interleukin-10 or portion or variant thereof and said polymer in a coating upon said device is capable of slow time release of interleukin-10 or portion or variant thereof.
10. The device of any one of claims 1-9, wherein said device comprises an expandable portion.

11. The device of claim 10, wherein said device is balloon-expandable stent and said expandable portion is a stent positioned about said balloon.

12. The device of claim 11, wherein said stent and balloon both include said polymer coating incorporating said interleukin-10 or a portion or variant thereof.

13. The device of any one of claims 10-12, wherein said expandable portion is a dilatation balloon expandable to pressures in the range of about 1 to 20 atmospheres.

14. The device of any one of claims 6-13, wherein said hydrogel polymer is selected from the group consisting of polycarboxylic acids, cellulosic polymers, gelatin, polyvinylpyrrolidone, maleic anhydride polymers, polyamides, polyvinyl alcohols, and polyethylene oxides.

15. The device of any one of claims 6-13, wherein said hydrogel polymer is polyacrylic acid.

16. The device of any one of claims 1-15, wherein said interleukin-10 comprises the amino acid sequence of SEQ ID NO:2, or a portion or variant thereof.

17. The device of claim 16, wherein said portion or variant thereof comprises a polypeptide selected from the group consisting of:
   a) a polypeptide which is at least about 60% identical to the amino acid sequence of SEQ ID NO:2; and
   b) a polypeptide which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:2.

18. The device of any one of claims 1-17, wherein the device further comprises at least one agent selected from the group consisting of: antithrombotics, anticoagulants,
antibiotics, antiplatelet agents, thrombolitics, antiproliferatives, steroidal and nonsteroidal antiinflammatories, agents that inhibit hyperplasia, agents that inhibit restenosis, smooth muscle cell inhibitors, growth factors, growth factor inhibitors, cell adhesion inhibitors, chemotherapeutic agents, and combinations thereof.

19. The device of claim 18, wherein the antithrombotic is selected from the group consisting of heparin, PPACK, enoxaprin, aspirin, hirudin, and combinations thereof.

20. The device of claim 18, wherein the antiproliferative is selected from the group consisting of monoclonal antibodies capable of blocking smooth muscle cell proliferation, heparin, angiopeptin, enoxaprin, and combinations thereof.

21. The device of claim 18, wherein the chemotherapeutic agent is taxol.

22. A vascular catheter for delivering mammalian interleukin-10 or a portion or variant thereof into tissue or occlusive formation at a desired location, comprising a catheter constructed for insertion into a vascular lumen having a catheter shaft and an expandable portion mounted on said catheter shaft, and at least a part of the exterior surface of the expandable portion comprises interleukin-10 or a portion or variant thereof.

23. The vascular catheter of claim 22, wherein the desired location is the vascular lumen.

24. The vascular catheter of any one of claims 22-23, wherein said expandable portion is expandable in response to controlled inflation pressure to fill the cross-section of the vascular lumen and engage the tissue or occlusive formation of said vascular lumen.

25. The vascular catheter of any one of claims 22-24, wherein the interleukin-10 or portion or variant thereof is contained in a polymer coating.

26. The vascular catheter of claim 25, wherein the polymer is selected from the group consisting of: a swellable hydrogel polymer and a hydrophobic polymer.
27. The vascular catheter of any one of claims 25-26, wherein the polymer is selected from the group consisting of: hydrosilicones, polysiloxanes, substituted polysiloxanes, polyurethanes, thermoplastic elastomers, ethylene vinyl acetate copolymers, polyolefin elastomers, polyamide elastomers, EPDM rubbers, fluorosilicones, polyethylene glycol (PEG), polysaccharides, phospholipids, and combinations thereof.

28. The vascular catheter of any one of claims 25-27, wherein the polymer is adhered to the catheter.

29. The vascular catheter of any one of claims 23-28, wherein said catheter is a dilatation catheter sized, constructed and arranged for insertion in a stenosed vascular lumen, and said expandable portion is an inflatable dilatation balloon adapted for brief inflation at pressures in the range for effecting widening of said stenosed vascular lumen, said elevated compressive pressure being in a range effective to simultaneously cause, with said compression of said hydrogel an administration of said interleukin-10 or a portion or variant thereof, widening of said vascular lumen.

30. The vascular catheter of any one of claims 20-22, wherein said hydrogel polymer is selected from the group consisting of polycarboxylic acids, cellulosic polymers, gelatin, polyvinylpyrrolidone, maleic anhydride polymers, polyamides, polyvinyl alcohols, and polyethylene oxides.

31. The vascular catheter of any one of claims 26-30, wherein said hydrogel polymer is polyacrylic acid.

32. The vascular catheter of any one of claims 22-31, wherein said interleukin-10 comprises the amino acid sequence of SEQ ID NO:2, or a portion or variant thereof.

33. The vascular catheter of claim 32, wherein said portion or variant thereof comprises a polypeptide selected from the group consisting of:
   a) a polypeptide which is at least about 60% identical to the amino acid sequence of SEQ ID NO:2; and
b) a polypeptide which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:2.

34. The vascular catheter of any one of claims 22-33, wherein the catheter further comprises at least one agent selected from the group consisting of: antithrombotics, anticoagulants, antibiotics, antiplatelet agents, thrombolytics, antiproliferatives, steroidal and nonsteroidal antiinflammatories, agents that inhibit hyperplasia, agents that inhibit restenosis, smooth muscle cell inhibitors, growth factors, growth factor inhibitors, cell adhesion inhibitors, chemotherapeutic agents, and combinations thereof.

35. The vascular catheter of claim 34, wherein the antithrombotic is selected from the group consisting of heparin, PPACK, enoxaprin, aspirin, hirudin, and combinations thereof.

36. The vascular catheter of claim 34, wherein the antiproliferative is selected from the group consisting of monoclonal antibodies capable of blocking smooth muscle cell proliferation, heparin, angiopeptin, enoxaprin, and combinations thereof.

37. The vascular catheter of claim 34, wherein the chemotherapeutic agent is taxol.

38. The vascular catheter of any one of claims 22-37, wherein said expandable portion is adapted for application of heat to said polymer material to control the rate of administration.

39. The vascular catheter of any one of claims 22-38, wherein said catheter further comprises a member, extending over said expandable portion to inhibit release of said interleukin-10 or a portion or variant thereof into body fluids during placement of said catheter.

39. The vascular catheter of claim 39, wherein said member is a retractable sheath.
40. The vascular catheter of any one of claims 22-39, wherein said expandable portion is constructed to withstand sufficient inflation pressure that application of said pressure to rapidly administer said interleukin-10 or portion or variant thereof simultaneously facilitates delivery of said interleukin-10 or portion or variant thereof into and through cracks in plaque formed by balloon angioplasty to reach smooth muscle tissue.

41. The vascular catheter of claim 40, wherein said pressure is in the pressure range of about 1 to 20 atmospheres.

42. The vascular catheter of claim 41, wherein said polymer is effective to release about 20% or more of said interleukin-10 or a portion or variant thereof during inflation in said pressure range.

43. The vascular catheter of any one of claims 22-42, wherein said expandable portion is constructed to withstand sufficient inflation pressure that application of said pressure to rapidly administer said interleukin-10 or a portion or variant thereof simultaneously facilitates delivery of said interleukin-10 or a portion or variant thereof into tissue or occlusive formation that lies deeper than the surface portion of the tissue or occlusive formation directly engaged by said expandable portion.

44. The vascular catheter of any one of claims 41-43, wherein the response characteristic of said hydrogel to said compressive pressure is effective to deliver said close of said interleukin-10 or a portion or variant thereof over a duration of about 10 minutes or less.

45. The vascular catheter of any one of claims 26-44, wherein said hydrogel polymer coating is about 10 to 50 microns thick in the swelled, uncompressed state.

46. The vascular catheter of any one of claims 22-45, wherein said expandable portion includes a stent, placeable in said body lumen by expansion thereof.

47. The vascular catheter of any one of claims 26-46, wherein the hydrogel releases about 50% of the interleukin-10 or a portion or variant thereof upon compression.
48. The vascular catheter of any one of claims 26-47, wherein the interleukin-10 or a portion or variant thereof is precipitated into the hydrogel.

49. The vascular catheter of any one of claims 22-48, wherein said catheter is constructed as a perfusion catheter having an expandable balloon.

50. The vascular catheter of any one of claims 22-492, wherein said expandable portion is constructed to withstand sufficient inflation pressure that application of said pressure to rapidly administer said interleukin-10 or portion or variant thereof simultaneously facilitates delivery of said interleukin-10 or portion or variant thereof into tissue or occlusive formation that lies deeper than the surface portion of the tissue or occlusive formation directly engaged by said expandable portion.

51. The vascular catheter of any one of claims 22-50, wherein said expandable portion includes a stent, placeable in said body lumen by expansion thereof.

52. The vascular catheter of claim 51, including a binding of said interleukin-10 or a portion or variant thereof and hydrogel polymer in a coating upon said stent for slow time release of interleukin-10 or portion or variant thereof remaining in said hydrogel polymer on said stent after said compression of said hydrogel polymer by said expansion.

53. The vascular catheter of claim 52, wherein said hydrogel polymer coating on said stent is a polyacrylic acid including an ammonium anion and said binding is an electrostatic binding.

54. The vascular catheter of any one of claims 51-53, wherein said stent is a balloon-expandable stent and said expandable portion is a stent positioned about said balloon.

55. The vascular catheter of claim 54, wherein said stent and balloon both include said swellable hydrogel coating incorporating said interleukin-10 or a portion or variant thereof.
56. A vascular balloon catheter for rapidly delivering a dose of mammalian interleukin-10 or portion or variant thereof into tissue or occlusive formation at a desired location of the wall of a vascular lumen, comprising:

   a catheter constructed for insertion into a vascular lumen having a catheter shaft and an expandable dilatation balloon mounted on said catheter shaft,

   at least a portion of the exterior surface of the expandable balloon comprising said dose of said interleukin-10 or portion or variant thereof.

57. The vascular balloon catheter of claim 56, wherein the interleukin-10 or portion thereof is contained in a polymer coating.

58. The vascular balloon catheter of claim 57, wherein the polymer is selected from the group consisting of: a swellable hydrogel polymer and a hydrophobic polymer.

59. The vascular balloon catheter of any one of claims 57-58, wherein the polymer is selected from the group consisting of: hydrogels, polysiloxanes, substituted polysiloxanes, polyurethanes, thermoplastic elastomers, ethylene vinyl acetate copolymers, polyolefin elastomers, polyamide elastomers, EPDM rubbers, fluorosilicones, polyethylene glycol (PEG), polysaccharides, phospholipids, and combinations thereof.

60. The vascular balloon catheter of any one of claims 57-59, wherein the polymer is adhered to the catheter.

61. The vascular balloon catheter of any one of claims 56-60, wherein said expandable balloon is expandable by an expansion controller to press against said tissue or occlusive formation at a compressive pressure in the range of about 1 to 20 atmospheres.

62. The vascular balloon catheter of claim 61, wherein said compressive pressure is elevated above the inflation pressure needed to fill the cross-section of the vascular lumen and engage the tissue or occlusive formation of said vascular lumen.

63. The vascular balloon catheter of any one of claims 58-62, wherein said hydrogel polymer is selected from the group consisting of polycarboxylic acids, cellulosic
polymers, gelatin, polyvinylpyrrolidone, maleic anhydride polymers, polyamides, polyvinyl alcohols, polyethylene oxides, and combinations thereof.

64. The vascular balloon catheter of any one of claims 58-62, wherein said hydrogel polymer is polyacrylic acid.

65. The vascular balloon catheter of any one of claims 56-64, wherein said catheter further comprises a sheath member, extendable over said balloon to inhibit release of said interleukin-10 or portion or variant thereof into body fluids during placement of said catheter.

66. The vascular balloon catheter of any one of claims 56-65, wherein said interleukin-10 comprises the amino acid sequence of SEQ ID NO:2, or a portion or variant thereof.

67. The vascular balloon catheter of claim 66, wherein said portion or variant thereof comprises a polypeptide selected from the group consisting of:
   a) a polypeptide which is at least about 60% identical to the amino acid sequence of SEQ ID NO:2; and
   b) a polypeptide which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:2.

68. The vascular balloon catheter of any one of claims 56-67, wherein the catheter further comprises at least one agent selected from the group consisting of: antithrombotics, anticoagulants, antibiotics, antiplatelet agents, thrombolytics, antiproliferatives, steroidal and nonsteroidal antiinflammatory agents, agents that inhibit hyperplasia, agents that inhibit restenosis, smooth muscle cell inhibitors, growth factors, growth factor inhibitors, cell adhesion inhibitors, chemotherapeutic agents, and combinations thereof.

69. The vascular balloon catheter of claim 68, wherein the antithrombotic is selected from the group consisting of heparin, PPACK, enoxaprin, aspirin, hirudin, and combinations thereof.
70. The vascular balloon catheter of claim 68, wherein the antiproliferative is selected from the group consisting of monoclonal antibodies capable of blocking smooth muscle cell proliferation, heparin, angiopeptin, enoxaprin, and combinations thereof.

71. The vascular balloon catheter of claim 68, wherein the chemotherapeutic agents is taxol.

72. The vascular balloon catheter of any one of claims 49-62, wherein the hydrogel releases about 50% of the interleukin-10 or portion or variant thereof upon compression.

64. The vascular balloon catheter of any one of claims 51-63, wherein the hydrogel includes up to about 20-30 mg of aqueous solution of interleukin-10 or portion or variant thereof.

65. The vascular balloon catheter of any one of claims 51-63, wherein the interleukin-10 or portion or variant thereof is precipitated into the hydrogel.

66. A bioprosthetic graft material comprising interleukin-10 or a portion or variant thereof, the graft material being capable of eluting the interleukin-10 or portion or variant thereof from the graft material.

67. The device, vascular catheter, vascular balloon catheter or bioprosthetic graft material of any one of claims 1-66, wherein said interleukin-10 or portion or variant thereof is capable of slow time release of interleukin-10 or portion or variant thereof.

68. An apparatus adapted to administer a biologically active agent to a patient comprising the device, vascular catheter, vascular balloon catheter or bioprosthetic graft material of any one of claims 1-67.

69. A method of treating or preventing vascular occlusion or obstruction comprising contacting the vascular lumen with interleukin-10 or portion or variant thereof.
70. A method of treating or preventing vascular occlusion or obstruction comprising contacting the vascular lumen with interleukin-10 or portion or variant thereof with the device of any one of claims 1-21.

71. A method of treating or preventing vascular occlusion or obstruction comprising contacting the vascular lumen with interleukin-10 or portion or variant thereof with the vascular catheter of any one of claims 22-55.

72. A method of treating or preventing vascular occlusion or obstruction comprising contacting the vascular lumen with interleukin-10 or portion or variant thereof with the vascular balloon catheter of any one of claims 56-65.

73. The method of any one of claims 69-72, wherein the method is performed following revascularization by a method selected from the group consisting of angioplasty, stenting, and bypass grafting.

74. A method of preventing blood vessel obstruction after blood vessel grafting comprising:
   a) isolating a blood vessel from a subject;
   b) contacting the blood vessel ex vivo with a compound selected from the group consisting of interleukin-10 protein or a portion or variant thereof and interleukin-10 nucleic acid or a portion or variant thereof; and
   c) returning the blood vessel into the subject.

75. The method of claim 74, wherein the blood vessel is returned to the subject by grafting at a heterogeneous location.

76. The method of any one of claims 74-75, wherein the blood vessel is saphenous vein.

77. The method of any one of claims 74-76, wherein the heterogeneous location is the heart.
78. The method of any one of claims 74-77, wherein the interleukin-10 protein comprises the amino acid sequence of SEQ ID NO:2, or a portion or variant thereof.

79. The method of claim 78, wherein said portion or variant thereof comprises a polypeptide selected from the group consisting of:
   a) a polypeptide which is at least about 60% identical to the amino acid sequence of SEQ ID NO:2; and
   b) a polypeptide which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:2.

80. The method of any one of claims 74-77, wherein the interleukin-10 nucleic acid comprises the amino acid sequence of SEQ ID NO:1, or a portion or variant thereof.

81. The method of claim 80, wherein said portion or variant thereof comprises a nucleic acid sequence selected from the group consisting of:
   a) a nucleic acid sequence which is at least about 60% identical to the amino acid sequence of SEQ ID NO:1; and
   b) a nucleic acid sequence which comprises at least about 10 contiguous amino acid residues of SEQ ID NO:1.

82. The method of claims 70-80, wherein the method further comprises administering a therapeutically effective amount of IL-10 or a portion or variant thereof.

83. A method of preventing, treating or alleviating symptoms associated with bypass graft occlusion, the method comprising contacting a graft with the device of any one of claims 1-21 or the vascular catheter of any one of claims 22-55 to administer a therapeutically effective amount of IL-10 or a portion or variant thereof.
Figure 1
Fig. 3

Act D (min) 0 0 30 30 60 60 120 120
IL-10 (10ng/ml) - - + - + - +

Figure 3

% Remaining mRNA

Act D (min) 0 30 60 120
TNF

Act D (min) 0 30 60 120
IP-10
Figure 6

B

C LPS IL-10 IL-10+LPS

Time (min) - 30 30 30 p-Stat3

Stat3

C

Time (hrs) C LPS IL-10 IL-10+LPS

SOC3

STAT3
### Figure 8

<table>
<thead>
<tr>
<th></th>
<th>Actinomycin D (1h)</th>
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<tbody>
<tr>
<td>SB203580 (20uM)</td>
<td>-</td>
</tr>
<tr>
<td>IL-10 (1h)</td>
<td>-</td>
</tr>
<tr>
<td>LPS (1h)</td>
<td>-</td>
</tr>
</tbody>
</table>

The table above shows the effects of different treatments on TNF and β-actin expression, with '+' indicating an increase and '-' indicating no change.

**Diagram:**
- **TNF**
- **β-actin**

The diagram visually represents the expression levels of TNF and β-actin under various conditions.
Figure 9

<table>
<thead>
<tr>
<th>CHX (5μg/ml)</th>
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<tbody>
<tr>
<td>UT</td>
</tr>
<tr>
<td>IP-10</td>
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<table>
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<tr>
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<tbody>
<tr>
<td>IP-10</td>
<td>β-actin</td>
<td></td>
</tr>
</tbody>
</table>

1. NT
2. α-IFNα/β
3. α-IgG
Figure 10

**p<0.001

Quiescent  Serum  TNF  IL-10  TNF+IL10
Figure 11

Control

IL-10

TNF

TNF+IL-10

IL-10

TNF+

IL-10

CTR

TNF

BrdU+ cells/ mm² #
Figure 13
<table>
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<tr>
<th>RPA probe 1</th>
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<th>Protected Fragment Size</th>
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<tr>
<td></td>
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<tr>
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<td>5' Truncation</td>
<td>Probe 1: 300</td>
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<tr>
<td></td>
<td>6xHis</td>
<td>Probe 2: 265</td>
</tr>
</tbody>
</table>

Figure 20: RNAse protection probes for use in distinguishing transfected and endogenous TNF mRNA