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(54) **SYSTEME ET PROCEDE D'APPORT DE CYTOKINES AU MOYEN DE CELLULES DE SECRETION DE CYTOKINES ENCAPSULEES**

(54) **SYSTEM AND METHOD FOR DELIVERY OF CYTOKINES USING ENCAPSULATED CYTOKINE-SECRETING CELLS**

(57) Procédé et système d'administration continue de cytokines à un patient au moyen d'une capsule biocompatible contenant des cellules de sécrétion de cytokines implantée directement dans le système nerveux central.

(57) A method and system for continuously delivering cytokines to a patient using a biocompatible capsule containing cytokine-secreting cells implanted directly into the CNS.

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(57) Abstract

A method and system for continuously delivering cytokines to a patient using a biocompatible capsule containing cytokine-secreting cells implanted directly into the CNS.

SYSTEM AND METHOD FOR DELIVERY OF CYTOKINES USING ENCAPSULATED CYTOKINE-SECRETING CELLS

TECHNICAL FIELD OF THE INVENTION

This invention relates to controlled, sustained delivery of cytokines directly to the CNS using encapsulated cytokine-secreting cells.

BACKGROUND OF THE INVENTION

Cytokines include interferons, such as IFN-ß, IFN-α and IFN-γ, which are useful in the treatment of a number of human viral infections and diseases, cancers and for affecting immunomodulation in the patient. Other cytokines contemplated here include lymphokines, interleukins (e.g. IL-10), and molecules such as the TGF-ß family of proteins.

Cytokines have been studied to treat a number of diseases including viral infections (rabies), tumors (hairy cell leukemia), and nervous system disorders of viral or suspected viral etiology (herpes zoster infections, multiple sclerosis, and subacute sclerosing panencephalitis (SSPE).

Because cytokines do not cross the blood brain barrier well, delivering drugs to their appropriate site of action without adverse side effects is a major challenge. Systemic delivery of cytokines for treatment has been problematic. In addition to inflammatory responses resulting from administration of cytokines, other side effects, including cough, asthenia (weakness), nausea, anorexia, mouth ulcers (aphthous stomatitis), fever, injection site inflammation, weight loss, diarrhea, insomnia and vomiting have been observed or are expected.

As cytokines have short half-lives, systemic delivery of cytokines typically require large dosages.

Such side effects have been observed in the treatment of multiple sclerosis with IFN-ß. For example, Betaseron™, an E.coli-produced unglycosylated IFN-ß from Berlex/Chiron was approved and launched in 1993 for treating relapsing or remitting multiple sclerosis ("MS"). IFN-ß has demonstrated efficacy in delaying onset of episodes, decreasing the severity of episodes, and slowing growth of lesions. Interferon beta is postulated to boost suppressor T-cell activity/number, thereby inhibiting a subset of myelin basic protein-specific T cells.

Systemic subcutaneous delivery of Betaseron™ was accompanied by significant side effects like inflammation, pain at the injection site, injection-site necrosis, and flu-like symptoms. In addition, patient receiving systemic delivery may develop antibodies to IFN-ß. Such side effects can be quite severe since injections are given every other day. In rare cases, side effects have included abnormal liver function and severe depression. The dosage of Betaseron™ administered is typically 8×10^6 IU or 250 µg every other day. Betaseron™ is administered with carrier proteins such as 15mg of albumin or dextrose.

MS is generally characterized by demyelination of CNS axons resulting in focal sclerotic lesions in the spinal cord, optic nerve and brain. Symptoms of MS may include muscle weakness, loss of coordination, vertigo, numbness, tingling, speech disturbances, and varying degrees of visual impairment. These initial symptoms often progress to acute muscle wasting, loss of vision, confinement to bed or wheelchair, aphasia, paralysis, and ultimately death. MS affects an estimated 250,000 people in the United States and is one of the most common causes of permanent neurological disability among young adults.

A glycosylated recombinant human IFN-ß, differing by one amino acid from Betaseron, is also expected to be available for treatment of patients that are suffering from chronic and progressive MS.

The encapsulated cell approach described here is expected to mitigate the foregoing difficulties by local delivery of a much lower dose of a continuously-synthesized cytokine from a retrievable implant directly to the CNS.

SUMMARY OF THE INVENTION

This invention provides novel methods and devices for the delivery of cytokines directly to the central nervous system ("CNS").

In one embodiment, this invention provides a novel approach employing polymer-encapsulated cells to release human cytokines directly to the CNS. The cells may be xenogeneic or allogeneic. Each device typically contains about 10^3 - 10^7 genetically-modified cells surrounded by a semipermeable membrane, and is implanted directly into the CNS, providing for slow continuous release of at least one cytokine. The preferred implant sites are intraventricular or intrathecal. Other implant sites are also contemplated, provided that delivery is to the central nervous system ("CNS"), and not through a route of administration that leads to the above-mentioned side effects. According to one of the methods of this invention, a cytokine is delivered at a dosage sufficient to maintain a measurable concentration in the cerebrospinal fluid ("CSF") that provides a therapeutic effect. We prefer a dosage of less than 25 μg cytokine per patient per day, preferably between 0.001 - 25 μg.

The semipermeable membrane prevents immunologic rejection of the cells and interposes a physical barrier between the encapsulated cells and the patient. Moreover, the device may be retrieved from the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a plasmid map of vector pcDNA3-IFNb1-123.

Figure 2 is a plasmid map of vector pcDNA3-IgSP-IFNb1-124.

DETAILED DESCRIPTION OF THE INVENTION

This invention contemplates delivery of proteins that, due to their cytokine-like properties, would be expected to produce the above-noted side effects when administered outside the CNS. Delivery of cytokines and like molecules directly to the CNS, may avoid their known and undesired side effects when

administered outside the CNS, as well as allow continuous and more consistent dosing.

In one embodiment of this invention, at least one cytokine is delivered intrathecally using encapsulated cells. Cells are genetically engineered to stably express and release recombinant human cytokines into the central nervous system ("CNS"). The living cells are encapsulated in one or more semipermeable polymer capsules and surgically inserted (under local anesthesia) in the spine, such that the cytokine is delivered into the CNS without having to cross the blood brain barrier.

This technique provides several advantages over other delivery routes:

(1) Drug can be delivered to the CNS directly, which will reduce unwanted peripheral side effects;

(2) Very small doses of drug (nanogram or small microgram quantities rather than milligrams) can be delivered compared with subcutaneous ("S.Q.") injections, also leading to fewer side effects;

(3) If cells are viable for long periods of time (such as six months), patients will be inconvenienced only twice a year instead of every day (or other frequent period) with S.Q. injections;

(4) Since viable cells continuously produce newly synthesized product, these cells should have advantages over pump delivery of drug stores, where drug is continuously degraded but not continuously replenished;

(5) Drug can be delivered more consistently than with other dosing methods(e.g., tablets, injections), thus improving efficacy and diminishing side effects.

As used herein "cytokine" generally means a soluble molecule that mediates interactions between cells and includes the family of molecules typically referred to as cytokines in the art. Specifically, cytokines contemplated here include lymphokines, interferons (IFNs), colony stimulating factors (CSFs), interleukins (ILs) (including IL-10), CD antigens and tumour necrosis factors (TNFs).

In particular, we prefer delivery of interferons IFN-ß, IFN-α, IL-10, and the TGF-β family.

Interferons are useful in the treatment of a number of human viruses, cancers and for immunomodulation. For example, the following diseases may be candidates for treatment with IFN-α or IFN-ß:

1) HIV replication in monocytes/macrophages
2) Multiple sclerosis
3) Hairy cell leukemia
4) Chronic viral infections
5) CML/AML
6) Rabies
7) Herpes zoster
8) SSPE
9) Jacob-Creutzfeld disease
10) Neuroblastoma
11) Glioblastoma multiforme, Malignant astrocytoma, or Malignant glioma

Previous attempts to treat MS with interferon injections administered either intrathecally (IT), intramuscularly (IM), subcutaneously (SQ) or intravenously (IV), used dosages much higher than contemplated in the present invention.

Many of the prior art approaches did not deliver cytokines directly into the CNS, and thus suffer the disadvantages discussed above.

Some prior art approaches did attempt delivery of IFN-ß to the CNS. See, e.g., (Jacobs et al., "Intrathecal Interferon in the Treatment of Multiple Sclerosis," Interferon Treatment of Neurologic Disorders, [Ed R.A. Smith] pp. 241-264 (1988), Milanese et al., J. Neurology, Neurosurgery and Psychiatry, 53, pp. 554-57 (1990), Confavreux et al., J. Neurology, Neurosurgery and Psychiatry, 49, pp. 1308-12 (1986).

These approaches had several disadvantages that are overcome by the novel approach of this invention.

First, delivery of the IFN-ß required numerous spinal taps, one for each dosing.

Second, the supply of IFN-ß was not continuous.

Third, the dosage range used was about 100-fold higher than that contemplated in the present invention -- leading to potential undesired side effects.

Any suitable cytokine may be delivered according to this invention. Primary cells that secrete such a cytokine may be used. Alternatively, cells may be genetically engineered to secrete such a cytokine, using techniques well known in the art.

The DNA sequences of many cytokines are known in the art. For example, there is a family of IFN-α molecules with known nucleic acid and amino acid sequences. See, e.g., U.S. patent number 4,530,901, incorporated herein by reference.

The nucleic acid and amino acid sequence of IFN-ß is also known. See U.S. patent 5,326,859, incorporated herein by reference.

Modified, truncated and mutein forms of these IFNs are well known and are contemplated by this invention. See, e.g., United States patent Nos. 5,376,567 and 4,588,585, incorporated herein by reference.

The DNA sequences of IL-10 and TGF-β are known.

Active fragments of the above-mentioned genes (i.e., those fragments of the genes having biological activity sufficient to achieve a therapeutic effect) are also contemplated. Also contemplated are cytokines modified by attachment of one or more polyethylene glycol (PEG) or other repeating polymeric moieties. Combinations of these proteins and polycistronic versions thereof are also contemplated.

In a preferred embodiment, full length recombinant human IFN-ß is delivered.

For IFN-ß, we prefer delivery of up to 1.4 µg/day/patient, more preferably between 0.005 - 1.4 µg/day, most preferably between 0.1-1.0 µg/day.

For IFN-α, we prefer delivery of up to 0.02 µg/day/patient, more preferably between 0.0009 - 0.02 µg/day, most preferably between 0.001-0.01 µg/day.

For IL-10, we prefer delivery of up to 21.3 µg/day/patient, preferably between 1.3-21.3 µg/day, most preferably between 5.0-10.0 µg/day.

For TGF-ß, we prefer delivery of up to 4.2 µg/day/patient, preferably between 0.042-4.2 µg/day, most preferably between 0.1-1.0 µg/day.

We prefer delivery to a site (e.g., the subarachnoid space or ventricles) that produces a measurable level of cytokine in the cerebrospinal fluid ("CSF").

When delivered to a site that produces a measureable CSF concentration, we prefer delivery of IFN-β sufficient to maintain a measurable concentration of up to 10 ng/ml in the CSF, preferably between 0.62-10.0 ng/ml in the CSF.

When delivered to a site that produces a measureable CSF concentration, we prefer delivery of IFN-α sufficient to maintain a measurable concentration of up to 0.15 ng/ml in the CSF, preferably between 0.009-0.15 ng/ml in the CSF.

When delivered to a site that produces a measureable CSF concentration, we prefer delivery of IL-10 sufficient to maintain a measurable concentration of up to 130 ng/ml in the CSF, preferably between 13-130 ng/ml in the CSF.

When delivered to a site that produces a measureable CSF concentration, we prefer delivery of TGF-β sufficient to maintain a measurable concentration of up to 2.6 ng/ml in the CSF, preferably between 0.42-2.6 ng/ml in the CSF.

A lower dosage is particularly useful because cytokines are often involved in cell-mediated immune responses. However, sometimes cell-mediated immunopathology can occur. For example, a granuloma may cause a bulky space-occupying lesion, impairing the function of sensitive tissues such as brain, retina and nerve.

The cell types that can be employed for encapsulated cell therapy within the scope of this invention include cells from allogeneic, autologous and xenogeneic sources. One of the advantages of this encapsulated approach rests with the immunoisolatory properties of the membranes of this invention, and their

ability to support cells that otherwise would not be appropriate for transplantation (i.e., non-human sources, immortalized and/or tumor cell lines).

A particular advantage to using xenogeneic over allogeneic cells is that in the unlikely event of membrane failure, the xenogeneic cells are more likely to be targeted for destruction by the immune system when compared to allogeneic cells. Furthermore, xenogeneic sources are easy to obtain and their use precludes the necessity for human tissue which is difficult to obtain and whose use is fraught with societal and ethical considerations. In addition, human tissue may contain adventitious agents that are more readily transmitted to the transplantation recipient. Finally, use of xenogeneic tissue and cell lines for transplantation in humans removes the risks associated with the handling and processing of human tissue.

The preferred cells chosen for the gene transfer technique are L6 or C2C12 mouse myoblast cells, baby hamster kidney (BHK) cells or rat insuloma cells (RIN) cells.

While BHK or RIN cells are preferred, a wide variety of cells may be used. These include well known, publicly available immortalized cell lines as well as dividing primary cell cultures. Examples of suitable publicly available cell lines include, chinese hamster ovary (CHO), mouse fibroblast (L-M), NIH Swiss mouse embryo (NIH/3T3), African green monkey cell lines (including COS-1, COS-7, BSC-1, BSC-40, BMT-10 and Vero), rat adrenal pheochromocytoma (PC12 and PC12A), AT3, AtT-20, C6 glioma, astrocytes and other fibroblast cell lines.

Primary cells that may be used include adrenal chromaffin cells, neural progenitor cells and neural stem cells (Reynolds and Weiss, Science, 255, pp. 1707-1710 (1992); Richards et al., PNAS 89, pp. 8591-8595 (1992); Ray et al., PNAS 90, pp. 3602-3606 (1993)), primary fibroblasts, Schwann cells, ß-TC cells, Hep-G2 cells, oligodendrocytes and their precursors, and the like.

A gene of interest (i.e., encoding an IFN-α, IFN-ß, IL-10, or TGF-β) can be inserted into a suitable expression vector by using standard techniques. It will be appreciated that more than one gene may be inserted into a

suitable expression vector. These techniques are well known to those skilled in the art.

The expression vector containing the gene of interest may then be used to transfect the cell line to be used in the methods of this invention. Standard transfection techniques such as calcium phosphate co-precipitation, DEAE-dextran transfection or electroporation may be utilized. Commercially available mammalian transfection kits may be purchased from e.g., Stratagene.

A wide variety of host/expression vector combinations may be used to express the gene encoding the desired cytokine.

Suitable promoters include, for example, the early and late promoters of SV40 or adenovirus and other known non-retroviral promoters capable of controlling gene expression.

Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences, such as various known derivatives of SV40 and known bacterial plasmids, e.g., pUC, pBlueScript™ plasmids from E. coli including pBR322, pCR1, pMB9, pUC, pBlueScript™ and their derivatives.

Expression vectors containing the geneticin (G418) or hygromycin drug selection genes (Southern, P.J. (1981), In Vitro, 18, p. 315, Southern, P.J. and Berg, P. (1982), J. Mol. Appl. Genet., 1, p. 327) are also useful. These vectors can employ a variety of different enhancer/promoter regions to drive the expression of both a biologic gene of interest (e.g., IFN-ß, IFN-α, IL-10 or TGF-β) and/or a gene conferring resistance to selection with toxin such as G418 or hygromycin B.

A variety of different mammalian promoters can be employed to direct the expression of the genes for G418 and hygromycin B and/or the biologic gene of interest. If cells of a CNS origin are used, preferably the promoter is selected from the following group:

the promoters of hDBH (human dopamine beta hydoxylase) (Mercer et al., Neuron, 7, pp. 703-716, (1991)), hTH (human tyrosine hydroxylase) (Kaneda, et al., Neuron, 6, pp. 583-594, (1991)), hPNMT (human phenylethanaolamine N-methyltransferase) (Baetge et al., PNAS, 85,

pp. 3648-3652, (1988)), mGFAP (mouse glial fibrillary acidic protein) (Besnard et al., J. Biol. Chem., 266, pp. 18877-18883, (1991)), myelin basic protein (MBP), mNF-L (mouse neurofilament-light subunit) (Nakahira et al., J. Biol. Chem., 265, pp. 19786-19791, (1990)), hPo (the human promoter for the gene encoding the major myelin glycoprotein in the peripheral nervous system) (Lemke et al., Neuron, 1, pp. 73-83, (1988)), mMT-1 (mouse metallothionen-1 promoter), rNSE (rat neuron-specific enolase) (Sakimura, et al., Gene, 60, pp. 103-113, 1987), and the like.

Examples of expression vectors that can be employed are the commercially available pRC/CMV, pRC/RSV, and pCDNA1NEO (InVitrogen).

One suitable vector is the pNUT expression vector. Baetge et al., Proc. Natl. Acad. Sci. USA, 83 pp. 5454-58 (1986).

Increased expression can be achieved by increasing or amplifying the copy number of the transgene encoding the desired biologically active molecule(s), using amplification methods well known in the art. Such amplification methods include, e.g., DHFR amplification (see, e.g., Kaufman et al., United States patent 4,470,461) or glutamine synthetase ("GS") amplification (see, e.g., United States patent 5,122,464, and European published application EP 338,841).

In a preferred embodiment, the human IFN-ß gene was expressed using the commercially available pcDNA-3 expression vector.

The IFN-ß expression vector was transfected into baby hamster kidney (BHK) cells using a standard calcium/phosphate transfection procedure and selected with increasing concentrations of methotrexate (1 to 200 µM) over 8 weeks to produce stable expressers. Following this selection, the BHK engineered cells were maintained *in vitro* in 50 µM methotrexate.

The encapsulation of cell lines engineered in this fashion allows for the selection of cells over several months for stable expression of the DHFR and the IFN-ß gene before transplantation. The selected cells can then be analyzed for IFN-β transgene stability and gene expression by Southern and Northern blot analysis, respectively.

According to the methods of this invention, other suitable molecules may also be delivered to the CNS. Such molecules include growth or trophic factors, other cytokines, lymphokines, hormones, and neurotransmitters. In particular, we prefer co-delivery of a molecule from the group selected of IGF-1, GGF2, NGF, GDNF, BDNF, NT-3, NT4/5, IFN-α and IFN-ß.

Co-delivery can be accomplished in a number of ways. Cells may be doubly transfected to secrete both molecules of interest. Alternatively, cells may be transfected with a single construct containing both genes. Also contemplated is encapsulation of two separately transfected cells or cell lines, each secreting one of the desired molecules. Separate cell lines can be encapsulated in separate devices and both devices can be implanted in the patient. The cells or cell lines may be the same or different.

This invention also contemplates use of a "suicide" gene in the transformed cells. Most preferably, the cell carries the Herpes simplex thymidine kinase gene (HSV-tk) as a safety measure, permitting the encapsulated cells to be killed *in vivo* by treatment with ganciclovir.

Use of a "suicide" gene is known in the art. See., e.g., Anderson, published PCT application WO 93/10218; Hamre, published PCT application WO 93/02556. The recipient's own immune system provides a first level of protection from adverse reactions to the implanted encapsulated cells if the cells are xenogeneic. In addition, the polymer capsule is preferably immunoisolatory (see infra). The presence of the TK gene (or other suicide gene) in the expression construct adds an additional level of safety to the recipient of the implanted cells.

In a preferred embodiment, the HSV-tk gene is inserted into the cytokine expression construct. This suicide gene allows elimination of the transfected cells upon ganciclovir administration. We prefer insertion of an 1800 bp fragment of the HSV-tk gene. A typical dosage of ganciclovir required to "kill" the cells is approximately 5 mg/kg.

The transduced cells are surrounded with a microporous or permselective membrane which permits the diffusion of small molecules such as nutrients and trophic factors into and out of the polymer capsule. The capsule

membrane also permits the molecule of interest to easily diffuse from the capsule into the surrounding host tissue or cerebrospinal fluid. Using the capsules of this invention, the implant may be retrieved if necessary or desired. Such retrievability may be essential in many clinical situations.

Numerous encapsulation devices are known, having various outer surface morphologies. Capsules have been categorized as Type 1 (T1), Type 2 (T2), Type 1/2 (T1/2) or Type 4 (T4) depending on their outer surface morphology. Such membranes are described, e.g., in Lacy et al., "Maintenance Of Normoglycemia In Diabetic Mice By Subcutaneous Xenografts Of Encapsulated Islets", Science, 254, pp. 1782-84 (1991), Dionne et al., PCT/US92/03327 and Baetge, WO 95/05452.

As used herein "a biocompatible capsule" means that the capsule, upon implantation in a host mammal, does not elicit a detrimental host response sufficient to result in the rejection of the capsule or to render it inoperable, for example through degradation.

As used herein "an immunoisolatory capsule" means that the capsule upon implantation into a mammalian host minimizes the deleterious effects of the host's immune system on the cells within its core. To be immunoisolatory, the jacket of the capsule should provide a physical barrier sufficient to prevent detrimental immunological contact between the isolated cells and the host's immune system. The thickness of this physical barrier can vary, but it will always be sufficiently thick to prevent direct contact between the cells and/or substances on either side of the barrier. The thickness of this region generally ranges between 5 and 200 microns; thicknesses of 10 to 100 microns are preferred, and thickness of 20 to 75 microns are particularly preferred.

Use of immunoisolatory capsules allows the implantation of xenogeneic cells or tissue, without a concomitant need to immunosuppress the recipient. Use of immunoisolatory capsules also allows use of unmatched cells (allografts).

The exclusion of IgG from the core of the vehicle is not the touchstone of immunoprotection, because in most cases IgG alone is insufficient to

produce cytolysis of the target cells or tissues. Thus, for immunoisolatory capsules, jacket nominal molecular weight cutoff (MWCO) values up to 1000 kD are contemplated. Preferably, the MWCO is between 50-700 kD, most preferably between 70-300 kD. See, e.g., WO 92/19195.

A variety of capsules are suitable for delivery of molecules according to this invention. Preferably the capsule of this invention will be similar to those described in U.S. patent nos. 5,158,881, 5,284,761, 5,389,533, 5,283,187, 4,976,859 and 4,968,733, incorporated herein by reference.

Useful biocompatible polymer capsules comprise (a) a core which contains a cell or cells, either suspended in a liquid medium or immobilized within an immobilizing biocompatible matrix, preferably comprising a hydrogel or extracellular matrix components, and (b) a jacket comprising a membrane which does not contain isolated cells, and which is biocompatible. Preferably the jacket is immunoisolatory and is sufficient to protect the cells in the core from detrimental immunological attack.

Many transformed cells or cell lines are most advantageously isolated within a capsule having a liquid core. For example, cells can be isolated within a capsule whose core comprises a nutrient medium, optionally containing a liquid source of additional factors to sustain cell viability and function.

Alternatively, the core may be composed of an immobilizing matrix, such as a hydrogel, which stabilizes the position of the cells in the device. The term "hydrogel" herein refers to a three dimensional network of cross-linked hydrophilic polymers. The network is in the form of a gel, substantially composed of water, preferably but not limited to gels being greater than 90% water.

Compositions which form hydrogels fall into three classes. The first class carries a net negative charge (e.g., alginate). The second class carries a net positive charge (e.g., collagen and laminin). Examples of commercially available extracellular matrix components include Matrigel™ and Vitrogen™. Fibroblasts generally survive well in a positively charged matrix and are thus suitably enclosed in extracellular-matrix type hydrogels. The third class is net neutral in charge (e.g., highly crosslinked polyethylene oxide, or polyvinylalcohol).

Any suitable matrix or spacer may be employed within the core, including precipitated chitosan, synthetic polymers and polymer blends, microcarriers and the like, depending upon the growth characteristics of the cells to be encapsulated. The core may also be formed from cells encapsulated in microspheres.

Alternatively, the capsule may have an internal scaffold. The scaffold may prevent cells from aggregating and improve cellular distribution within the device. See PCT publication WO96/02646.

Various polymers and polymer blends can be used to manufacture the capsule jacket, including polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones (including polyethersulfones), polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

The capsule can be any configuration appropriate for maintaining biological activity and providing access for delivery of the product or function, including for example, cylindrical, rectangular, disk-shaped, patch-shaped, ovoid, stellate, or spherical. Moreover, the capsule can be coiled or wrapped into a mesh-like or nested structure. If the capsule is to be retrieved after it is implanted, configurations which tend to lead to migration of the capsules from the site of implantation, such as spherical capsules, are not preferred. Certain shapes, such as rectangles, patches, disks, cylinders, and flat sheets offer greater structural integrity and are preferable where retrieval is desired.

In one preferred embodiment, the implantable capsule is of a sufficient size and durability for complete retrieval after implantation. Such macrocapsules have a core of a preferable minimum volume of about 1 to 10µl and depending upon use are easily fabricated to have a volume in excess of 10O µl.

In a preferred hollow fiber configuration, the fiber will have an inside diameter of less than 1500 microns, preferably less than 300-600 microns. In either geometry, the hydraulic permeability will be in the range of 1-100 mls/min/M^2/mmHg, preferably in the range of 25 to 70 mls/min/M^2/mmHg.

The glucose mass transfer coefficient of the capsule, defined, measured and calculated as described by Dionne et al., ASAIO Abstracts, p. 99 (1993), and Colton et al., The Kidney, eds., Brenner BM and Rector FC, pp. 2425-89 (1981) will be greater than 10^{-6} cm/sec, preferably greater than 10^{-4} cm/sec.

Methods of making biocompatible semipermeable hollow fiber membranes are disclosed in United States patents 5,284,761 and 5,158,881, 4,976,859 and 4,968,733, herein incorporated by reference. See also WO 95/0542.

Any suitable method of sealing the capsules may be used, including the employment of polymer adhesives and/or crimping, knotting and heat sealing. These sealing techniques are known in the art. In addition, any suitable "dry" sealing method can also be used. In such methods, a substantially non-porous fitting is provided through which the cell-containing solution is introduced. Subsequent to filling, the capsule is sealed. Such a method is described in copending United States application Serial No. 08/082,407, herein incorporated by reference.

Preferably the device has a tether that aids in retrieval. Such tethers are known. See, e.g., WO 92/19195.

In one preferred embodiment, the encapsulation procedure is as follows: The hollow fibers are fabricated from polyether sulfone (PES) with an outside diameter of 720 μm and a wall thickness of 100 μm (AKZO-Nobel Wüppertal, Germany).

In some studies we contemplate using an inner support in the device to provide increased tensile strength.

Fiber material is first cut into 5 cm long segments and the distal extremity of each segment were sealed with a photopolymerized acrylic glue (LCM-25, ICI). Following sterilization with ethylene oxide and outgassing, the fiber segments are loaded with a suspension of about 2×10^5 transfected cells/μl in a collagen solution (Zyderm® soluble bovine collagen) via a Hamilton syringe and a 25 gauge needle through an attached injection port. The proximal end of the capsule is sealed with the same acrylic glue.

A silicone tether (Specialty Silicone Fabrication, Taunton, USA) (ID: 690 μm; OD: 1.25 μm) is placed over the proximal end of the fiber allowing easy manipulation and retrieval of the device.

The methods and devices of this invention are intended for use in a mammalian host, recipient, patient, subject or individual, preferably a primate, most preferably a human.

Implantation of the BAO is performed under sterile conditions. Generally, the BAO is implanted at a site in the host which will allow appropriate delivery of the secreted product or function to the host and of nutrients to the encapsulated cells or tissue, and will also allow access to the BAO for retrieval and/or replacement.

A number of different implantation sites are contemplated for the devices and methods of this invention. These implantation sites include the central nervous system, including the brain, and spinal cord. Preferred sites in the brain include the striatum, the cerebral cortex, subthalamic nuclei and nucleus Basalis of Maynert. Other preferred sites are the cerebrospinal fluid, most preferably the subarachnoid (intrathecal) space and the lateral ventricles.

According to one embodiment of this invention, capsular delivery of IFN-ß, synthesized in vivo, to the brain ventricles, brain parenchyma, the intrathecal space or other suitable CNS location, in a dosage described supra, is desirable. In this embodiment, IFN-β has potential applications based on its anti-viral, anti-cancer, anti-tumor and immunomodulatory activities. Such therapies potentially include malignant diseases such as osteosarcoma, glioma, and Hodgkin's disease, tumor therapy, viral infections, and demyelinating diseases, such as multiple sclerosis.

The actual dosage of IFN-ß can be varied by implanting a fewer or greater number of capsules. We prefer implanting between 1 and 10 capsules, most preferably between 1 and 5 capsules.

Alternatively, the dose can be varied by increasing/decreasing the number of cells per capsule, as well as the output per cell (including choosing cells with a different output of IFN-β), or any other suitable method.

According to another embodiment of this invention, capsular delivery of IFN-α synthesized in vivo, to the brain ventricles, brain parenchyma, the intrathecal space or other suitable CNS location, in a dosage described supra, is desirable. The actual dosage of IFN-α can be varied by implanting a fewer or greater number of capsules or other suitable method.

Preferably, this embodiment is used in the treatment or prophylaxis of acute leukaemia, Hodgkin's disease, hepatitis, herpes keratitis, adenovirus conjunctivitis, ocular infections, infections caused by Epstein-Barr virus, cytomeglovirus, varicella zoster, herpes simplex virus-1, herpes labialis, chronic hepatitis B infections, warts, condyloma acuminatum, juvenile laryngeal papillomatosis, hairy cell leukemia, coryza, subacute sclerosing panencephalitis (SSPE), amyotrophic lateral sclerosis, and malignant gliomas.

According to another embodiment of this invention, capsular delivery of IL-10 synthesized in vivo to the brain ventricles, parenchyma, the intrathecal space or other suitable CNS location in a dosage described supra, is desirable. The actual dosage of IL-10 can be varied by implanting a fewer or greater number of capsules. Preferably, this embodiment is used in the treatment of inflammatory disease.

According to another embodiment of this invention, capsular delivery of TGF-β synthesized in vivo to the brain ventricles, parenchyma, the intrathecal space or other suitable CNS location in a dosage described supra, is desirable. The actual dosage of TGF-β can be varied by implanting a fewer or greater number of capsules. Preferably, this embodiment is used in the treatment of multiple sclerosis, or inflammatory diseases.

EXAMPLES

Construction of Interferon β1 Expression Vectors

Construction of pcDNA3-IFNß1-123 (pre-IFNß1) Expression Vector:

Plasmid pLG104R containing the pre-interferon ß1 cDNA was obtained from American Type Culture Collection (ATCC, Rockville, Maryland).

Due to the lack of convenient restriction sites in pLG104R, the preIFNβ1 coding region was generated by polymerase chain reaction (PCR) using oligonucleotides ohIFNß1-219 (SEQ. ID NO: 1: 5'-CCCAAGCTTCGCCACCATGACCAACAAGTGTCT-3') and ohIFNß1-220 (SEQ. ID NO: 2: 5'-CCCGGATCCTCAGTTTCGGAGGTAACCTGT-3') To facilitate directional cloning of the amplified pre-IFNß1 coding region, synthetic restriction enzyme sites HindIII (5'-AAGCTT-3') and BamHI (5'-GGATCC-3') were engineered into the 5' ends of oligonucleotides ohIFNß1-219 and ohIFNß1-220, respectively. To enhance the translational efficiency, a consensus ribosome binding site, called Kozak sequence (5'-CGCCACC-3'), was inserted immediately 5' of the translation start codon ATG in OhIFNß1-219.

One hundred nanograms of pLG104R plasmid was added to a 50 µl PCR reaction mixture containing 10 mM Tris.HCl (pH 8.3), 50 mM KCl, 800 of each nM dNTP, 2 mM $MgCl_2$, 400 nM of primers ohIFNß1-219 and ohIFNß1-220, and 2.5 units of Thermus aquatics (Taq) DNA polymerase (Boehringer Mannheim, Germany). The PCR reaction mixtures were subjected to 30 amplification cycles consisting of: denaturation, 94°C 30 seconds; annealing, 50°C 30 seconds; and extension, 72°C 30 seconds. The 589 bp pre-IFNß1 PCR fragment was digested with restriction endonucleases BamHI and HindIII and resolved on an 1% Trivie agarose gel (TrivieGen). The 589-bp HindIII/BamHI DNA fragment was excised and purified using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME). pcDNA3 expression vector was also digested with BamHI and HindIII and purified from 1% SeaPlaque agarose (FMC BioProducts, Rockland, ME) using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME). The ligation mixture was transformed into E. Coli DH5α (Gibco BRL, Gaithersburg, MD).

A cracking gel procedure (Promega Protocols and Applications Guide, 1991) was used to screen out the positive sub-clones. The identify of the correct clone was further verified by BamHI/HindIII double digestion. The positive sub-clone for the pre-IFNß1 was designated pcDNA3-IFNß1-123. See Figure 1.

Construction of pcDNA3-IgSP-IFNß1-124 (IgSP-mature IFNß1) Expression Vector:

Recombinant PCR methodology was used to generate the IgSP-mature IFNß1 fusion gene. Oligonucleotides ohIFNß1-221 (SEQ ID NO: 3: 5'-CCCAAGCTTGCGTCACCCCTAGAGTCGAGCTGT-3') and ohIFNß1-220 (SEQ ID NO: 2: 5'-CCCGGATCCTCAGTTTCGGAGGTAACCTGT-3') are specific for the IgG signal peptide sequence (IgSP) and the mature IFNβ1 sequence, respectively, and contain synthetic HindIII and BamHI restriction sites at the 5' end, respectively. Oligonucleotides ohIFNß1-222 (SEQ ID NO: 4: 5'-GTTGTAGCTCATCCTCTTGAACTCCAGGGG-3') and ohIFNß1-223 (SEQ ID NO: 5: 5'-GAGTTCAAGAGGATGAGCTACAACTTGCTT-3') are complementary to each other. Furthermore, oligonucleotide ohIFNß1-222 has its 5' 16 nucleotides identical to the IgSP sequence and its 3' 18 nucleotides identical to the mature IFNß1; and vice versa for ohIFNß1-223. The first two PCR reactions were carried out using oligonucleotide pairs ohIFNß1-221/ohIFNß1-222 and ohIFNß1-223/ohIFNß1-220 on templates pBS-IgSP-hPOMCΔACTH-029 and pLG104R plasmids, respectively. One hundred ng of template DNA was added to a 50 µl PCR reaction mixture containing 10 mM Tris.HCl (pH 8.3), 50 mM KCl, 800 of each nM dNTP, 2 mM MgCl2, 400 nM of primers #1 and #2, and 2.5 units of Taq DNA polymerase (Boehringer Mannheim, Germany). The PCR reaction mixtures were subjected to 30 amplification cycles consisting of: denaturation, 94°C for 30 seconds; annealing, 50°C 30 seconds; and extension, 72°C 30 seconds. The PCR products were resolved on 1% TrivieGel (TrivieGen). Two agarose plugs containing each one of the first PCR products were transferred to a tube containing 50 µl of PCR reaction mixtures identical to the one described above with the exception that the oligonucleotides ohIFNß1-220 and ohIFNß1-221 were used.

The second PCR reaction was subjected to 30 amplification cycles consisting of: denaturation, 94°C for 30 seconds (first cycle 2 minutes); annealing, 60°C 30 seconds (second to fourth cycles 37 °C 2 minutes); and extension, 72°C 30 seconds (last cycle 2 minutes). The 725 bp IgSP-mature IFNβ1 fusion PCR product and the cloning vectors pcDNA3 were digested with BamHI and HindIII

restriction enzymes and subsequently purified from 1% SeaPlaque agarose gel using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME). The ligation mixture was transformed into E. coli DH5α (Gibco BRL, Gaithersburg, MD).

A cracking gel procedure (Promega Protocols and Applications Guide, 1991) was used to screen out the positive sub-clones. The identity of the correct clone was further verified by BamHI/HindIII double digestion. The positive sub-clone for the IgSP-mature IFNβ1 was designated pcDNA3-IgSP-IFNß1-124. See Figure 2.

Transformation of BHK Cells

BHK cells were transformed as follows:

The final plasmid constructions discussed above were all amplified in a standard *E.coli* strain (HB1O1) and purified by the Qiagen-Plasmid Kit (Kontron).

The final plasmid is transfected into a line of baby hamster kidney cells (BHK) using standard calcium phosphate methodology. Gene amplification is performed in increasing concentrations of methotrexate (1-200 μM) over 8 weeks to produce stable amplified cell lines. Following this selection, the engineered BHK cells are maintained in vitro in 50-200 μM MTX. IFN expression is observed in the absence of drug selection over three months inclusive, when assayed by Northern Blot analysis, bioassay, or ELISA.

The encapsulation of cell lines engineered in this fashion allows for the selection of cells over several months for stable expression of the DHFR gene and gene of interest before transplantation. The selected cells can then be analyzed for transgene stability and gene expression by Southern and Northern blot analysis, respectively.

The level of human IFN expression from each capsule is assayed (using ELISA or bioassay) before implantation. The protein is biologically active as determined by a bioassay.

Encapsulation

The encapsulation procedure is as follows: The hollow fibers are fabricated from polyether sulfone (PES) with an outside diameter of 720 μm and a wall thickness of a 100 μm (AKZO-Nobel Wüppertal, Germany). These fibers are described in United States patents 4,976,859 and 4,968,733, herein incorporated by reference. In some studies, we prefer a PES#5 membrane which has a MWCO of about 280 kd. In other studies, we contemplate using a PES#8 membrane which has a MWCO of about 90 kd.

The devices we contemplate for these studies comprise:

1) a semipermeable poly (ether sulfone) hollow fiber membrane fabricated by AKZO Nobel Faser AG;
2) a hub membrane segment;
3) a light cured methacrylate (LCM) resin leading end; and
4) a silicone tether.

The preferred semipermeable PES#5 membrane has the following characteristics:

Internal Diameter	500±30 μm
Wall Thickness	100 ±15 μm
Force at Break	100±15 cN
Elongation at Break	44±10%
Hydraulic Permeability (ml/min m^2 mmHg)	63±8
nMWCO (dextrans)	280±20 kD

The components of the device are commercially available. The LCM glue is available from Ablestik Laboratories (Newark, DE); Luxtrak Adhesives LCM23 and LCM24). The tether material is available from Specialty Silicone Fabricators (Robles, CA). The tether dimensions are 0.79 mm OD x 0.43 mm ID x length 202 mm.

Fiber material is first cut into 5 cm long segments and the distal extremity of each segment are sealed with a photopolymerized acrylic glue (LCM-25, ICI).

Following sterilization with ethylene oxide and outgassing, the fiber segments are loaded with approximately 1 x 10^6 transfected cells in a collagen

solution (Zyderm® soluble bovine collagen) via a Hamilton syringe and a 25 gauge needle through an attached injection port. The proximal end of the capsule is sealed with the same acrylic glue. The collagen matrix may also be formed from Zyplast™ or any other suitable matrix forming material.

A silicone tether (Specialty Silicone Fabrication, Taunton, MA) (ID: 690 μm; OD: 1.25 μm) is placed over the proximal end of the fiber allowing easy manipulation and retrieval of the device.

Implantation

Patients receive one or more 5 cm long devices, implanted as follows:

Surgical procedure:

Infection prophylaxy with 2g cefazolin (Kefzol®) IV. Premedication with midazolam (Dormicum®) IV.

Under local anesthesia (xylocain 1%) a cranio-caudal skin incision of 3 cm is performed at the L4-L5 level. The subcutaneous tissue is sectioned to the dorsal fascia.

The subarachnoid space is punctured with a 25G Tuohy needle and 12 ml of CSF is withdrawn. A guide wire is introduced through the needle, and the needle is then retrieved. A dilator is introduced over the guide wire to widen the *ligamentum flavum*. The dilator is retrieved, and a cannula (4F) is introduced over the guide wire. The guide wire is then retrieved. The capsule is then pushed through the cannula and positioned in the subarachnoid space. Finally, the cannula is retrieved. See, e.g., WO 94/15663 and U.S. patent 5,487,739, incorporated herein by reference. If more than one capsule is implanted, the same procedure is repeated.

The silicone tether is fixed at the lumbar fascia with 4-0 polypropylene (Prolene®). The skin is closed with interrupted 4-0 nylon suture (Dermalon®).

For the retrieval of the device at 3 months, the skin is re-opened at the same location and the 4-0 polypropylene is sectioned. The capsule or capsules are retrieved by gently pulling on the silicone tether. The biocompatibility, the histology and the cytokine release of the explanted capsule or capsules is examined before the re-implantation of one or more new devices in the patient, following the same protocols.

Evaluation and follow-up

The patients are evaluated for side effects such as cough, weight loss, stomatitis, asthenia, and fever.

Sequences

The following is a summary of the sequences set forth in the Sequence Listing:

SEQ ID NO: 1-- oligonucleotide ohIFNß1-219

SEQ ID NO: 2-- oligonucleotide ohIFNß1-220

SEQ ID NO: 3-- oligonucleotide ohIFNß1-221

SEQ ID NO: 4-- oligonucleotide ohIFNß1-222

SEQ ID NO: 5-- oligonucleotide ohIFNß1-223

The foregoing description has been for the purpose of illustration and description only. This description is not intended to limit the invention to the precise form exemplified. It is intended that the scope of the invention be defined by the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CytoTherapeutics, Inc.

(ii) TITLE OF INVENTION: SYSTEM AND METHOD FOR DELIVERING CYTOKINES USING ENCAPSULATED CYTOKINE-SECRETING CELLS

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: FISH & NEAVE
(B) STREET: 1251 Ave. of the Americas
(C) CITY: New York
(D) STATE: NY
(E) COUNTRY: USA
(F) ZIP: 10020-1104

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/651,900
(B) FILING DATE: 21-MAY-1996

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Massaro, Jane A.
(B) REGISTRATION NUMBER: 34,218
(C) REFERENCE/DOCKET NUMBER: CTI-36 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 212 596 9000
(B) TELEFAX: 212 596 9090

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCAAGCTTC GCCACCATGA CCAACAAGTG TCT 33

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCCGGATCCT CAGTTTCGGA GGTAACCTGT 30

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCCAAGCTTG CGTCACCCCT AGAGTCGAGC TGT 33

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTTGTAGCTC ATCCTCTTGA ACTCCAGGGG 30

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAGTTCAAGA GGATGAGCTA CAACTTGCTT 30

WE CLAIM:

1. A method for continuous delivery of at least one cytokine to a patient comprising implanting at least one biocompatible capsule containing encapsulated cells directly into the central nervous system of the patient, the encapsulated cells delivering less than 25 μg of cytokine per day to the patient.

2. The method of claim 1 wherein the capsule is implanted into the intrathecal space.

3. The method of claim 1 wherein the cytokine is selected from the group consisting of IFN-ß, IFN-α, IL-10, and TGF-β.

4. A method for continuous delivery of IFN-ß or muteins or active fragments thereof to a patient comprising implanting a biocompatible capsule containing encapsulated cells directly into the central nervous system of the patient, the encapsulated cells producing up to 1.4 μg IFN-β per day.

5. A method for continuous delivery of IFN-α or muteins or active fragments thereof to a patient comprising implanting a biocompatible capsule containing encapsulated cells directly into the central nervous system of the patient, the encapsulated cells producing up to 0.02 μg IFN-α per day.

6. A method for continuous delivery of IL-10 to a patient comprising implanting a biocompatible capsule containing encapsulated cells directly into the central nervous system of the patient, the encapsulated cells producing up to 21.3 μg IL-10 per day.

7. A method for continuous delivery of TGF-β to a patient comprising implanting a biocompatible capsule containing encapsulated cells

directly into the central nervous system of the patient, the encapsulated cells producing up to 4.2 μg TGF-β per day.

8. A system for delivering IFN-β or muteins or active fragments thereof, directly to the CNS of a patient comprising one or more biocompatible capsules, each capsule comprising

a) a core comprising IFN-β-secreting cells;

b) a biocompatible jacket surrounding and encapsulating said core;

said system delivering up to 1.4 μg IFN-β per day.

9. A system for delivering IFN-α or muteins or active fragments thereof, directly to the CNS of a patient comprising one or more biocompatible capsules, each capsule comprising

a) a core comprising IFN-α-secreting cells;

b) a biocompatible jacket surrounding and encapsulating said core;

said system delivering up to 0.02 μg IFN-α per day.

10. A system for delivering IL-10 or muteins or active fragments thereof, directly to the CNS of a patient comprising one or more biocompatible capsules, each capsule comprising

a) a core comprising IL-10-secreting cells;

b) a biocompatible vehicle surrounding and encapsulating said core;

said system delivering up to 21.3 μg IL-10 per day.

11. A system for delivering TGF-β or muteins or active fragments thereof, directly to the CNS of a patient comprising one or more biocompatible capsules, each capsule comprising

a) a core comprising TGF-β-secreting cells;

b) a biocompatible vehicle surrounding and encapsulating said core;

said system delivering up to 4.2 μg TGF-β per day.

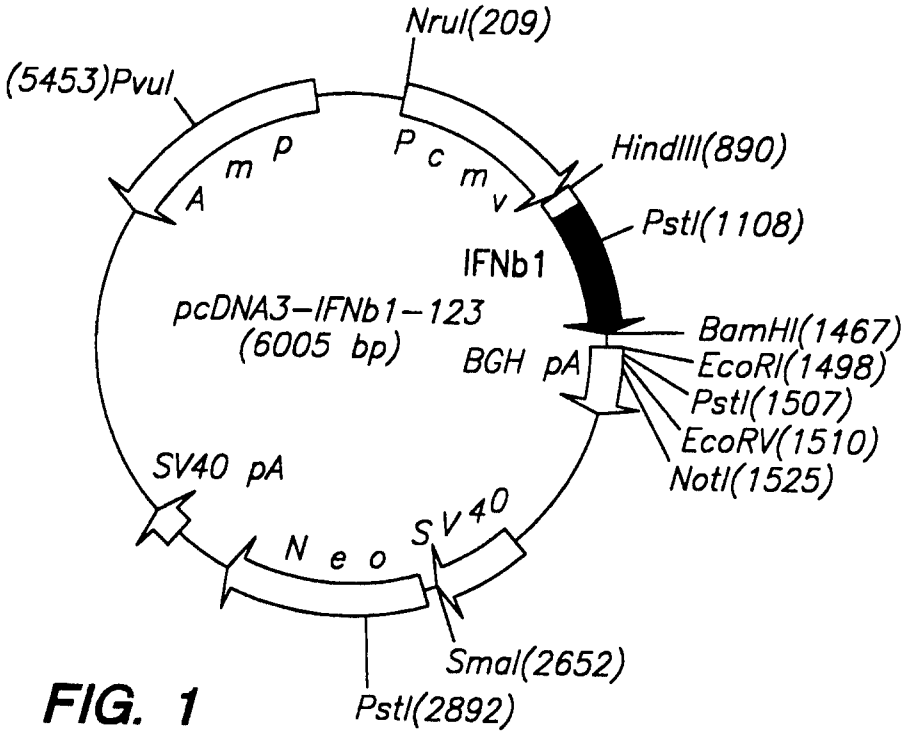
12. A method for continuous delivery of IFN-ß, or muteins or active fragments thereof, to a patient comprising implanting encapsulated cells directly into the central nervous system of the patient, the encapsulated cells producing the IFN-ß or mutein or active fragment thereof at a concentration of up to 10.0 ng/ml in the CSF.

13. A method for continuous delivery of IFN-α, or muteins or active fragments thereof, to a patient comprising implanting encapsulated cells directly into the central nervous system of the patient, the encapsulated cells producing the IFN-α or mutein or active fragment thereof at a concentration of up to 0.15 ng/ml in the CSF.

14. A method for continuous delivery of IL-10, or muteins or active fragments thereof, to a patient comprising implanting encapsulated cells directly into the central nervous system of the patient, the encapsulated cells producing the IL-10 or mutein or active fragment thereof at a concentration of up to 130 ng/ml in the CSF.

15. A method for continuous delivery of TGF-β, or muteins or active fragments thereof, to a patient comprising implanting encapsulated cells directly into the central nervous system of the patient, the encapsulated cells

producing the TGF-β or mutein or active fragment thereof at a concentration of up to 2.6 ng/ml in the CSF.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PstI(1108)
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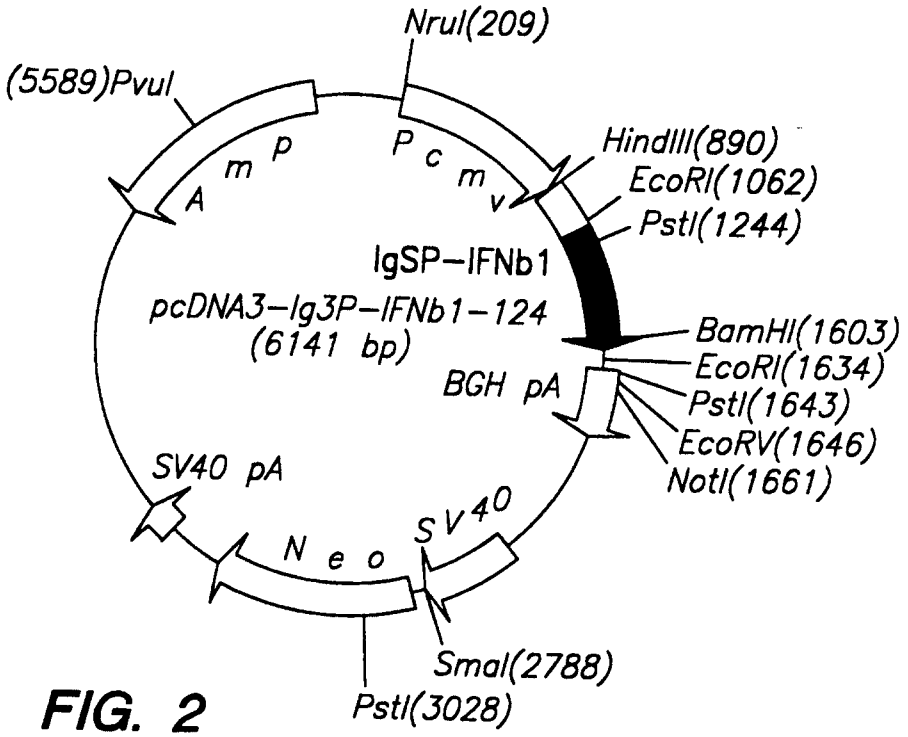
BamHI(1467)
EcoRI(1498)
PstI(1507)
EcoRV(1510)
NotI(1525)
BGH pA
SV40 pA
SmaI(2652)
PstI(2892)

FIG. 1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HindIII(890)
EcoRI(1062)
PstI(1244)
P c m v
A m p
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pcDNA3–Ig3P–IFNb1–124
(6141 bp)
BamHI(1603)
EcoRI(1634)
PstI(1643)
EcoRV(1646)
NotI(1661)
BGH pA
SV40 pA
N e o
S V 4 0
SmaI(2788)
PstI(3028)

FIG. 2