(54) Title: THERAPEUTIC EFFECT OF HEAT SHOCK PROTEINS IN PREVENTING AMYLIN AGGREGATION IN TYPE 2 DIABETES MELLITUS

(57) Abstract: The present invention concerns methods and compositions related to increasing heat shock protein (HSP) levels in pancreatic B cells to reduce amylin aggregation in individuals with Type 2 Diabetes. In specific cases, gold nanoparticles are provided to pancreatic B cells with the nanoparticles either harboring or lacking one or more HSPs, and the cells are then heated, such as with near-infrared light, for example. In particular targeting cases, antibodies to a pancreatic B cell-specific antigen are also employed.
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THERAPEUTIC EFFECT OF HEAT SHOCK PROTEINS IN PREVENTING AMYLIN AGGREGATION IN TYPE 2 DIABETES MELLITUS

TECHNICAL FIELD

[0001] This patent application claims priority to U.S. Provisional Patent Application Serial No. 61/432,793, filed on January 14, 2011, the entire contents of which is herein specifically incorporated by reference in its entirety.

[0002] The present invention generally concerns at least the fields of molecular biology, cell biology, and medicine. In particular aspects, the field concerns treatment of type 2 diabetes using heat and/or heat shock proteins.

BACKGROUND OF THE INVENTION

I. Conformational diseases and heat shock proteins

[0003] Proteins that fold into a compact structure contain a large number of hydrophobic residues in their sequences. In the correctly folded structure, these residues are buried inside the protein core and are protected against non-native interactions. The presence of hydrophobic residues makes these proteins very susceptible to aggregation when unfolded or partially folded (Chiti et al., 2002). An increasing number of neurodegenerative disorders are associated with the existence of misfolded proteins and the presence of non-native structures described as oligomers, protein aggregates, fibrils, or plaques. Furthermore, "conformational diseases" (namely Parkinson's, Alzheimer's, and Type 2 Diabetes mellitus (T2DM)) are associated with the dysfunctional aggregation of endogenous proteins that not only result in nonfunctional proteins but more importantly in toxic aggregates (Hayden et al., 2005). To avoid this detrimental aggregation, a cell uses a high proportion of its energy in refolding or preventing self-association of incompletely folded proteins. Responsible for this important task are HSPs or molecular chaperones many of which are ATP-dependent. HSPs are in contact with most cellular proteins from the moment of their production to assist with proper folding and later on to ensure the maintenance of native protein conformation during stress. They also help with the recovery of misfolded proteins and as a last resort they are involved in the trafficking of misfolded proteins to facilitate protein degradation by the ubiquitin-proteasome system.
Multiple attempts have been made to decrease self-association of misfolded proteins, for example by using monoclonal antibodies that interact at strategic sites where protein unfolding is initiated in order to prevent protein aggregation in neurodegenerative diseases (Beka, 2002). Other investigators have tried to increase the levels of HSPs by transfection or using transgenic animal models to demonstrate that HSPs counteract disease progression by acting as suppressors of toxic protein aggregates in neurodegenerative diseases. Others have tried HSP-inducing drugs that act by the modulation or activation of heat shock transcription factors (HSF) for the treatment of cancer and neurodegenerative diseases (Westerheide and Moriomoto, 2005). However, little has been done in regards to the activity of HSPs in preventing the formation of toxic misfolded aggregates of islet amyloid polypeptide implicated in the progressive deficit in β-cell mass in T2DM.

//. Loss of pancreatic β-cell mass due to toxic amylin aggregates

Matveyenko and Butler (2006) reported that there is already a 50% deficit in β-cell mass in patients with impaired fasting glucose and a 70% deficit in patients with overt T2DM. Therefore, it seems that loss of β-cell mass is an important event in the pathophysiology of T2DM (Matveyenko and Butler, 2006).

Islet amyloid polypeptide (IAPP), also called amylin, is a 37-amino acid peptide that is co-secreted with insulin by pancreatic β-cells in a 1:10 ratio. It is normally released in response to meals and appears to lower glucagon secretion and the rate of appearance of glucose after a meal. Human, feline and non-human primate forms of amylin are the only ones with the capacity to oligomerize and form aggregates due to the hydrophobicity of amino acid residues 20-29. Janson et al. (1999) demonstrated that amylin aggregates containing less than 6000 molecules (called intermediate-sized amyloid particles) are toxic to pancreatic islet cell membranes. Their cytotoxicity is mediated through disruption of cell membrane permeability by the interaction of amylin aggregates with the membranes. Once amylin aggregates become larger (>10⁶ molecules) the hydrophobic regions of amylin are hidden inside the core of the aggregate and their capacity to interpolate within the membrane is reduced (Janson et al., 1999). However, accumulation of mature islet amyloid is responsible for space-occupying lesions associated with secretory and absorptive defects (Hayden et al., 2005). Cells exposed to "aged" human IAPP (h-IAPP) solutions were surrounded and encased by these mature amyloid fibrils with no effect on the adjacent cell membranes (Janson et al., 1999).
Matveyenko and Butler concluded that amylin-induced β-cell apoptosis is responsible for the loss of β-cell mass in T2DM, ultimately leading to decreased insulin secretion (Mayveyenko and Butler, 2006). Insulin resistance and hyperglycemia not only induce insulin production but also increased IAPP production, which will demand higher concentrations of molecular chaperones to help with folding activity. This higher demand for HSPs within an already exhausted β-cell, due to hyperglycemic stress, may result in β-cell overload ultimately leading to an accumulation of misfolded amylin aggregates and toxicity.

[0007] Pancreatic β-cells respond to the accumulation of unfolded or misfolded proteins by increasing their degradation through the ubiquitin-proteasome system. After existing proteases have been consumed, the cell will respond with survival and/or apoptotic pathways, depending on the nature of the stress. Survival responses include the up-regulation of genes encoding endoplasmic reticulum chaperones or HSPs in order to increase protein folding activity and prevent more protein aggregation. The second response includes the reduction of new protein synthesis in order to prevent further accumulation of unfolded proteins. Finally, transcriptionally-activated apoptosis occurs when the ER is chronically overwhelmed and can no longer deal with the overload of unfolded proteins (Hayden et al., 2005). The ability of HSPs to protect pancreatic β-cells against cell damage makes them an attractive therapeutic target in the treatment of T2DM.

///. Heat shock proteins and type 2 diabetes mellitus

[0008] HSPs were highly conserved during evolution and are essential for cell survival. They are primarily found intracellularly, but they are also found in the extracellular compartment. The major heat shock protein families are HSP 60, 70 and 90. Hsp72 (also called Hsp70), the most important member of the HSP70 family, is highly induced by stress. Kurucz et al. (2002) reported a decreased expression of Hsp72 in skeletal muscle of T2DM patients that correlated with insulin resistance (Kurucz et al., 2002). On the other hand, it has been reported that pancreatic islets from T2DM patients have greater immunostaining for Hsp70 than the islets from non-diabetic humans (Laybutt et al., 2007). This up-regulation appears to confer protection to pancreatic β-cells against stress. Protein misfolding and amylin fibrils may be stress signals for induction of the heat shock response. Additional support for this hypothesis comes from complementary observations that HSP activators such as puromycin and azetidine inhibit protein
synthesis and prematurely terminate nascent chains leading to misfolded proteins, induce the heat shock response (Westerheide and Morimoto, 2005).

[0009] Evans et al. (2006) demonstrated in vitro the anti-aggregation activity of Hsp70. Their results proved that the combination of Hsp70/40 is more effective in preventing amyloid β peptide aggregation (characteristic of Alzheimer's disease). They also reported the chaperone activity of Hsp90 in blocking amyloid β self-assembly (Evans et al., 2006). Little has been done to prove the chaperone activity of the inducible Hsp72, for example, in preventing amylin aggregation in T2DM.

**BRIEF SUMMARY OF THE INVENTION**

[0010] The present invention is directed to methods and compositions that regard the therapeutic effect of heat shock proteins in T2DM in a mammal, including a human, dog, cat, or horse, for example. In a specific embodiment, the present invention is directed to methods and compositions that regard the therapeutic effect of heat shock proteins in preventing amylin aggregation in T2DM in a mammal. In certain embodiments, the invention concerns a particular problem in T2DM, which is pancreatic Beta cell death due to amylin toxicity. No therapies are available yet to target this problem; therefore, the use of heat and/or heat shock proteins is an innovative therapeutic approach. In particular embodiments, augmented levels of Hsp prevent misfolding and toxicity of amylin oligomers, including further misfolding and toxicity of amylin oligomers, thus conserving β-cell mass and ultimately improving insulin secretion.

[0011] In embodiments of the invention, compositions are employed to enhance levels of heat shock proteins in pancreatic B cells. The compositions may be comprised of one or more components, including but not limited to heat shock proteins, antibodies, and/or heatable carriers. The composition may include a heat shock protein, a nucleic acid encoding a heat shock protein, an agent that enhances the expression of a heat shock protein, or a combination of any of the foregoing. The heatable carriers may comprise one or more of nanoparticles, chitosan, and liposomes, for example. In specific embodiments, the heatable carriers are employed alone for delivery to the pancreas, and upon their heating they increase the levels of Hsps in the pancreas. In other cases, the heatable carriers are used for delivering one or more Hsps to the pancreas and the heatable carrier then may or may not be heated upon direct or indirect localization to the pancreas. The heatable carrier may comprise one or more tissue
targeting moieties that target the heatable carrier to a pancreatic B cell in a subject. In some
embodiments, the tissue targeting moiety is an antibody that binds to a pancreatic B cell. The
antibodies may be employed in the invention to specifically target the compositions to pancreatic
B cells. Any method of delivery to a subject is contemplated. In one embodiment, the antibody
is laparoscopically delivered to the pancreas.

[0012] In particular embodiments, the invention regards the use of heat shock proteins and/or thermo stress in the treatment of T2DM. In certain cases, thermo stress is
achieved by utilizing gold nanoshells delivered to pancreatic Beta cells (and optionally
specifically targeted thereto) that are then irradiated with near-infrared pulsed-laser. Also, one
could use bigger nanocarriers based on chitosan (Guo et al., 2010) targeted to pancreatic B-cells,
which in certain cases also carry gold nanorods to serve as a NIR thermotherapy device to
induce the synthesis of HSPs; they may also include a fluorescent probe for real-time B-cell
imaging, in specific embodiments. This not only would help to prevent B-cell death but would
also allow the evaluation of B-cell mass prior and after treatment, for example.

[0013] The use of heat to treat islet amyloid deposition and to prevent B-cell death
is a useful approach in the management of T2DM. Moreover, the generation of gold
nanoparticles conjugated to polyclonal/monoclonal antibodies and delivery (such as
intravenously) to a subject resulting in selective uptake in pancreatic B-cells and exposure to
near infrared laser (NIR) is useful as an adjuvant therapy for T2DM. This would ultimately
improve B-cell survival and insulin response to hyperglycemia, in particular aspects.

[0014] In certain cases, the present invention provides a novel use of nanoparticles,
including gold nanoshells, because most of their current uses are reported in the treatment of
cancer by inducing localized hyperthermia to kill cancer cells. In contrast, in the present
invention, these nanoshells increase the levels of chaperones that will assist with folding and
prevent aggregation of unfolded amylin. This ultimately improves pancreatic Beta cell survival
and should be clinically used as a preventive and therapeutic intervention for T2DM.

[0015] In specific embodiments, gold nanoshells are generated to specifically
target human pancreatic Beta cells, and in certain cases the nanoparticles are administrated
intravenously to patients. In certain cases, a particular time and amount of laser energy is
utilized for the heatable carriers to produce thermal stress with minimum of toxicity. It has been
demonstrated that NIR energy has the highest tissue penetration because water, blood and other tissues are relatively transparent in this spectrum; therefore, in certain embodiments this type of energy is used to heat the nanoshells. A wavelength of 808 nm at a power intensity of 4W/cm² (Gobin et al., 2007) may be employed, in certain aspects, although surrounding ranges of these parameters may also or alternatively be employed. Animal studies are useful to determine intensity and time of exposure of the pancreatic tissue, for example.

[0016] For certain diagnostic and therapeutic embodiments, one can utilize multifunctional nanocarriers, for example based on chitosan, that are provided to pancreatic B-cells and, in certain cases will comprise gold nanorods to serve as a NIR thermotherapy device and a fluorescent probe for real-time B-cell imaging.

[0017] In specific embodiments of the invention, the toxicity of amylin in pancreatic Beta cells comprises an IC50 of amylin between 8 and 11 uM. It is determined that pancreatic Beta cells exposed to heat at 43°C for 30 minutes have better survival rates than the controls.

[0018] In certain aspects, a transgenic mouse model is employed that accumulates human amylin in the pancreas. By 10 weeks of age, these hIAPP mice develop diabetes with a deficit in B-cell mass due to B-cell apoptosis (Huang et al., 2007). In particular cases, this animal model is used to characterize the exemplary nanoshells and/or chitosan nanocarriers. In some cases, the rod shape and size is optimized and the required concentration range is determined of rods for sufficient heating to induce stress response without toxicity. Also, the duration, frequency and intensity of infrared irradiation is determined.

[0019] In certain embodiments, the present invention is employed in conjunction with another therapy for the individual, such as one that includes treatment of T2DM and/or symptoms thereof.

[0020] In particular aspects, the nanoparticle is a rod, sphere, ellipsoid, elliptical disk, rectangular disk, or square, for example.

[0021] In one embodiment of the invention, there is a method of treating an individual for Type II diabetes, comprising: 1) delivering a therapeutically effective amount of a heatable carrier to the pancreas of the individual; and 2) heating the carrier. In a specific
embodiment, the carrier comprises nanoparticles, chitosan, poly(amidoamine) dendrimer or a lipid-based carrier. In certain aspects, the carrier is delivered directly to the pancreas. In some embodiments, the carrier further comprises an antibody that binds to a target polypeptide on the B cells and the direct delivery comprises targeting of the particle to pancreatic B cells using the antibody. The target polypeptide may comprise Na-K-ATPase, in certain embodiments, and the antibody binds the FXYD2 domain of the regulatory subunit of Na-K-ATPase, in some cases. In specific embodiments, the antibody binds to a region in the N-terminal 1-20, 1-15, or 1-11 amino acids of the FXYD2 domain.

[0022] Direct delivery comprises delivery of the carrier by laparoscopy to the pancreas, in some cases, although in certain embodiments the carrier is delivered indirectly to the pancreas. Nonlimiting examples of delivery methods include intravenous administration to the individual, oral administration, or via endoscopic retrograde cholangiopancreatography (ERCP).

[0023] In particular embodiments of the invention, the nanoparticle is a rod, sphere, ellipsoid, elliptical disk, rectangular disk, or square, and in specific embodiments the nanoparticle is a gold nanoparticle.

[0024] In a specific embodiment of the invention, the carrier comprises one or more Heat Shock Proteins (HSPs) or fragments thereof, or a nucleic acid encoding one more HSPs or fragments thereof. In some cases, the carrier comprises HSP72, HSP27, HSP78, GRP78, fragments thereof, or a combination thereof. In certain aspects, the carrier further comprises a label, such as fluorescent, radioactive, or a nanoparticle.

[0025] In one embodiment of the invention, there is a method of treating an individual for Type II diabetes, comprising delivering to the pancreas of the individual a therapeutically effective amount of a composition comprising: a) HSP72, HSP27, HSP78, GRP78, fragments thereof, or a combination thereof; and b) one or more moieties selected from the group consisting of an antibody that binds a target polypeptide on pancreatic B cells, a nanoparticle, a lipid-based carrier, chitosan, or poly(amidoamine) dendrimer. In a specific embodiment, the composition is delivered to the pancreas directly, such as by targeting of the composition to pancreatic B cells using the antibody, for example. Direct delivery may comprise laparoscopy, although the composition may be delivered to the pancreas indirectly.
such as by intravenous administration. In embodiments of the invention, methods include the step of heating the pancreas.

[0026] In another embodiment, there is a composition, comprising: a) HSP72, HSP27, HSP78, GRP78, fragments thereof, or a combination thereof; and b) one or more moieties selected from the group consisting of antibody that binds a target polypeptide on pancreatic B cells, a nanoparticle, a lipid-based carrier (such as a liposome), chitosan, and poly(amidoamine) dendrimer. In one case the antibody is directed to the FXYD2 domain of the regulatory subunit of Na-K-ATPase. In certain aspects of the composition, the nanoparticle is a rod, sphere, ellipsoid, elliptical disk, rectangular disk, or square. In specific aspects, the composition comprises the nanoparticle and the antibody, such as wherein the nanoparticle is conjugated to the antibody, for example.

[0027] In specific aspects of the composition, the nanoparticle is a nanorod that is conjugated with an antibody to the FXYD2 domain of the regulatory subunit of Na-K-ATPase. In certain specific aspects, the antibody binds to a region in the first 1-20, 1-15, or 1-11 amino acids of the FXYD2 domain. The composition may be further defined as comprising the nanoparticle and chitosan, such as wherein the nanoparticle is seeded in the chitosan. In some cases, the nanoparticle is a gold nanoparticle. In some cases of the composition, it comprises a detectable label, such as one that is fluorescent, radioactive, or nanoparticles.

[0028] In one embodiment of the invention, there is a kit comprising the composition of the invention, said composition housed in a suitable container. In certain cases, the kit further comprises an additional therapy for diabetes.

[0029] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described hereinafter which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and specific embodiment disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the
invention, both as to its organization and method of operation, together with further objects and
advantages will be better understood from the following description when considered in
connection with the accompanying figures. It is to be expressly understood, however, that each
of the figures is provided for the purpose of illustration and description only and is not intended
as a definition of the limits of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] For a more complete understanding of the present invention, reference is
now made to the following descriptions taken in conjunction with the accompanying drawings.

[0031] FIG. 1. h-IAPP toxicity in Beta-TC-6 cells determined by trypan blue
exclusion assay in which live cells exclude trypan blue, while dead cells that take up trypan blue
appear blue when observed under white light. Data are representative of three independently
performed experiments with similar results.

[0032] FIG. 2. h-IAPP IC50 determined in the Beta-TC-6 cell line using the MTS
cell proliferation assay. Different cell concentrations were used. The mean h-IAPP IC50 was
calculated at 10.43 ± 1.92 µM. Data are the sum of four independently performed experiments
with similar results.

[0033] FIG. 3 provides a western showing that thermal stress activates the
expression of Hsp72 and Hsp25. Beta-TC-6 cells were heat shocked at 43°C for 30min, 45min
or 60min or maintained at 37°C for 30min or 60min (control). Twenty-four hours later, cells
were lysed and equal concentrations of cell lysate (3C^g) was added to a 10% SDS-PAGE and
probed with anti-Hsp72 (top panel) or anti-Hsp25 (bottom panel). Data are representative of
three independently performed experiments with similar results.

[0034] FIG. 4 demonstrates that heat shock treatment reduces h-IAPP toxicity
against Beta-TC-6 cells. Beta-TC-6 cells were heat shocked at 43°C for 30 min (HS) or
maintained at 37°C (Ctrl). Twenty-four hours later, cells were treated with various
concentrations of h-IAPP. After a further 24 h of incubation, cell viability was assessed using the
MTS assay. Data are the mean percentage of live cells (+ SD) and is the sum of three
independently performed experiments. *p<0.05 vs h-IAPP treated cells.
FIG. 5 demonstrates that h-IAPP transgenic but not m-IAPP C. elegans develop toxic aggregates. IAPP plasmids expressing in pharynx, body wall muscles and neurons were co-injected at a concentration of 20ng\(\pm\)1 each. (A) Body wall muscle expression; h-IAPP show more fluorescence and more aggregates than m-IAPP model. (B) Pharynx expression; h-IAPP model show high fluorescence in the pharynx tissue that could correspond to the presence of protein aggregates. (C) h-IAPP expressed in head muscles is distributed into discrete foci, while m-IAPP has a more soluble distribution pattern. (D) h-IAPP show more intense fluorescence in vulva muscles than m-IAPP model. Data are representative of at least eight independently performed experiments with similar results.

FIG. 6. Injection of h-IAPP transgenic C. elegans with Hsp72 promoter significantly reduces the appearance of toxic h-IAPP aggregates. h-IAPP transgenic mice were injected with control plasmid (left panel) or Hsp72 plasmid (right panel) and the appearance of h-IAPP aggregates was observed using a 60x microscope. Data are representative of at least eight independently performed experiments with similar results.

FIG. 7. Transfection of Beta-TC-6 cells with Hsp72 significantly reduces h-IAPP-induced toxicity. Beta-TC-6 cells were transfected with Hsp72 plasmids and incubated for 72 h in a 37°C incubator. Cells were then treated with h-IAPP. After a further 24 h of incubation the number of live cells were counted using a fluorescence microscope. Data are the mean number of live cells (+ SD) from 5 different areas under 60x magnification, and is a representative of three independently performed experiments with similar results.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. For purposes of the present invention, the following terms are defined below.

As used herein, the use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." Some embodiments of the invention may consist of or consist essentially of one or more elements, method steps, and/or methods of the invention. It is contemplated that any method or
composition described herein can be implemented with respect to any other method or composition described herein.

[0040] The term "heatable" as used herein refers to a carrier that is able to enter a tumor and deliver a specific amount of heat.

[0041] The term "nanoparticle" as used herein refers to particles sized between 1 and 100 nanometers, in certain embodiments.

[0042] The term "therapeutically effective amount" as used herein refers to an amount that results in an improvement or remediation of one or more symptoms of T2DM.

IV. General Embodiments of the Invention

[0043] In embodiments of the invention, heat shock treatment is used as a therapy for the treatment of diabetic patients, including T2DM. In at least certain embodiments of type 2 diabetes mellitus (T2DM), the misfolded islet amyloid polypeptide hormone (also called amylin) forms toxic aggregates that destroy pancreatic β-cells (Hayden et al., 2005). In certain embodiments of the invention, there are new pharmacological approaches to minimize pancreatic β-cell destruction in T2DM patients by reducing and/or preventing amylin aggregation by molecular chaperones such as HSPs. In certain aspects of the invention, increased levels of HSPs, for example induced by heat shock therapy, improve pancreatic β-cell survival in T2DM. In particular cases, heat shock treatment is used as a therapy, including a concomitant therapy, for the treatment of diabetic patients in which loss of β-cells worsens insulin resistance.

V. Heat Shock Proteins (HSPs)

[0044] In some embodiments of the invention, one, two, three, four, or more HSPs are provided to an individual for the treatment of Type II diabetes, such as provided to the pancreas in the individual. In certain cases, the HSPs are provided as proteins or protein fragments, although in alternative embodiments the HSPs are provided in nucleic acid form. In certain aspects, the HSPs are provided in a composition comprising an antibody, nanoparticle, liposome, chitoson, or a combination thereof.
In embodiments of the invention, one or more of a variety of HSPs are provided to an individual with Type II diabetes. In specific embodiments, the one or more HSPs are capable of acting as chaperones on amylin in pancreatic B cells, thereby reducing amylin aggregation in the pancreatic B cells. In certain cases, the one or more HSPs are capable of inhibiting apoptosis of a pancreatic B cell. In specific cases, however, the one or more HSPs are selected from the group consisting of HSP72 (also called Hsp72, Hspal, or Hsp70 1); HSP27 (having alternate names of 28 kDa heat shock protein, estrogen regulated 24 kDa protein; heat shock 27kDa protein 1; heat shock protein beta 1; HSP28; SRP27, and stress responsive protein 27); GRP75 (having alternate names of Heat shock 70 KD protein 9; Heat shock 70 KD protein 9B; HSPA9; Mortalin, perinuclear; MOT; MOT2; p66 mortalin; PBP74; Peptide binding protein 74; and Stress 70 protein, mitochondrial); and GRP78 (having alternate names of Endoplasmic reticulum luminal Ca\textsuperscript{2+} binding protein grp78; glucose related protein 78KD; heat shock 70kD protein 5; and immunoglobulin heavy chain binding-protein).

Heat shock protein 70 KD 1A (HSP72) protein sequence is provided in SEQ ID NO: 1 and the mRNA sequence is provided in SEQ ID NO: 2 (open reading frame (ORF) is 244-2169 nt). HSP 27 protein sequence is provided in SEQ ID NO: 3, and the mRNA sequence is provided in SEQ ID NO: 4 (ORF is 156-773 nt). GRP75 protein sequence is provided in SEQ ID NO: 5 (NP_004125.3) and the DNA sequence is provided in SEQ ID NO: 6 (ORF is 109-2148; NM_004134. GRP78 protein sequence is provided in SEQ ID NO: 7 (NP_005338.1) and the DNA sequence is provided in SEQ ID NO: 8 (ORF is 257-2221; NM_005347.3).

In a specific embodiment, a fragment of one or more particular HSPs is employed in the invention. The fragment may include the amylin-binding site and/or chaperone activity domains, in specific embodiments. The fragment may include the C-terminus or the N-terminus of the HSP. The fragment may comprise at least 5-20 consecutive amino acids of the corresponding HSP sequence, at least 5-25, at least 5-30, at least 5-35, at least 5-40, at least 5-45, at least 5-50, at least 5-55, at least 5-60, at least 5-65, at least 5-70, at least 5-75, at least 10-20, at least 10-25, at least 10-30, at least 10-35, at least 10-40, at least 10-45, at least 10-50, at least 10-55, at least 10-60, at least 10-65, at least 10-70, at least 10-75, at least 15-20, at least 15-25, at least 15-30, at least 15-35, at least 15-40, at least 15-45, at least 15-50, at least 15-55, at least 15-60, at least 15-65, at least 15-70, at least 15-75, at least 20-25, at least 20-30, at least 20-


[0049] In some cases, the fragment is identical in sequence to the corresponding wild-type HSP sequence, although in specific embodiments the fragment has between 70% and 75% identity to the corresponding wild-type sequence, between 75% and 80%, between 80% and 85%, between 85% and 90%, between 90% and 95%, or between 95% and 99% identity to the corresponding wild-type HSP sequence. In some cases, the fragment has 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the corresponding wild-type HSP protein. In some embodiments, there are one, two, three, four, five, or more amino acid changes compared to the corresponding wild-type HSP protein sequence, and in specific embodiments the amino acid changes are conservative amino acids changes.
In alternative embodiments, the HSP employed comprises HSP70 or HSP73 or fragments thereof. HSP70 and/or HSP73 may be utilized in conjunction with one or more of HSP72, HSP27, GRP75, GRP78, or as an alternative to one or more of HSP72, HSP27, GRP75, GRP78.

In embodiments of the invention, the carrier comprises one or more molecules of an HSP, and in cases where there are two or molecules of an HSP with the carrier, the two or more molecules may be different HSPs.

VI. Carrier and other Moieties

In embodiments of the invention, the levels and/or activity of HSPs are increased in pancreatic B cells of diabetic individuals. In some methods of the invention, endogenous HSPs have levels and/or activity increased in pancreatic B cells, whereas in some cases one delivers exogenous HSPs to the pancreas to thereby increase the level in pancreatic B cells. In certain aspects, HSP levels in pancreatic B cells are increased by both increasing endogenous levels and adding exogenous HSPs to the cellular level of the pancreatic B cells.

In certain cases, pancreatic B cell HSP levels are increased by delivering a moiety capable of being heated at the pancreas and applying heat to the pancreas. In specific embodiments, the moiety is capable of being heated at the pancreas by near infrared (NIR) light, for example. NIR energy is useful in certain aspects because of its high penetration and high absorbance by gold nanoshells. In certain aspects, the moiety exhibits enhanced near infrared light absorption. In specific embodiments, the moiety is a nanoparticle that strongly absorbs near infrared (NIR) light and locally heats the pancreatic cells, including pancreatic B cells. Such local heating of pancreatic B cells will increase HSP levels (including HSP72, HSP27, GRP75, and/or GRP78, for example) therein, which will act as chaperone to amylin and will prevent or reduce amylin aggregation toxicity.

In certain embodiments of the invention, compositions comprise nanoparticles, lipid-based compounds, and/or chitosan, for example. Such compositions act as carriers for the HSPs, in certain cases, although in other cases the compositions lack HSPs and themselves are delivered to the pancreas and heated. Examples of chitosan-based nanoparticles are known in the art, such as are described in Liu et al. (2007).
[0055] Nanoparticles may be assembled by standard means in the art, although in specific embodiments they are assembled by the methodology of Prevo et al. (2008); Huang et al. (2008); Adegboyega et al. (2007); Cherukuri and Curley (2010); Gobin (2007); or Guo (2010), for example.

[0056] Other moieties that may be included in the invention are antibodies, which may be employed to directly target the compositions to pancreatic B cells of the diabetic individual.

VII. Gold nanorods and their use in plasmonic photothermal therapy (PPTT)

[0057] Physiological media such as water, blood and tissue, are relatively transparent in the NIR region of the spectrum, allowing deep tissue penetration (Prevo et al., 2008). Noble metal nanoparticles, due to the phenomenon of surface plasmon resonance, exhibit an enhanced visible and near-infrared light absorption. The use of these plasmonic nanoparticles that strongly absorb NIR light has introduced a more specific and efficient cancer therapy strategy to locally heat and kill cancer cells within tumors located deep within body tissue. This type of treatment is called photothermal heating or plasmonic photothermal therapy (PPTT) (Huang et al., 2008). It has been demonstrated that by adjusting the particle number, size, and laser energy, investigators were able to selectively induce cell death or transiently modify cellular functions without causing cell destruction. In embodiments of the invention, PPTT is employed in T2DM not to kill pancreatic β-cells, but to stress them and stimulate their heat shock response in order to elucidate the potential role of thermal stress in the protection of pancreatic β-cells against amylin deposition. This is an innovative use of gold nanorods, since most of their current uses are reported in the treatment of cancer by inducing localized hyperthermia to kill cancer cells. The use of photothermal heating to denature proteins in laser wound closure has also been reported (Prevo et al., 2008).

[0058] Thus, in certain aspects there is the use of gold nanorods that strongly absorb NIR pulsed-laser energy to induce localized hyperthermia in pancreatic β-cells not to kill the cells but instead to stress them in order to increase the levels of HSPs (the molecular chaperones that will assist with folding and prevent aggregation of unfolded amylin). This would ultimately improve pancreatic β-cell survival and is clinically useful as a preventive and/or therapeutic intervention for T2DM.
VIII. Specific markers of pancreatic β-cells

[0059] In certain embodiments of the invention, a marker on pancreatic B cells is employed such that the carrier composition is targeted to the pancreatic B cells. A marker that is specific for pancreatic B cells may be employed in the invention.

[0060] Flamez et al. (2010) identified a specific β-cell biomarker that contains the FXYD domain of the regulatory subunit of the Na+-K+-ATPase. The intrinsic properties of the pump are regulated by association with tissue-specific small single-span membrane proteins belonging to the FXYD family (Flamez et al., 2010). There are three splice variants of FXYD2 and two of these are expressed in human pancreatic islets (FXYD2ya and FXYD2yb). FXYD2ya is produced in β-cells but not alpha or delta cells, making this a specific target for β-cells. Antibodies recognizing the first 11 amino acids from the amino terminal extracellular domain of mouse FXYD2ya (which differs from humans in four amino acids) are conjugated to nanorods allowing their binding to β-cells. Absorption of NIR pulsed laser energy by antibody-bound nanorods will induce the heat shock response to protect β-cells from the toxic effect of amylin exposure.

[0061] Monoclonal antibodies directed against the first 11 amino acids from the amino terminal extracellular domain of human FXYD2ya may be employed, in specific embodiments. This is a specific β-cell biomarker that contains the FXYD domain of the regulatory subunit of the Na+-K+-ATPase. The intrinsic properties of the pump are regulated by association with tissue-specific small single-span membrane proteins belonging to the FXYD family. There are three splice variants of FXYD2 and two of these are expressed in human pancreatic islets (FXYD2ya and FXYD2yb). FXYD2ya is produced in β-cells, but not alpha or delta cells, making this a specific target for β-cells. In some cases, antibodies recognizing the first 11 amino acids from the amino terminal extracellular domain of FXYD2ya are conjugated to nanorods, thereby allowing their binding to β-cells. See Flamez et al. (2010).

IX. Application of Heat in the Invention

[0062] In embodiments of the invention, a composition is delivered directly or indirectly to the pancreas, and the pancreas is heated directly, although in certain cases the pancreas is heated indirectly. In some cases, the heat is applied as a steady pulse, although in some cases the heat is applied as an intermittent pulse. The level of heat applied will be such
that HSP proteins and/or their expression are thereby enhanced but not to the level of killing the localized cells. In specific embodiments of the invention, an increase in expression that is two-fold or greater is encompassed in the invention, including three-fold, four-fold, five-fold, 10-fold, 25-fold, 50-fold, 75-fold, 100-fold, 150-fold, 200-fold, 250-fold, or greater. In specific embodiments, the duration of heating is a specific range, for example no less than 5 seconds, for example but no longer than two hours, for example.

[0063] The type of heat that is applied to the pancreas may be of a particular kind. In specific embodiments, near infrared heat is employed. Gold nanorods that strongly absorb NIR light are utilized in certain embodiments as a strategy to locally heat and increase the levels of heat shock proteins within the pancreatic tissue. Increased levels of heat shock proteins in the pancreas therapeutically prevent amylin aggregation and consequently prevent amylin toxicity in pancreatic beta cells, in particular aspects of the invention.

X. Antibodies and Antibody Preparation

[0064] In certain embodiments of the invention, antibodies are employed that target compositions of the invention to the pancreas of the diabetic individual. In certain aspects, the antibodies target the compositions of the invention to pancreatic B cells of the diabetic individual. In specific embodiments, the antibodies bind to a target polypeptide on the surface of the pancreatic B cells, and in particular cases the target polypeptide is unique to the surface of pancreatic B cells. In specific embodiments, the target polypeptide is an extracellular domain of a membrane protein of the pancreatic B cell.

[0065] In certain cases of the invention, the target polypeptide comprises Na-K-ATPase of the pancreatic B cell. In particular, the antibody binds a region of the FXYD2 domain of the regulatory subunit of the Na-K-ATPase. The FXYD2 protein sequence (GenBank® NP_001 120961.1, and is incorporated by reference herein) is provided in SEQ ID NO:9, and the FXYD2 nucleic acid sequence (GenBank® NM_001 127489.1, and is incorporated by reference herein) is provided in SEQ ID NO:10. In certain embodiments, the antibody binds to a region of the FXYD2 domain, for example, within the N-terminal 1-20 amino acids of the FXYD2 domain, although in some cases it binds within the N-terminal 1-15 amino acids or N-terminal 1-11 amino acids of the FXYD2 domain, for example. According to Flamez et al. (2010), these regions are identified as beta cell-specific in pancreatic beta cells.
[0066] As used herein, the term "antibody" is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE. Generally, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting.

[0067] The term "antibody" is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')2, single domain antibodies (DABs), Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means for preparing and characterizing antibodies are also well known in the art (See, e.g., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; incorporated herein by reference).

[0068] The skilled artisan recognizes that any type of antibodies may be employed in the invention so long as they target pancreatic B cells. Provided below are general descriptions of how to generate different yet exemplary types of antibodies.

A. Polyclonal antibodies

[0069] Polyclonal antibodies to the target polypeptide may be raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the polypeptide (or respective fragment thereof) and an adjuvant. In certain embodiments, it is useful to conjugate the target polypeptide or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g. keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, for example using a bifunctional or derivatizing agent, for example maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glytaraldehyde, succinic anhydride, SOCl₂, or R¹N=C=NR, where R and R¹ are different alkyl groups.

[0070] Animals are immunized against the immunogenic conjugates or derivatives by combining 1 mg of 1 µg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freud's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freud's complete adjuvant by subcutaneous injection at multiple sites. 7 to 14 days later the animals are
bled and the serum is assayed for anti-target polypeptide antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal boosted with the conjugate of the same target polypeptide, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum can be used to enhance the immune response.

B. Monoclonal antibodies

[0071] Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

[0072] For example, the anti-target polypeptide monoclonal antibodies of the invention may be made using the hybridoma method first described by Kohler & Milstein, Nature 256:495 (1975), or may be made by recombinant DNA methods [Cabilly, et al, U.S. Pat. No. 4,816,567], for example.

[0073] In the hybridoma method, a mouse or other appropriate host animal, such as hamster is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)].

[0074] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.
Preferred myeloma cells are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 cells available from the American Type Culture Collection, Rockville, Md. USA, for example.

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the target polypeptide. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson & Pollard, Anal. Biochem. 107:220 (1980), for example.

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods. Goding, Monoclonal Antibodies: Principles and Practice, pp.59-104 (Academic Press, 1986). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies of the invention is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host
cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison, et al., Proc. Nat. Acad. Sci. 81, 6851 (1984), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of an anti-target polypeptide monoclonal antibody herein.

[0081] Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention, or they are substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for a target polypeptide and another antigen-combining site having specificity for a different antigen.

[0082] Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[0083] For diagnostic applications, the antibodies of the invention may be labeled with a detectable moiety, in certain embodiments. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as $^3$H, $^{14}$C, $^{32}$P, $^{35}$S, or $^{125}$I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; biotin; radioactive isotopic labels, such as, e.g., $^{125}$I, $^{32}$P, $^{14}$C, or $^3$H, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase.

[0085] The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987), for example.

[0086] Competitive binding assays rely on the ability of a labeled standard (which may be a target polypeptide or an immunologically reactive portion thereof) to compete with the test sample analyte (target polypeptide) for binding with a limited amount of antibody. The amount of target polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

[0087] Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three part complex. David & Greene, U.S. Pat. No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

C. Humanized antibodies

[0088] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature 321, 522-525 (1986); Riechmann et al, Nature 332, 323-327 (1988); Verhoeyen et al, Science 239, 1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are
chimeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0089] It is important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e. the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. For further details see U.S. application Ser. No. 07/934,373 filed Aug. 21, 1992, which is a continuation-in-part of application Ser. No. 07/715,272 filed Jun. 14, 1991.

D. Human antibodies

[0090] Human monoclonal antibodies can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor, J. Immunol. 133, 3001 (1984), and Brodeur, et al., Monoclonal Antibody Production Techniques and Applications, pp.51-63 (Marcel Dekker, Inc., New York, 1987).

[0091] It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (JH) gene in chimeric and germ-

Alternatively, the phage display technology (McCafferty et al, Nature 348, 552-553 [1990]) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle.

Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g. Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3, 564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al, Nature 352, 624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al, J. Mol. Biol. 222, 581-597 (1991), or Griffith et al, EMBO J. 12, 725-734 (1993). In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al, Bio/Technol. 10, 779-783 [1992]). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This techniques allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage
antibody repertoires (also known as "the mother-of-all libraries") has been described by Waterhouse et al., Nucl. Acids Res. 21, 2265-2266 (1993), and the isolation of a high affinity human antibody directly from such large phage library is reported by Griffith et al., EMBO J. (1994), in press. Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable capable of restoring a functional antigen-binding site, i.e. the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT patent application WO 93/06213, published Apr. 1, 1993). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

E. Mini-antibodies

[0094] "Mini-antibodies" or "minibodies" are also contemplated for use with the present invention. Minibodies are sFv polypeptide chains which include oligomerization domains at their C-termini, separated from the sFv by a hinge region. Pack et al. (1992) Biochem 31:1579-1584. The oligomerization domain comprises self-associating alpha-helices, e.g., leucine zippers, that can be further stabilized by additional disulfide bonds. The oligomerization domain is designed to be compatible with vectorial folding across a membrane, a process thought to facilitate in vivo folding of the polypeptide into a functional binding protein. Generally, minibodies are produced using recombinant methods well known in the art. See, e.g., Pack et al. (1992) Biochem 31:1579-1584; Cumber et al. (1992) J Immunology 149B:120-126.

F. Antibody-like binding peptidomimetics

[0095] Antibody-like binding peptidomimetics are also contemplated in the present invention. Liu et al. Cell Mol Biol (Noisy-le-grand). 2003 Mar;49(2):209-16 describe "antibody like binding peptidomimetics" (ABiPs), which are peptides that act as pared-down antibodies and have certain advantages of longer serum half-life as well as less cumbersome synthesis methods.
G. Bispecific antibodies

[0096] Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for a target polypeptide, the other one is for any other antigen, and preferably for another pancreatic B-cell-specific antigen.

[0097] Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Millstein and Cuello, Nature 305, 537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in PCT application publication No. WO 93/08829 (published May 13, 1993), and in Traunecker et al., EMBO 10, 3655-3659 (1991).

[0098] According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CHI) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the
other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in copending application Ser. No. 07/931,811 filed Aug. 17, 1992.

[0099] For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology 121, 210 (1986).

H. Heteroconjugate antibodies

[0100] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373; EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

XL Lipid Compositions

[0101] In certain embodiments, the present invention concerns a novel composition comprising one or more lipids associated with at least one composition for providing to a pancreatic B cell. In specific cases, the lipid is heatable. A lipid is a substance that is characteristically insoluble in water and extractable with an organic solvent. Lipids include, for example, the substances comprising the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which are well known to those of skill in the art which contain long chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes. Of course, compounds other than those specifically described herein that are understood by one of skill in the art as lipids are also encompassed by the compositions and methods of the present invention.

[0102] A lipid may be naturally occurring or synthetic (i.e., designed or produced by man). However, a lipid is usually a biological substance. Biological lipids are well known in the art, and include for example, neutral fats, phospholipids, phosphoglycerides, steroids,
terpenes, lysolipids, glycosphingolipids, glycolipids, sulphatides, lipids with ether and ester-linked fatty acids and polymerizable lipids, and combinations thereof.

A. Lipid Types

[0103] A neutral fat may comprise a glycerol and a fatty acid. A typical glycerol is a three carbon alcohol. A fatty acid generally is a molecule comprising a carbon chain with an acidic moiety (e.g., carboxylic acid) at an end of the chain. The carbon chain may of a fatty acid may be of any length, however, it is preferred that the length of the carbon chain be of from about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, to about 30 or more carbon atoms, and any range derivable therein. However, a preferred range is from about 14 to about 24 carbon atoms in the chain portion of the fatty acid, with about 16 to about 18 carbon atoms being particularly preferred in certain embodiments. In certain embodiments the fatty acid carbon chain may comprise an odd number of carbon atoms, however, an even number of carbon atoms in the chain may be preferred in certain embodiments. A fatty acid comprising only single bonds in its carbon chain is called saturated, while a fatty acid comprising at least one double bond in its chain is called unsaturated.

[0104] Specific fatty acids include, but are not limited to, linoleic acid, oleic acid, palmitic acid, linolenic acid, stearic acid, lauric acid, myristic acid, arachidic acid, palmitoleic acid, arachidonic acid ricinoleic acid, tuberculosteric acid, lactobaciUic acid. An acidic group of one or more fatty acids is covalently bonded to one or more hydroxyl groups of a glycerol. Thus, a monoglyceride comprises a glycerol and one fatty acid, a diglyceride comprises a glycerol and two fatty acids, and a triglyceride comprises a glycerol and three fatty acids.

[0105] A phospholipid generally comprises either glycerol or an sphingosine moiety, an ionic phosphate group to produce an amphipathic compound, and one or more fatty acids. Types of phospholipids include, for example, phosphoglycerides, wherein a phosphate group is linked to the first carbon of glycerol of a diglyceride, and sphingophospholipids (e.g., sphingomyelins), wherein a phosphate group is esterified to a sphingosine amino alcohol. Another example of a sphingophospholipid is a sulfatide, which comprises an ionic sulfate group that makes the molecule amphipathic. A phopholipid may, of course, comprise further chemical
groups, such as for example, an alcohol attached to the phosphate group. Examples of such alcohol groups include serine, ethanolamine, choline, glycerol and inositol. Thus, specific phosphoglycerides include a phosphatidyl serine, a phosphatidyl ethanolamine, a phosphatidyl choline, a phosphatidyl glycerol or a phosphotidyl inositol. Other phospholipids include a phosphatidic acid or a diacetyl phosphate. In one aspect, a phosphatidylcholine comprises a dioleoylphosphatidylcholine (a.k.a. cardiolipin), an egg phosphatidylcholine, a dipalmitoyl phosphalidycholine, a monomyristoyl phosphatidylcholine, a monopalmitoyl phosphatidylcholine, a monostearoyl phosphatidylcholine, a monooleoyl phosphatidylcholine, a dibutryl phosphatidylcholine, a diacetyl phosphate, a monosialoganglioside, GM1), which comprises about 2, about 3, about 4, about 5, about 6, to about 7 or so sugar groups, that may be in a branched chain, attached to the primary hydroxyl group. In other embodiments, the glycolipid is a ceramide (e.g., lactosylceramide).

[0106] A glycolipid is related to a sphingogospholipid, but comprises a carbohydrate group rather than a phosphate group attached to a primary hydroxyl group of the sphingosine. A type of glycolipid called a cerebroside comprises one sugar group (e.g., a glucose or galactose) attached to the primary hydroxyl group. Another example of a glycolipid is a ganglioside (e.g., a monosialoganglioside, a GMI), which comprises about 2, about 3, about 4, about 5, about 6, to about 7 or so sugar groups, that may be in a branched chain, attached to the primary hydroxyl group. In other embodiments, the glycolipid is a ceramide (e.g., lactosylceramide).

[0107] A steroid is a four-membered ring system derivative of a phenanthrene. Steroids often possess regulatory functions in cells, tissues and organisms, and include, for example, hormones and related compounds in the progestagen (e.g., progesterone), glucocorticoid (e.g., Cortisol), mineralocorticoid (e.g., aldosterone), androgen (e.g., testosterone) and estrogen (e.g., estrone) families. Cholesterol is another example of a steroid, and generally serves structural rather than regulatory functions. Vitamin D is another example of a sterol, and is involved in calcium absorption from the intestine.

[0108] A terpene is a lipid comprising one or more five carbon isoprene groups. Terpenes have various biological functions, and include, for example, vitamin A, coenzyme Q and carotenoids (e.g., lycopene and β-carotene).
B. Charged and Neutral Lipid Compositions

[0109] In certain embodiments, a lipid component of a composition is uncharged or primarily uncharged. In one embodiment, a lipid component of a composition comprises one or more neutral lipids. In another aspect, a lipid component of a composition may be substantially free of anionic and cationic lipids, such as certain phospholipids (e.g., phosphatidylcholine) and cholesterol. In certain aspects, a lipid component of an uncharged or primarily uncharged lipid composition comprises about 95%, about 96%, about 97%, about 98%, about 99% or 100% lipids without a charge, substantially uncharged lipid(s), and/or a lipid mixture with equal numbers of positive and negative charges.

[0110] In other aspects, a lipid composition may be charged. For example, charged phospholipids may be used for preparing a lipid composition according to the present invention and can carry a net positive charge or a net negative charge. In a non-limiting example, diacetyl phosphate can be employed to confer a negative charge on the lipid composition, and stearylamine can be used to confer a positive charge on the lipid composition.

C. Making Lipids

[0111] Lipids can be obtained from natural sources, commercial sources or chemically synthesized, as would be known to one of ordinary skill in the art. For example, phospholipids can be from natural sources, such as egg or soybean phosphatidylcholine, brain phosphatidic acid, brain or plant phosphatidylcholine, heart cardiolipin and plant or bacterial phosphatidylethanolamine. In another example, lipids suitable for use according to the present invention can be obtained from commercial sources. For example, dimyristyl phosphatidylycholine ("DMPC") can be obtained from Sigma Chemical Co., dicetyl phosphate ("DCP") is obtained from K & K Laboratories (Plainview, NY); cholesterol ("Choi") is obtained from Calbiochem Behring; dimyristyl phosphatidyglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). In certain embodiments, stock solutions of lipids in chloroform or chloroform/methanol can be stored at about 20°C. Preferably, chloroform is used as the only solvent since it is more readily evaporated than methanol.
D. Lipid Composition Structures

[0112] In a preferred embodiment of the invention, the composition for delivery to the pancreatic B cell may be associated with a lipid. A composition associated with a lipid may be dispersed in a solution containing a lipid, dissolved with a lipid, emulsified with a lipid, mixed with a lipid, combined with a lipid, covalently bonded to a lipid, contained as a suspension in a lipid, contained or complexed with a micelle or liposome, or otherwise associated with a lipid or lipid structure. A lipid or lipid/pancreatic B cell-associated composition of the present invention is not limited to any particular structure. For example, they may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in either size or shape. In another example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. In another non-limiting example, a lipofectamine (Gibco BRL) or Superfect (Qiagen) are also contemplated.

[0113] In certain embodiments, a lipid composition may comprise about 1%, about 2%, about 3%, about 4% about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100%, or any range derivable therein, of a particular lipid, lipid type or non-lipid component such as a protein, nucleic acids or other material disclosed herein or as would be known to one of skill in the art. In a non-limiting example, a lipid composition may comprise about 10% to about 20% neutral lipids, and about 33% to about 34% of a cerebroside, and about 1% cholesterol. In another non-limiting example, a liposome may comprise about 4% to about 12% terpenes, wherein about 1% of the micelle is specifically lycopene, leaving about 3% to
about 11% of the liposome as comprising other terpenes; and about 10% to about 35% phosphatidyl choline, and about 1% of a drug. Thus, it is contemplated that lipid compositions of the present invention may comprise any of the lipids, lipid types or other components in any combination or percentage range.

E. Emulsions

A lipid may be comprised in an emulsion. A lipid emulsion is a substantially permanent heterogeneous liquid mixture of two or more liquids that do not normally dissolve in each other, by mechanical agitation or by small amounts of additional substances known as emulsifiers. Methods for preparing lipid emulsions and adding additional components are well known in the art (e.g., Modern Pharmaceutics, 1990, incorporated herein by reference).

For example, one or more lipids are added to ethanol or chloroform or any other suitable organic solvent and agitated by hand or mechanical techniques. The solvent is then evaporated from the mixture leaving a dried glaze of lipid. The lipids are resuspended in aqueous media, such as phosphate buffered saline, resulting in an emulsion. To achieve a more homogeneous size distribution of the emulsified lipids, the mixture may be sonicated using conventional sonication techniques, further emulsified using microfluidization (using, for example, a Microfluidizer, Newton, Mass.), and/or extruded under high pressure (such as, for example, 600 psi) using an Extruder Device (Lipex Biomembranes, Vancouver, Canada).

F. Micelles

A lipid may be comprised in a micelle. A micelle is a cluster or aggregate of lipid compounds, generally in the form of a lipid monolayer, and may be prepared using any micelle producing protocol known to those of skill in the art (e.g., Canfield et al., 1990; El-Gorab et al., 1973; Colloidal Surfactant, 1963; and Catalysis in Micellar and Macromolecular Systems, 1975, each incorporated herein by reference). For example, one or more lipids are typically made into a suspension in an organic solvent, the solvent is evaporated, the lipid is resuspended in an aqueous medium, sonicated and then centrifuged.

G. Liposomes

In particular embodiments, a lipid comprises a liposome. A "liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the
generation of enclosed lipid bilayers or aggregates. Liposomes may be characterized as having vesicular structures with a bilayer membrane, generally comprising a phospholipid, and an inner medium that generally comprises an aqueous composition.

[0118] A multilamellar liposome has multiple lipid layers separated by aqueous medium. They form spontaneously when lipids comprising phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). Lipophilic molecules or molecules with lipophilic regions may also dissolve in or associate with the lipid bilayer.

[0119] In certain less preferred embodiments, phospholipids from natural sources, such as egg or soybean phosphatidylcholine, brain phosphatidic acid, brain or plant phosphatidylinositol, heart cardiolipin and plant or bacterial phosphatidylethanolamine are preferably not used as the primary phosphatide, i.e., constituting 50% or more of the total phosphatide composition or a liposome, because of the instability and leakiness of the resulting liposomes.

[0120] In particular embodiments, a lipid and/or composition of the invention may be, for example, encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the composition, entrapped in a liposome, complexed with a liposome, etc.

H. Making Liposomes

[0121] A liposome used according to the present invention can be made by different methods, as would be known to one of ordinary skill in the art. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure.

[0122] For example, a phospholipid (Avanti Polar Lipids, Alabaster, AL), such as for example the neutral phospholipid dioleoylphosphatidylcholine (DOPC), is dissolved in tert-butanol. The lipid(s) is then mixed with the composition and/or other component(s). Tween 20 is added to the lipid mixture such that Tween 20 is about 5% of the composition's weight. Excess tert-butanol is added to this mixture such that the volume of tert-butanol is at least 95%.
The mixture is vortexed, frozen in a dry ice/acetone bath and lyophilized overnight. The lyophilized preparation is stored at -20°C and can be used up to three months. When required the lyophilized liposomes are reconstituted in 0.9% saline. The average diameter of the particles obtained using Tween 20 for encapsulating the composition is about 0.7 to about 1.0 µm in diameter.

[0123] Alternatively, a liposome can be prepared by mixing lipids in a solvent in a container, e.g., a glass, pear shaped flask. The container should have a volume ten times greater than the volume of the expected suspension of liposomes. Using a rotary evaporator, the solvent is removed at approximately 40°C under negative pressure. The solvent normally is removed within about 5 min to 2 hours, depending on the desired volume of the liposomes. The composition can be dried further in a desiccator under vacuum. The dried lipids generally are discarded after about 1 week because of a tendency to deteriorate with time.

[0124] Dried lipids can be hydrated at approximately 25 50 mM phospholipid in sterile, pyrogen free water by shaking until all the lipid film is resuspended. The aqueous liposomes can be then separated into aliquots, each placed in a vial, lyophilized and sealed under vacuum.

[0125] In other alternative methods, liposomes can be prepared in accordance with other known laboratory procedures (e.g., see Bangham et al., 1965; Gregoriadis, 1979; Deamer and Uster 1983, Szoka and Papahadjopoulos, 1978, each incorporated herein by reference in relevant part). These methods differ in their respective abilities to entrap aqueous material and their respective aqueous space to lipid ratios.

[0126] The dried lipids or lyophilized liposomes prepared as described above may be dehydrated and reconstituted in a solution of inhibitory peptide and diluted to an appropriate concentration with a suitable solvent, e.g., DPBS. The mixture is then vigorously shaken in a vortex mixer. Unencapsulated additional materials, such as agents including but not limited to hormones, drugs, nucleic acid constructs and the like, are removed by centrifugation at 29,000 g and the liposomal pellets washed. The washed liposomes are resuspended at an appropriate total phospholipid concentration, e.g., about 50 200 mM. The amount of additional material or active agent encapsulated can be determined in accordance with standard methods. After determination of the amount of additional material or active agent encapsulated in the liposome
preparation, the liposomes may be diluted to appropriate concentrations and stored at 4°C until use. A pharmaceutical composition comprising the liposomes will usually include a sterile, pharmaceutically acceptable carrier or diluent, such as water or saline solution.

[0127] The size of a liposome varies depending on the method of synthesis. Liposomes in the present invention can be a variety of sizes. In certain embodiments, the liposomes are small, e.g., less than about 100 nm, about 90 nm, about 80 nm, about 70 nm, about 60 nm, or less than about 50 nm in external diameter. In preparing such liposomes, any protocol described herein, or as would be known to one of ordinary skill in the art may be used. Additional non-limiting examples of preparing liposomes are described in U.S. Patent Nos. 4,728,578, 4,728,575, 4,737,323, 4,533,254, 4,162,282, 4,310,505, and 4,921,706; International Applications PCT/US85/01161 and PCT/US89/05040; U.K. Patent Application GB 2193095 A; Mayer et al., 1986; Hope et al., 1985; Mayhew et al. 1987; Mayhew et al., 1984; Cheng et al., 1987; and Liposome Technology, 1984, each incorporated herein by reference).

[0128] A liposome suspended in an aqueous solution is generally in the shape of a spherical vesicle, having one or more concentric layers of lipid bilayer molecules. Each layer consists of a parallel array of molecules represented by the formula XY, wherein X is a hydrophilic moiety and Y is a hydrophobic moiety. In aqueous suspension, the concentric layers are arranged such that the hydrophilic moieties tend to remain in contact with an aqueous phase and the hydrophobic regions tend to self associate. For example, when aqueous phases are present both within and without the liposome, the lipid molecules may form a bilayer, known as a lamella, of the arrangement XY YX. Aggregates of lipids may form when the hydrophilic and hydrophobic parts of more than one lipid molecule become associated with each other. The size and shape of these aggregates will depend upon many different variables, such as the nature of the solvent and the presence of other compounds in the solution.

[0129] The production of lipid formulations often is accomplished by sonication or serial extrusion of liposomal mixtures after (I) reverse phase evaporation (II) dehydration-rehydration (III) detergent dialysis and (IV) thin film hydration. In one aspect, a contemplated method for preparing liposomes in certain embodiments is heating sonicating, and sequential extrusion of the lipids through filters or membranes of decreasing pore size, thereby resulting in the formation of small, stable liposome structures. This preparation produces
liposomal/carrier/HSP of liposomes only of appropriate and uniform size, which are structurally stable and produce maximal activity. Such techniques are well-known to those of skill in the art (see, for example Martin, 1990).

[0130] Once manufactured, lipid structures can be used to encapsulate compounds that are toxic (e.g., chemotherapeutics) or labile (e.g., nucleic acids) when in circulation. The physical characteristics of liposomes depend on pH, ionic strength and/or the presence of divalent cations. Liposomes can show low permeability to ionic and/or polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and/or results in an increase in permeability to ions, sugars and/or drugs. Liposomal encapsulation has resulted in a lower toxicity and a longer serum half-life for such compounds (Gabizon et al., 1990).

[0131] Liposomes interact with cells to deliver agents via four different mechanisms: Endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and/or neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic and/or electrostatic forces, and/or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and/or by transfer of liposomal lipids to cellular and/or subcellular membranes, and/or vice versa, without any association of the liposome contents. Varying the liposome formulation can alter which mechanism is operative, although more than one may operate at the same time.

[0132] Numerous disease treatments are using lipid based gene transfer strategies to enhance conventional or establish novel therapies, in particular therapies for treating hyperproliferative diseases. Advances in liposome formulations have improved the efficiency of gene transfer in vivo (Templeton et al., 1997) and it is contemplated that liposomes are prepared by these methods. Alternate methods of preparing lipid-based formulations for nucleic acid delivery are described (W0 99/18933).
In another liposome formulation, an amphipathic vehicle called a solvent dilution microcarrier (SDMC) enables integration of particular molecules into the bi-layer of the lipid vehicle (U.S. Patent 5,879,703). The SDMCs can be used to deliver lipopolysaccharides, polypeptides, nucleic acids and the like. Of course, any other methods of liposome preparation can be used by the skilled artisan to obtain a desired liposome formulation in the present invention.

1. Liposome Targeting

Association of the composition with a liposome may improve biodistribution and other properties of the composition. For example, liposome mediated nucleic acid delivery and expression of foreign DNA in vitro has been very successful (Nicolau and Sene, 1982; Fraley et al., 1979; Nicolau et al., 1987). The feasibility of liposome mediated delivery and expression of foreign DNA in cultured chick embryo, HeLa and hepatoma cells has also been demonstrated (Wong et al., 1980). Successful liposome mediated gene transfer in rats after intravenous injection has also been accomplished (Nicolau et al., 1987).

It is contemplated that a liposome composition of the invention may comprise additional materials for delivery to a tissue. For example, in certain embodiments of the invention, the lipid or liposome may be associated with an antibody that targets the liposome to a pancreatic B cell.

Targeted delivery is achieved by the addition of antibodies or other ligands without compromising the ability of these liposomes to deliver large amounts of the composition of the invention. It is contemplated that this will enable delivery to specific cells, tissues and organs. The targeting specificity of the ligand-based delivery systems are based on the distribution of the ligand receptors on different cell types. The targeting ligand may either be non-covalently or covalently associated with the lipid complex, and can be conjugated to the liposomes by a variety of methods. For example, bifunctional cross-linking reagents have been extensively used for a variety of purposes including preparation of affinity matrices, modification and stabilization of diverse structures, identification of ligand and receptor binding sites, and structural studies. Exemplary methods for cross-linking ligands to liposomes are described in U.S. Patent 5,603,872 and U.S. Patent 5,401,511, each specifically incorporated
Various ligands can be covalently bound to liposomal surfaces through the cross-linking of amine residues.

[0137] Liposomes, in particular, multilamellar vesicles (MLV) or unilamellar vesicles such as microemulsified liposomes (MEL) and large unilamellar liposomes (LUVET), each containing phosphatidylethanolamine (PE), have been prepared by established procedures. The inclusion of PE in the liposome provides an active functional residue, a primary amine, on the liposomal surface for cross-linking purposes. Ligands such as epidermal growth factor (EGF) have been successfully linked with PE-liposomes. Ligands are bound covalently to discrete sites on the liposome surfaces. The number and surface density of these sites will be dictated by the liposome formulation and the liposome type. The liposomal surfaces may also have sites for non-covalent association. To form covalent conjugates of ligands and liposomes, cross-linking reagents have been studied for effectiveness and biocompatibility. Cross-linking reagents include glutaraldehyde (GAD), bifunctional oxirane (OXR), ethylene glycol diglycidyl ether (EGDE), and a water soluble carbodiimide, preferably 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Through the complex chemistry of cross-linking, linkage of the amine residues of the recognizing substance and liposomes is established.

[0138] Thus, in certain embodiments of the present invention, in order to enhance the transduction of cells or to increase transduction of target cells, antibodies are associated with the lipid complex. Such methods are known in the art. For example, liposomes have been described further that specifically target cells of the mammalian central nervous system (U.S. Patent 5,786,214, incorporated herein by reference). The liposomes are composed essentially of N glutarylphosphatidylethanolamine, cholesterol and oleic acid, wherein a monoclonal antibody specific for neuroglia is conjugated to the liposomes. It is contemplated that a monoclonal antibody or antibody fragment may be used to target delivery to specific cells, tissues, or organs in the animal, such as the pancreas.

J. Lipid Administration

[0139] The actual dosage amount of a lipid composition (e.g., a liposome-pancreatic B cell composition) administered to a patient can be determined by physical and physiological factors such as body weight, severity of condition, idiopathy of the patient and on
the route of administration. With these considerations in mind, the dosage of a lipid composition for a particular subject and/or course of treatment can readily be determined.

[0140] The present invention can be administered intravenously, intradermally, intraarterially, intraperitoneally, intraleSIONally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, rectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, intravesically, mucosally, intrapericardially, orally, topically, locally and/or using aerosol, injection, infusion, continuous infusion, localized perfusion bathing target cells directly or via a catheter and/or lavage.

XII. Pharmaceutical Preparations

[0141] Pharmaceutical compositions of the present invention comprise an effective amount of one or more compositions of the invention dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of an pharmaceutical composition that contains at least one composition or additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

[0142] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the pharmaceutical compositions is contemplated.
The composition may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. The present invention can be administered intravenously, intradermally, transdermally, intrathecally, intraarterially, intraperitoneally, intranasally, intravaginally, intrarectally, topically, intramuscularly, subcutaneously, mucusally, orally, topically, locally, inhalation (e.g., aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (e.g., liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference).

The composition may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as formulated for parenteral administrations such as injectable solutions, or aerosols for delivery to the lungs, or formulated for alimentary administrations such as drug release capsules and the like.

Further in accordance with the present invention, the composition of the present invention suitable for administration is provided in a pharmaceutically acceptable carrier with or without an inert diluent. The carrier should be assimilable and includes liquid, semi-solid, i.e., pastes, or solid carriers. Except insofar as any conventional media, agent, diluent or carrier is detrimental to the recipient or to the therapeutic effectiveness of a the composition contained therein, its use in administrable composition for use in practicing the methods of the present invention is appropriate. Examples of carriers or diluents include fats, oils, water, saline solutions, lipids, liposomes, resins, binders, fillers and the like, or combinations thereof. The
composition may also comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (e.g., methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

[0146] In accordance with the present invention, the composition is combined with the carrier in any convenient and practical manner, i.e., by solution, suspension, emulsification, admixture, encapsulation, absorption and the like. Such procedures are routine for those skilled in the art.

[0147] In a specific embodiment of the present invention, the composition is combined or mixed thoroughly with a semi-solid or solid carrier. The mixing can be carried out in any convenient manner such as grinding. Stabilizing agents can be also added in the mixing process in order to protect the composition from loss of therapeutic activity, i.e., denaturation in the stomach. Examples of stabilizers for use in the composition include buffers, amino acids such as glycine and lysine, carbohydrates such as dextrose, mannose, galactose, fructose, lactose, sucrose, maltose, sorbitol, mannitol, etc.

[0148] In further embodiments, the present invention may concern the use of a pharmaceutical lipid vehicle compositions that include the composition and an aqueous solvent. As used herein, the term "lipid" will be defined to include any of a broad range of substances that is characteristically insoluble in water and extractable with an organic solvent. This broad class of compounds are well known to those of skill in the art, and as the term "lipid" is used herein, it is not limited to any particular structure. Examples include compounds which contain long-chain aliphatic hydrocarbons and their derivatives. A lipid may be naturally occurring or synthetic (i.e., designed or produced by man). However, a lipid is usually a biological substance. Biological lipids are well known in the art, and include for example, neutral fats, phospholipids, phosphoglycerides, steroids, terpenes, lysolipids, glycosphingolipids, glycolipids, sulphatides, lipids with ether and ester-linked fatty acids and polymerizable lipids, and combinations thereof. Of course, compounds other than those specifically described herein that are understood by one of skill in the art as lipids are also encompassed by the compositions and methods of the present invention.
[0149] One of ordinary skill in the art would be familiar with the range of techniques that can be employed for dispersing a composition in a lipid vehicle. For example, the composition may be dispersed in a solution containing a lipid, dissolved with a lipid, emulsified with a lipid, mixed with a lipid, combined with a lipid, covalently bonded to a lipid, contained as a suspension in a lipid, contained or complexed with a micelle or liposome, or otherwise associated with a lipid or lipid structure by any means known to those of ordinary skill in the art. The dispersion may or may not result in the formation of liposomes.

[0150] The actual dosage amount of a composition of the present invention administered to an animal patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. Depending upon the dosage and the route of administration, the number of administrations of a preferred dosage and/or an effective amount may vary according to the response of the subject. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[0151] In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

[0152] In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight,
weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above.

A. Alimentary Compositions and Formulations

[0153] In preferred embodiments of the present invention, the composition is formulated to be administered via an alimentary route. Alimentary routes include all possible routes of administration in which the composition is in direct contact with the alimentary tract. Specifically, the pharmaceutical compositions disclosed herein may be administered orally, buccally, rectally, or sublingually. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft- shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

[0154] In certain embodiments, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz et al., 1997; Hwang et al., 1998; U.S. Pat. Nos. 5,641,515; 5,580,579 and 5,792, 451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof; an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or combinations thereof; a lubricant, such as, for example, magnesium stearate; a sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with
shellac, sugar, or both. When the dosage form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Gelatin capsules, tablets, or pills may be enterically coated. Enteric coatings prevent denaturation of the composition in the stomach or upper bowel where the pH is acidic. See, e.g., U.S. Pat. No. 5,629,001. Upon reaching the small intestines, the basic pH therein dissolves the coating and permits the composition to be released and absorbed by specialized cells, e.g., epithelial enterocytes and Peyer's patch M cells. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

[0155] For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally- administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically- effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

[0156] Additional formulations which are suitable for other modes of alimentary administration include suppositories. Suppositories are solid dosage forms of various weights and shapes, usually medicated, for insertion into the rectum. After insertion, suppositories soften, melt or dissolve in the cavity fluids. In general, for suppositories, traditional carriers may include, for example, polyalkylene glycols, triglycerides or combinations thereof. In certain embodiments, suppositories may be formed from mixtures containing, for example, the active ingredient in the range of about 0.5% to about 10%, and preferably about 1% to about 2%.
B. Parenteral Compositions and Formulations

[0157] In further embodiments, composition may be administered via a parenteral route. As used herein, the term "parenteral" includes routes that bypass the alimentary tract. Specifically, the pharmaceutical compositions disclosed herein may be administered for example, but not limited to intravenously, intradermally, intramuscularly, intraarterially, intrathecally, subcutaneous, or intraperitoneally U.S. Pat. Nos. 6,7537,514, 6,613,308, 5,466,468, 5,543,158; 5,641,515; and 5,399,363 (each specifically incorporated herein by reference in its entirety).

[0158] Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy injectability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (i.e., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0159] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic.
with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in isotonic NaCl solution and either added hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologies standards.

[0160] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. A powdered composition is combined with a liquid carrier such as, e.g., water or a saline solution, with or without a stabilizing agent.

C. Miscellaneous Pharmaceutical Compositions and Formulations

[0161] In other preferred embodiments of the invention, the active compound may be formulated for administration via various miscellaneous routes, for example, topical (i.e., transdermal) administration, mucosal administration (intranasal, vaginal, etc.) and/or inhalation.

[0162] Pharmaceutical compositions for topical administration may include the active compound formulated for a medicated application such as an ointment, paste, cream or powder. Ointments include all oleaginous, adsorption, emulsion and water-solubly based compositions for topical application, while creams and lotions are those compositions that include an emulsion base only. Topically administered medications may contain a penetration
enhancer to facilitate adsorption of the active ingredients through the skin. Suitable penetration enhancers include glycerin, alcohols, alkyl methyl sulfoxides, pyrrrolidones and luarocapram. Possible bases for compositions for topical application include polyethylene glycol, lanolin, cold cream and petrolatum as well as any other suitable absorption, emulsion or water-soluble ointment base. Topical preparations may also include emulsifiers, gelling agents, and antimicrobial preservatives as necessary to preserve the active ingredient and provide for a homogenous mixture. Transdermal administration of the present invention may also comprise the use of a "patch". For example, the patch may supply one or more active substances at a predetermined rate and in a continuous manner over a fixed period of time.

[0163] In certain embodiments, the pharmaceutical compositions may be delivered by eye drops, intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering compositions directly to the lungs via nasal aerosol sprays has been described e.g., in U.S. Pat. Nos. 5,756,353 and 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga et ah, 1998) and lysophosphatidyl-glycerol compounds (U.S. Pat. No. 5,725, 871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroethylene support matrix is described in U.S. Pat. No. 5,780,045 (specifically incorporated herein by reference in its entirety).

[0164] The term aerosol refers to a colloidal system of finely divided solid of liquid particles dispersed in a liquefied or pressurized gas propellant. The typical aerosol of the present invention for inhalation will consist of a suspension of active ingredients in liquid propellant or a mixture of liquid propellant and a suitable solvent. Suitable propellants include hydrocarbons and hydrocarbon ethers. Suitable containers will vary according to the pressure requirements of the propellant. Administration of the aerosol will vary according to subject's age, weight and the severity and response of the symptoms.

XIII. Combination Therapy

[0165] In certain embodiments of the invention, the methods and compositions of the invention are administered to an individual who has taken or is taking Type II diabetes therapy, including therapy for one or more complications from diabetes. The present invention
generally concerns treatment for Type 2 diabetes, and the individual may or may not have been treated or is being treated for T2DM.

[0166] The skilled artisan recognizes that individuals with Type II diabetes may be asymptomatic or they may have one or more symptoms as follows: blurred vision; erectile dysfunction; fatigue; frequent or slow-healing infections; increased appetite, thirst, and/or urination. Type 2 diabetes can be diagnosed by measuring fasting blood glucose level, hemoglobin A1c test, oral glucose tolerance test, and/or random (non-fasting) blood glucose level.

[0167] In certain embodiments of the invention, the methods and compositions of the invention are administered to an individual who has taken or is taking therapy for one or more complications from diabetes. Such therapy may include any means to lower high blood glucose levels, including by one or more drugs, exercise, and/or diet, for example. Medications to treat diabetes include alpha-glucosidase inhibitors; biguanides; exenatide, mitiglinide, pramlintide, sitagliptin, and saxagliptin; meglitinides; sulfonylureas; and/or thiazolidinediones. Medications to prevent complications may also include an ACE inhibitor, statin drugs, or aspirin. The diabetic individual may also be treated for cataracts; vascular damage; diabetic retinopathy; foot sores or ulcers; glaucoma; high blood pressure; high cholesterol; kidney disease or kidney failure; or macular edema, for example.

[0168] In certain embodiments, an individual at risk for developing Type II diabetes or at risk for developing additional complications from diabetes are treated with methods or compositions of the invention. Such individuals at risk include those with an age greater than 45 years; HDL cholesterol of less than 35 mg/dL or triglyceride level of greater than 250 mg/dL; high blood pressure; history of gestational diabetes; polycystic ovarian syndrome; impaired glucose tolerance; and/or having a race/ethnicity including African Americans, Hispanic Americans, and Native Americans, for example.

[0169] The combination therapy that is in addition to the methods or compositions of the present invention may be delivered before, during, and/or after administration of the present invention.
XIV. **Kits of the Invention**

[0170] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, a composition of the invention may be comprised in a kit. The kits will thus comprise, in suitable container means, one or more of an antibody, nanoparticle, HSP, chitosan, or lipid-based compound, such as a liposome. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing the composition and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow molded plastic containers into which the desired vials are retained, for example.

[0171] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. In specific cases, the container means may itself be a syringe, pipette, and/or other such like apparatus, from which the formulation may be applied to the pancreas, for example. However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

[0172] Irrespective of the number and/or type of containers, the kits of the invention may also comprise, and/or be packaged with, an instrument for assisting with the injection/administration and/or placement of the ultimate composition within the body of an individual, including to the pancreas. Such an instrument may be a syringe, pipette, forceps, and/or any such medically approved delivery vehicle.
EXAMPLES

[0173] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

HEAT SHOCK TREATMENT PREVENTS DEATH OF CULTURED MOUSE PANCREATIC B-CELLS AND IN VIVO CAENORHABDITIS ELEGANS MODEL BY PREVENTING THE FORMATION OF TOXIC AMYLIN AGGREGATES

Materials and Methods

[0174] Transgenic C. elegans model expressing h-IAPP in vivo. Due to the fact that animal diabetes model systems take approximately 18-24 months to develop diabetes, the inventors developed a human-IAPP (h-IAPP) C. elegans model to determine h-IAPP toxicity in a living organism. Animals which chronically express h-IAPP and mouse-IAPP (m-IAPP) as a control were first generated. These animals were tagged with YFP (yellow fluorescent protein) in pharynx, body wall muscles, neurons and intestines under the control of promoters lev-1, tnt-4, aex-3 and ges-1, respectively. Plasmids were co-injected into the pha-l(e2123); him-5(el490); Ute-l(ce314) hermaphrodites at concentrations of 37.5ng/µl each, along with SOng/µl of the pha-1 rescuing plasmid pBXl. pha-1 is a temperature-sensitive embryonic lethal mutation, which allows worms to grow normally at 15°C; however, at 20°C the mutation is 100% embryonic lethal. Thus, transgenic animals that carry pBXl (pha-1 rescue) are selected due to their ability to grow at 20°C. Animals acutely expressing h-IAPP and m-IAPP were next generated, and were tagged with YFP, in pharynx, body wall muscles and neurons under the control of the Hsp16 promoter (a heat-induced chaperone promoter that activates Hsp16 expression).
[0175] Tissue-specific expression of h-IAPP and m-IAPP cDNA. Transgenes were generated by PCR amplification of h-IAPP and m-IAPP cDNA clones (Origene) that were subcloned into BamHI sites of expression vector pSX95.77YFP by In-Fusion PCR cloning system (Clontech, Mountain View, CA) using human-amylin forward (5’-CGACTCTAGGATCCATGGGCATCCTGAAGCTGCAAG-3’; SEQ ID NO: 11) and reverse (5’-CCAATCCCCGGGATCCAAACTGCCAGC-3’; SEQ ID NO: 12) primers for h-IAPP cloning and m-amylin forward (5’-CGACTCTAGGATCCATATGCATCTCTCCACTCCAGGCAG-3’; SEQ ID NO: 13) and reverse (5’-CCAATCCCCGGGATCCAAACGAGTAAAGAAATCCAGG-3’; SEQ ID NO: 14) primers for m-IAPP cloning. Positive clones were identified by colony PCR and sequenced. The resulting constructs were then digested with Sal I, blunt-ended and then ligated with the Gateway Vector Conversion Reading frame Cassette C1 (Life Technologies) to generate Gateway destination vectors pNG1 and pNG2 that contained the h-IAPP and m-IAPP clones, respectively. Entry clones pLR22, pLR25 and pLR35 contained the C. elegans promoter regions lev-1 1, tnt-4 and aex-3, respectively. All these entry clones contained Gateway ATTL sites, which allowed promoter sequences in pLR22, pLR25 and pLR35 to be recombined, using the LR and pNG2, to generate C. elegans transgenic tissue-specific plasmids. Three h-IAPP plasmids: pPR3 (lev-1 1 promoter), pPR4 (tnt-4 promoter) and pPR5 (aex-3 promoter); and three m-IAPP plasmids: pPR8 (lev-1 1 promoter), pPR9 (tnt-4 promoter) and pPRIO (aex-3 promoter) were generated. Positive plasmids were identified by restriction enzyme pattern digestion.

[0176] Measurement of lifespan studies in transgenic C. elegans model expressing h-IAPP in vivo. For lifespan studies, animals were synchronized by using a dissecting microscope to identify fourth larval stage (L4) hermaphrodites based on the appearance of a dark half circle in the vulva. Studies of aging begin on day zero by placing ten L4 hermaphrodites on a small petri dish; h-IAPP and m-IAPP transgensics were identified using a fluorescence microscope. Hermaphrodites were transferred to a new dish every day during the reproductive period to prevent larvae from growing into full adults and being confused with the experimental animals. Using a dissecting microscope, it was determined whether animals were alive or dead by tapping the plate. If the animal did not respond then the worm’s head was gently tapped with a platinum pick. If there was no response, the worm was scored as dead and removed from the plate. Self-fertilization was assessed by detecting the presence of larvae on the
Petri dish. The period between day 1 and the last day of self-progeny production was referred to as the self-fertilization span. Larvae were synchronized by hypochlorite treatment and transgenic animals were identified using a fluorescence microscope. Seventy-two hours after treatment, larvae stage or adult stage were determined and compared with the control.

[0177] Cell culture and transfection conditions. Mouse insulinoma Beta-TC-6 cells were grown in HEPES-buffered Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% of heat-inactivated FBS (Gibco, Grand Island, NY), 100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco). Cells were maintained in a humidified 5% CO2 atmosphere at 37°C. For transfection experiments, Beta-TC-6 cells were plated on 96-well and 6-well tissue culture plates at a density of 6 x 10^4 or 1 x 10^6 cells per well, respectively, and allowed to attach overnight. When 90-95% confluency is reached, cells were transfected with h-IAPP cDNA clone (vector pCMV6-XL5, Origene, Rockville, MD) or m-IAPP cDNA clone (vector pCMV6-Kan/NEO, Origene). Transfection complex were prepared with Opti-MEM (Gibco), and Lipofectamine 2000 (Life Technologies, Grand Island, NY), according to the manufacturer's instructions. Medium was changed after 6h and cells were incubated for 72h. At the end of the transfection period, cells plated on 96-well plates were used to evaluate cell viability by MTS assay and cells plated on 6-well plates were harvested for Western blot analysis to detect the protein levels of h-IAPP and m-IAPP. Empty vector pCMV6 served as a transfection control.

[0178] To evaluate the protective role of Hsp72 against h-IAPP-induced β-cell death, Hsp72 was co-transfected with h-IAPP in Beta-TC-6 cells. Human Hsp72 cDNA clone (vector pEGFP, Addgene, Cambridge, MA) and h-IAPP cDNA clone (vector pCMV6-XL5, Origene) was used to prepare transfection complex as explained above. At the end of the transfection period, cells plated on 96-well plates were used to evaluate cell viability by MTS assay and cells plated on 6-well plates were harvested for Western blot analysis to detect the protein levels of h-IAPP and Hsp72. Empty vectors pCMV6 and pEGFP-C3 will serve as transfection controls.

[0179] Cell viability assay. Cell viability was measured by MTS assay (Promega). MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] according to the manufacturer's instructions. Briefly, 60µl of MTS
solution was added to each well and incubated for 4 h at 37°C, 5% CO2. The absorbance was read at 490nm on a spectrophotometer Spectra Max Plus 384. Cell viability was expressed as the percentage of MTS reduction with respect to the controls.

[0180] Western blot analysis, h-amylin and m-amylin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), human Hsp72 (Enzo Life Sciences, Farmingdale, NY), β-actin antibodies (Abeam, Cambridge, MA) and horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were used. Protein bands were visualized by chemiluminescence (SuperSignal West Femto Substrate, Thermo Scientific, Dubuque, IA), and films were developed and an image processor was used to compare relative expression levels.

[0181] Amylin treatment of mouse pancreatic β-cells and human pancreatic islets. According to the results of Lorenzo et al. (1994), islet toxicity is first detected at concentrations of 5μM human amylin and almost complete islet cell death occurs after 24 hours of incubation with 20μM amylin in both rat and human islet cell cultures. For this reason one can conduct amylin toxicity assays with freshly prepared human amylin solution (in water), at concentrations of 5, 10, 20 and 40μM. Note that the solution should be prepared immediately before the assay and used within 5 min of being dissolved. According to the results reported by Janson et al. (1999), freshly dissolved h-IAPP is cytotoxic to β-cells while the "aged" h-IAPP in the amyloid form (aqueous solution stored at 20°C for 7 days) is not cytotoxic. Human amylin peptide is obtained from Bachem (Torrance, California).

Results

[0182] FIG. 1 shows the toxicity of amylin at different concentrations in murine pancreatic beta cells determined by trypan blue exclusion assay. Mouse insulinoma Beta-TC-6 cells (ATCC, Manassas, VA) were seeded on a 96-well plate and exposed to different concentrations of exogenous human islet amyloid polypeptide ("h-IAPP"; Bachem, King of Prussia, PA). Toxicity was detected at a concentrations greater than 5μM h-IAPP (determined by trypan blue exclusion assay) and complete cell death occurred at 40μM.

[0183] In order to have a quantitative measurement of h-IAPP-induced toxicity, the inventors calculated the IC50 for h-IAPP. For this purpose, lyophilized h-IAPP was dissolved in
distilled water to prepare a 640 µM stock solution and immediately added it to the culture medium of Beta-TC-6 cells, reaching final concentrations of 40 µM, 20 µM, 10 µM, 5 µM, 1 µM, 0.5 µM, 0.1 µM, vehicle, or medium (FIG. 2). The next day cell viability was determined using the MTS cell proliferation assay (Promega Corp., Madison, WI), which detects the conversion of MTS into formazan by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product is proportional to the number of living cells. h-IAPP IC50 was demonstrated to be 10.43±1.9 µM (FIG. 2).

[0184] In particular embodiments of the invention, heat shock treatment increases the levels of Hsp72 (as an exemplary HSP of the invention) in β-cells preventing the formation of amylin fibrils and β-cell death. Heat shock is used as a prophylactic treatment in order to induce higher levels of Hsp72 before exposure to toxic amylin, in certain aspects. In this way, it is demonstrated that intracellular HSPs help to deal with increased levels of exogenous amylin. In particular cases, there are increased levels of Hsp72, reduced formation of amylin fibrils and less β-cell death after prophylactic treatment compared with controls not receiving heat shock. Also, in some embodiments the mitigator effect of heat treatment in β-cells already exposed to toxic amylin is demonstrated. In particular cases, exposure of β-cells to amylin followed by heat shock treatment attenuates the toxic response. Specifically these cells have a better chance of survival due to reduced formation of amylin fibrils compared with controls not receiving heat shock. One can prove that the beneficial effect of heat shock treatment depends on the induction of Hsp72 expression by using quercetin, which is one of the most effective inhibitors of heat-induced Hsp72 expression (Wang et al., 2009); quercetin blocks the prophylactic and mitigator effects of heat shock, in particular embodiments.

[0185] To test the hypothesis that heat shock treatment will mitigate the toxic effect of h-IAPP, we first determined which heat shock treatment resulted in maximal expression of Hsp25 and Hsp72 in Beta-TC-6 cells. Western blot was performed to measure the levels of Hsp72 and Hsp25 (Hsp 25 is the murine equivalent of human Hsp27). Levels of Hsp72 and Hsp 25 in cells were assessed by standard western blot utilizing rabbit anti-mouse Hsp72 polyclonal antibody and rabbit anti-mouse Hsp25 polyclonal antibody (Assay Designs), respectively. Peroxidase-conjugated goat anti-rabbit IgG was used as a secondary antibody (Jackson ImmunoResearch). Protein bands were visualized by chemiluminescence, films were developed and an image processor was used to visualize the results. It was demonstrated that Hsp72 and
Hsp25 expression was absent under basal conditions and their expression was up-regulated after heat shock treatment. The highest levels were found after 45min of heat shock at 43°C (FIG. 3).

[0186] **Heat shock treatment to prevent death of cultured mouse β-cells due to toxic amylin aggregates.** Studies were then conducted to established that induction of the heat shock proteins protected β-cells against exogenous h-IAPP toxicity.

[0187] Beta-TC-6 cells were plated on a 96-well plate at 6x10^4 cells/well and cultured for 24h. On day 2, cells were heat shocked at 43°C for 30min and then returned to the incubator for 24h to allow for Hsp72 expression. On day 3, different concentrations of h-IAPP solution were applied to the supernatant of the heat shocked cells reaching final concentrations of 40µM, 20µM, 10µM, 8µM, 5µM, 3µM, 1µM, vehicle, or medium. After 24h of incubation cell viability was assessed. We demonstrated that heat shock treatment effectively mitigated the toxic effect of h-IAPP in β-cells (FIG. 4).

**Heat shock proteins to prevent the formation of toxic amylin aggregates in a c. elegans model**

[0188] The foregoing results were next validated in a complex animal system, the *c. elegans* model. The inventors developed a human-IAPP (h-IAPP) and mouse-IAPP (m-IAPP) *c. elegans* model to determine h-IAPP toxicity. This is because h-IAPP, but not m-IAPP induces aggregates in the *c. elegans* and results in its ultimate death. It was demonstrated that the h-IAPP transgenic model showed high fluorescence in coelomocytes (cells that have a similar function to the macrophages of vertebrates, but are in a fixed position), spermatheca, vulva muscles, body wall muscles, pharynx and anal depressor muscle; while the m-IAPP transgenic model did not show significant aggregate in coelomocytes, body wall muscles and pharynx, nor in vulva, spermatheca and anal depressor (FIG. 5). It was next demonstrated that heat shock (HS) treatment of h-IAPP *c. elegans* for 30 min at 33°C significantly reduced the number of visible h-IAPP toxic aggregates (FIG. 6). To prove that we can deliver Hsp72 as a therapy against the toxic effect of h-IAPP in β-cells, it was demonstrated that transfection of Beta-TC-6 cells with Hsp72 plasmids significantly reduces h-IAPP-induced toxicity (FIG. 7).
EXAMPLE 2

HEAT SHOCK TREATMENT CAN BE ACHIEVED UTILIZING GOLD NANORODS SPECIFICALLY TARGETED TO MOUSE PANCREATIC B-CELLS AND IRRADIATED WITH A NIR PULSED-LASER TO INDUCE LOCALIZED HYPERTHERMIA

[0189] In certain embodiments of the invention, targeted gold nanorods irradiated with NIR induce the synthesis of Hsp72 in β-cells in response to the thermal stress.

[0190] Synthesis of gold nanorods and antibody conjugation: Gold nanorods (axial diameter 10nm, wavelength) conjugated with rabbit polyclonal antibodies directed against the first 11 amino acids from the amino terminal extracellular domain of mouse FXYD2ya (Eurogentec Inc. San Diego, CA) (Flamez et al., 2010) are generated by Nanopartz Inc. (Loveland, CO).

[0191] Cell culture and cellular delivery of antibody-conjugated gold nanorods: studies are carried out in a 24-well plate (15.6 mm internal diameter/well) as described above and allowed overnight to attach. DMEM is taken out and cells are rinsed with phosphate buffered saline (PBS). Cells are incubated for 120 min with medium alone, medium containing InM of naked gold nanorods or with medium containing InM of conjugated nanorods to allow nanorod endocytosis as demonstrated in (Adegnoyega et al., 2007; Cherukuri and Curley, 2010). Following incubation, the medium are aspirated; the cells gently washed three times with PBS and fresh medium added to each well. The cells are then irradiated as described below. All treatments are performed in triplicate and studies repeated 3-5 times.

[0192] Determination of the optimal duration of NIR irradiation: Irradiation is accomplished using an Integrated Fiber Array Packet, FAP-I System, with a wavelength of 808 nm (Coherent, Santa Clara, CA) at a power density of 4 W/cm² (Gobin, 2007) and a spot size of 85.6 mm diameter in order to cover all wells in one column at a time for 5, 10 or 15 min through the culture medium. Cell viability and Hsp72 levels are determined 24, 48 and 72 h after laser irradiation using the same assays as described above. The optimal time is the one with the least number of dead cells and highest Hsp72 levels, in particular cases.
Photothermal therapy: Protocols may be the same as in Example 1, but instead of heat shock the cells are treated with gold nanorods and NIR for a period of time determined in the previous section.

Results are calculated as means ± SEM. Comparisons of cytotoxicity (reduction in the number of murine β-cells after heat shock treatments or nanorod irradiation treatment) relative to the control are analyzed by one-way analysis of variance (ANOVA) with Student-Newman-Keuls test for identifying differences among means. Comparison of levels of Hsp72 and amylin aggregate formation in cell medium is qualitative and is assessed with imaging processing software. Statview 5.0 software will be used. Controls for each of the experiments were described in individual experimental sections.

In certain embodiments, targeting gold nanorods to β-cells increases the efficiency of nanorod endocytosis. Although nanorods suspended in the medium could make contact with the cells and uptake could occur by nonspecific endocytosis, washing the cell monolayer prior to irradiation will remove the nanorods that have just landed on the cells and are not specifically attached, in particular cases. Therefore, one can expect a significantly larger uptake of nanorods by β-cells and a concomitant increase in Hsp72 levels after irradiation compared to the naked gold nanorods. In specific cases, antibodies may be humanized to decrease provocation of an immune response in humans.

It has already been established that T2DM treatment should target both insulin sensitivity and deterioration and loss of β-cell mass. The use of heat to offset the detrimental effects of islet amyloid deposition and to prevent β-cell death is an innovative approach in the management of T2DM. Moreover, the creation of gold nanoparticles conjugated to polyclonal/monoclonal antibodies and delivered intravenously to a patient resulting in selective uptake in pancreatic β-cells and exposure to NIR is useful as an adjuvant therapy for T2DM. This would ultimately improve β-cell survival and insulin response to hyperglycemia. Optimization of the rod shape and size and the determination of the required concentration of rods for sufficient heating to induce a stress response without toxicity may be employed. Also, the duration, frequency and intensity of NIR irradiation may be determined experimentally or using computational models, in specific embodiments.
The use of multifunctional nanocarriers for cell imaging and NIR photothermal therapy is a novel approach that is under investigation for cancer treatment (Guo et al., 2010). In the case of T2DM, multifunctional nanocarriers based on chitosan (Guo et al., 2010) targeted to pancreatic β-cells using monoclonal antibodies and carrying gold nanorods to serve as a NIR theromtherapy device to induce the synthesis of HSPs, and optionally includes a fluorescent probe for real-time β-cell imaging, constitutes a useful strategy that not only helps to prevent β-cell death but also allows the evaluation of β-cell mass prior and during the treatment. FIG. 6 is an example of a regimen of heat treatment of pancreatic B-cells.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.


Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present invention. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.
CLAIMS

What is claimed is:

1. A method of treating an individual for Type II diabetes, comprising:
   1) delivering a therapeutically effective amount of a heatable carrier to
      the pancreas of the individual; and
   2) heating the carrier.

2. The method of claim 1, wherein the carrier comprises nanoparticles, chitosan, poly(amidoamine) dendrimer or a lipid-based carrier.

3. The method of claim 1, wherein the carrier is delivered directly to the pancreas.

4. The method of claim 3, wherein the carrier further comprises an antibody that binds to a target polypeptide on the B cells and the direct delivery comprises targeting of the particle to pancreatic B cells using the antibody.

5. The method of claim 4, wherein the target polypeptide comprises Na-K-ATPase.

6. The method of claim 4, wherein the antibody binds the FXYD2 domain of the regulatory subunit of Na-K-ATPase.

7. The method of claim 6, wherein the antibody binds to a region in the N-terminal 1-20 amino acids of the FXYD2 domain.

8. The method of claim 6, wherein the antibody binds to a region in the N-terminal 1-15 amino acids of the FXYD2 domain.

9. The method of claim 6, wherein the antibody binds to a region in the N-terminal 1-11 amino acids of the FXYD2 domain.
10. The method of claim 3, wherein the direct delivery comprises delivery of the carrier by laparoscopy to the pancreas.

11. The method of claim 1, wherein the carrier is delivered indirectly to the pancreas.

12. The method of claim 11, wherein the indirect delivery is by intravenous administration to the individual.

13. The method of claim 2, wherein the nanoparticle is a rod, sphere, ellipsoid, elliptical disk, rectangular disk, or square.

14. The method of claim 2, wherein the nanoparticle is a rod.

15. The method of claim 2, wherein the nanoparticle is a gold nanoparticle.

16. The method of claim 1, wherein the carrier comprises one or more Heat Shock Proteins (HSPs) or fragments thereof.

17. The method of claim 16, wherein the carrier comprises HSP72, HSP27, HSP78, GRP78, fragments thereof, or a combination thereof.

18. The method of claim 1, wherein the carrier further comprises a label.

19. The method of claim 18, wherein the label is fluorescent, radioactive, or a nanoparticle.

20. The method of claim 16, wherein the carrier comprises nucleic acid that encodes one or more Heat Shock Proteins (HSPs) or fragments thereof.

21. The method of claim 16, wherein the carrier comprises one or more compositions that increase expression of heat shock protein in the individual.
22. The method of claim 21, wherein the compositions comprise proteasome inhibitors.

23. The method of claim 22, wherein the proteasome inhibitors are selected from the group consisting of Z-Leu-Leu-Leu-H (aldehyde) [MG 132], lactacystin, Z-Leu-Leu-Nva-H (aldehyde) [MG 115], Z-ne-Glu(OtBu)-Ala-Leu-H (aldehyde) [PSI], epoxomicin, bortezomib, disulfiram, epigallocatechin-3-gallate, salinosporamide A, carfilzomib, ONX 0912, CEP-18770, ubiquitin aldehyde, MLN9708, and a combination thereof.

24. A method of treating an individual for Type II diabetes, comprising delivering to the pancreas of the individual a therapeutically effective amount of a composition comprising:

   a) HSP72, HSP27, HSP78, GRP78, fragments thereof, or a combination thereof; and

   b) one or more moieties selected from the group consisting of an antibody that binds a target polypeptide on pancreatic B cells, a nanoparticle, a lipid-based carrier, chitosan, or poly(amidoamine) dendrimer.

25. The method of claim 24, wherein the composition is delivered to the pancreas directly.

26. The method of claim 25, wherein the direct delivery to the pancreas comprises targeting of the composition to pancreatic B cells using the antibody.

27. The method of claim 25, wherein the direct delivery comprises laparoscopy.

28. The method of claim 24, wherein the composition is delivered to the pancreas indirectly.
29. The method of claim 28, wherein the indirect delivery is by intravenous administration.

30. The method of claim 24, further comprising the step of heating the pancreas.

31. The method of claim 24, wherein the HSP72, HSP27, HSP78, GRP78, fragments thereof, or a combination thereof are provided to the individual in nucleic acid or amino acid form.

32. A composition, comprising:

   a) HSP72, HSP27, HSP78, GRP78, fragments thereof, or a combination thereof; and

   b) one or more moieties selected from the group consisting of antibody that binds a target polypeptide on pancreatic B cells, a nanoparticle, a lipid-based carrier, chitosan, and poly(amidoamine) dendrimer.

33. The composition of claim 32, wherein the antibody is directed to the FXYD2 domain of the regulatory subunit of Na-K-ATPase.

34. The composition of claim 32, wherein the nanoparticle is a rod, sphere, ellipsoid, elliptical disk, rectangular disk, or square.

35. The composition of claim 32, wherein the lipid-based carrier is a liposome.

36. The composition of claim 32, further defined as comprising the nanoparticle and the antibody.

37. The composition of claim 36, wherein the nanoparticle is conjugated to the antibody.

38. The composition of claim 37, wherein the nanoparticle is a nanorod that is conjugated with an antibody to the FXYD2 domain of the regulatory subunit of Na-K-ATPase.
39. The composition of claim 36, wherein the antibody binds to a region in the first 1-20 amino acids of the FXYD2 domain.

40. The composition of claim 27, further defined as comprising the nanoparticle and chitosan.

41. The composition of claim 35, further defined as the nanoparticle being seeded in the chitosan.

42. The composition of claim 32, wherein the nanoparticle is a gold nanoparticle.

43. The composition of claim 32, further comprising a detectable label.

44. The composition of claim 43, wherein the detectable label is fluorescent, radioactive, or nanoparticles.

45. The composition of claim 32, wherein the HSP72, HSP27, HSP78, GRP78, fragments thereof, or a combination thereof are in nucleic acid or amino acid form.

46. A kit comprising the composition of claim 32, said composition housed in a suitable container.

47. The kit of claim 46, further comprising an additional therapy for diabetes.
FIG. 1