METHODS AND COMPOSITIONS FOR TREATMENT OF GRAFT REJECTION AND PROMOTION OF GRAFT SURVIVAL

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ABSTRACT
Embodiments herein illustrate methods of treating, reducing or preventing transplantation rejection and/or side effects associated with transplantation. Some embodiments relate to compositions and methods for inhibition of graft rejection and promotion of graft survival. Other embodiments relate to modulation of cellular activities, including graft rejection, promotion of graft survival, graft versus host rejection and conditions commonly associated with graft rejection. Yet other embodiments relate to the inhibitory compounds comprising naturally occurring and man-made inhibitors of serine protease, derivatives and fragments of the carboxy-terminus of alpha1-antitrypsin and inducers of other alpha1-antitrypsin activities and uses thereof.

Day 7

H&E

Allogeneic

Day 15

IHC (Insulin)

Autologous

Allogeneic
Figs. 1A-1B

A

![Blood glucose levels for Control and AAT treated groups over days from transplantation.](chart)

B

![Timeline showing rejection phases under different AAT treatments.](chart)
Figs. 1C-1D

**Immunization-induced** Abs (detectable day -1)

**Treatment-induced** Abs (detectable day 18)

C

D

<table>
<thead>
<tr>
<th>Treatment: ALB</th>
<th>+</th>
<th>-</th>
<th>-</th>
<th>-</th>
</tr>
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<tbody>
<tr>
<td>AAT</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Detectable Abs</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Immunization</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
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</table>
Figs. 2A – 2D

A

![Bar graph showing total cells x 10^6 at 24 and 48 hours from ThG injection.]

B

![Bar graph showing percent total cells with various treatments.]

C

![Bar graph showing elastase activity with different AAT treatments.]

D

![Flow cytometry scatter plots comparing Saline and AAT treatments.]

- **Elicited M.**
- Neutrophils
- Elicited Mφ

Data points include:
- Saline
- AAT
- No AAT
- Native AAT
- Oxidized AAT
Figs. 3A – 3B

A

Monocytes/elicited Mφ

- O - Saline
- • - AAT

CD3⁺ cells

CD3⁺YNK cells (day 5)

Neutrophils

NK cells

Days from injection

B

CD3⁺NK cells (day 5)

Saline

AAT

NK Cells

CD3
Fig. 3C

Day 7

H&E
AAT

Day 15

IHC (Insulin)

Fig. 4A

Nitric oxide

% Induction

AAT mg/ml

Fig. 4B

Viability

% Viable cells

AAT mg/ml
Figs. 4G-4H

G

H

% CD45^+ MHC II^+ cells

CT
IFN/IL-1
IFN/IL-1/AAT

% Insulin positive

MHC II

CD45

Relative cell frequency

10^0 10^1 10^2 10^3 10^4

STZ
STZ/AAT
Figs. 5A-5B

A

Percent TNFα

CT  AAT  TACEi

supernatant

Fold increase in TNFα

CT  AAT  TACEi

surface

***

B

Relative cell frequency

10^0  10^1  10^2  10^3  10^4

TNFα
Figs. 5C-5D

C

![Figure C: Glucose mg/dl bar chart](image)

D

![Figure D: TNFα induction bar chart](image)
Fig. 6D

Fig. 7A

IL-1β/IFN-γ-induced mAAT expression in islets

Fig. 7B

RT-PCR mAAT/GAPDH
Fold from CT
Figs. 7C-7D

C

\[ \% \text{ change} \quad \text{mAAT/GAPDH mRNA} \]

\[ \begin{array}{cccccccc}
0 & 1 & 2 & 3 & 4 & 5 & 7 \\
\end{array} \]

Days from grafting

D

CT  Oncostatin M  mAAT
Fig. 8C

**Constant stimuli: AAT gradient**

CT

IL-1β (10 ng/ml) / IFNγ (5 ng/ml)

**Constant AAT: Stimuli gradient**

IL-1β "100" = 50 ng/ml

IFNγ "100" = 25 ng/ml

CT

AAT (16 μg/ml)

Fig. 8D

**Constant stimuli: AAT gradient**

CT

IL-1β (10 ng/ml) / IFNγ (5 ng/ml)

**Constant AAT: Stimuli gradient**

IL-1β "100" = 50 ng/ml

IFNγ "100" = 25 ng/ml

CT

AAT (16 μg/ml)
Fig. 9A

Percent graft survival

- ≥ 30-day therapy (n = 12)
- 21-day therapy (n = 6)
- 14-day therapy (n = 6)
- Control (n = 6)

Weeks from transplantation

0 1 2 3 4 5 6 7 8 9 10 11 12
Figure 9B

First graft

Mouse

hAAAT

1 (a) 52 days
2 (a) 30 days
3 (a) 30 days
4 (b) 14 days
5 (a) 52 days
6 (a) 52 days
7 (b) 21 days

Same strain

Same strain

DBA/2

DBA/2

Balb/c

3rd strain

CBA/Ca (H-2^d)

CBA/Ca (H-2^d)

CBA/Ca (H-2^d)

CBA/Ca (H-2^d)

Graft Removal

Hypoglycemia

Treatement Withdrawal

(a) First graft donor strain: DBA/2 (H-2^d)
(b) First graft donor strain: Balb/c (H-2^d)
Fig. 12A

Treatments:
- foxp3
- TGFβ1
- CTLA-4

Days from transplant:
1 3 5 7

AAT
72

ALB
Fig. 12B

Day 14
- foxp3
- CTLA4
- IL-10

Day 30
- G
- K
- G

Fig. 12B
Fig. 13C
Islet allotransplant (cuff site)

AAT

IHC: Anti-GFP (foxp3) DAPI

x400
Fig. 14

<table>
<thead>
<tr>
<th>AAT µg/ml</th>
<th>CD14 (RT-PCR)</th>
<th>hAAT (genomic)</th>
<th>Insulin (RT-PCR)</th>
<th>VEGF (RT-PCR)</th>
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<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<td>25</td>
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<tr>
<td>100</td>
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</tbody>
</table>
Fig. 15

IL-10/G-actin mRNA

CD3/G-actin mRNA

CD86/G-actin mRNA
Fig. 16A

graphs showing Splenocytes response to various concentrations of ConA, ALB, and AAT.

Cell number [fold from CT]

IFNγ ng/ml

ConA µ g/ml

0.0 0.6 1.2

1.0 1.1 1.2

0.5 mg/ml

ALB/AAT
Fig. 17

Evoked (LPS)

Steady-state

Fold change from untreated

Evoked (sterile inflammation)

IL-1Ra IL-6 IL-10 IL-17 IL-23 IFN-γ MCP-1 MIP-2

TRP/AT TRP/Saline Saline Un-treated

IL-10

P = 0.0187

P = 0.0069

IL-6 ng/ml
Fig. 18A

<table>
<thead>
<tr>
<th>AAT</th>
<th>Graft (GFP&lt;sup&gt;+&lt;/sup&gt;/+)</th>
<th>Recipient DLN (GFP&lt;sup&gt;-/-&lt;/sup&gt;)</th>
<th>Hour</th>
<th>GFP (Genomic)</th>
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<tr>
<td></td>
<td></td>
<td>+</td>
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<td>9</td>
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<tr>
<td></td>
<td></td>
<td>+</td>
<td>24</td>
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</table>
Fig. 19

IL-1β/IFNγ

AAT
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RELATED APPLICATIONS

[0001] This application is a continuation in part of U.S. application Ser. No. 11/916,521 filed on Jun. 7, 2006, which is a U.S. National Stage Entry of PCT/US2006/022436, filed Jun. 7, 2006, which claims the benefit of U.S. provisional patent application Ser. No. 60/687,850, filed on Jun. 7, 2005, all of which are incorporated herein by reference in their entirety.

FEDERALLY FUNDED RESEARCH

[0002] The studies disclosed herein were supported in part by grant number AI-15614 from the National Institutes of Health. The U.S. government may have certain rights to practice the subject invention.

FIELD

[0003] Embodiments of the present invention relate to compositions and methods for treatment of conditions in need of or having a transplant. In particular, embodiments of the present invention relate to compositions and methods for treatment of conditions associated with transplantsations in a subject, for example, graft rejection. More particularly, the present invention relates to compositions and uses of alpha1-antitrypsin (α1-antitrypsin) and agents with α1-antitrypsin-like activity and/or compositions and uses of serine protease inhibitors.

BACKGROUND

Serine Proteases

[0004] Serine proteases serve an important role in human physiology by mediating the activation of vital functions. In addition to their normal physiological function, serine proteases have been implicated in a number of pathological conditions in humans. Serine proteases are characterized by a catalytic triad consisting of aspartic acid, histidine, and serine at the active site.

[0005] Naturally occurring serine protease inhibitors have been classified into families primarily on the basis of the disulfide bonding pattern and the sequence homology of the reactive site. Serine protease inhibitors, including the group known as serpins, have been found in microbes, in the tissues and fluids of plants, animals, insects and other organisms. At least nine separate, well-characterized proteins are now identified, which share the ability to inhibit the activity of various proteases. Several of the inhibitors have been grouped together, namely α1-antitrypsin-protease inhibitor, secretary leukocyte protease inhibitor or SLPI, antithrombin III, antichymotrypsin, Cl-inhibitor, and α2-antiplasmin, which are directed against various serine proteases, i.e., leukocyte elastase, thrombin, cathepsin G, chymotrypsin, plasminogen activators, and plasmin. These inhibitors are members of the α1-antitrypsin-protease inhibitor class. The protein α2-macroglobulin inhibits members of all four classes of endogenous proteases: serine, cysteine, aspartic, and metalloproteases. However, other types of protease inhibitors are class specific. For example, the α1-antitrypsin-protease inhibitor (also known as (α1-antitrypsin or AAT) and inter-alpha-trypsin inhibitor inhibit only serine proteases, α1-cys-

teine protease inhibitor inhibits cysteine proteases, and α1-antichymotrypsin inhibits collagenolytic enzymes of the metalloenzyme class.

[0006] The normal plasma concentration of AAT ranges from 1.3 to 3.5 mg/ml although it can behave as an acute phase reactant and increase 3-4 fold during host response to inflammation and/or tissue injury such as with pregnancy, acute infection, and tumors. It easily diffuses into tissue spaces and forms a 1:1 complex with target proteases, principally neutrophil elastase. Other enzymes such as trypsin, chymotrypsin, cathepsin G, plasmin, thrombin, tissue kallikrein, and factor Xa can also serve as substrates. The enzyme/inhibitor complex is then removed from circulation by binding to serpin-enzyme complex (SEC) receptor and catabolized by the liver and spleen. AAT appears to represent an important part of the defense mechanism against activity by serine proteases.

[0007] α1-antitrypsin is one of few naturally occurring mammalian serine protease inhibitors currently approved for the clinical therapy of protease imbalance. Therapeutic α1-antitrypsin has been commercially available since the mid 1980's and is prepared by various purification methods (see for example Bolken et al., U.S. Pat. No. 4,629,567; Thompson et al., U.S. Pat. Nos. 4,760,130; 5,616,693; WO 98/56821). Prolastin is a trademark for a purified variant of α1-antitrypsin and is currently sold by Talecris Company (U.S. Pat. No. 5,610,285 Lebing et al., Mar. 11, 1997). Recombinant unmodified and mutant variants of α1-antitrypsin produced by genetic engineering methods are also known (U.S. Pat. No. 4,711,848); methods of use are also known, e.g., (α1-antitrypsin gene therapy/delivery (U.S. Pat. No. 5,399,346).

Graft Rejection

[0008] There are many diseases that culminate in organ dysfunction or failure. Representative non-limiting examples include renal failure due to diabetes melitus, hypertension, urinary output obstruction, drug-induced toxicity, or hypoperfusion, as well as cardiac dysfunction due to ischemic coronary artery disease, cardiomyopathy/infection, or valvulopathy. Pulmonary diseases include substantial damage due to chronic obstructive pulmonary disease (COPD, including chronic bronchitis and emphysema), AAT deficiency, cystic fibrosis, and interstitial fibrosis. Under certain conditions, the only therapeutic option for treatment of a subject may be organ transplantation. Pancreatic-islet transplantation provides diabetic patients with the only option for a tightly controlled blood glucose level, as evidenced by the prevention of diabetic complications. In the case of islet, post-transplant inflammation, which precedes immune rejection, is a critical determinant of graft survival. This early inflammation is mediated by cells other than the impeding allospecific immune cells.

[0009] One challenge to therapeutic transplantation is the damaging effects of the host immune system on the transplant. MHC molecules exist on the surfaces of cells and the particular structures of MHC molecules are typically unique for each individual (with the exception of identical twins, where the MHC molecule complements are identical). The immune system is programmed to attack foreign or "non-self" MHC-bearing tissues. For these reasons, when an organ or tissue is transplanted into a recipient, an effort is made to optimize the degree of tissue matching between donor and recipient. MHC antigens are characterized for the recipient and donors. Matching a donor to an allograft recipient by
MHC structure reduces the magnitude of the rejection response. An archetypal example is blood group matching. Most transplants are allografts that occur between non-identical members of the same species. Since these matches are imperfect, there is an expected graft rejection immune response associated with allografts. Current methods used, in order to enhance graft survival, include medications to suppress the immune response which can result in graft rejection. These medications are referred to as immunosuppressant or antigraft rejection drugs, such as prednisone, cyclosporine A, and cyclophosphamide, to name a few. As mentioned above, local inflammation is experienced immediately after grafting, and cells that are particularly sensitive to non-specific inflammation, such as islets, can endure graft dysfunction more severely than other types.

Despite advances in the field of antigen rejection therapy, graft maintenance remains a challenge since the available antirejection therapies are imperfect. For example, immunosuppression enhances the risk for opportunistic infection or neoplasia. Toxicities abound and include, but are not limited to, diabetes, organ dysfunction, renal failure, hepatic dysfunction, hematological defects, neuromuscular and psychiatric side effects, and many others. Therefore, there is a need for a more effective anti-rejection medical treatment that prolongs graft survival and improves the quality of life.

Bone marrow transplantation is a unique kind of transplant where immune cells from a donor are transferred into a recipient, thereby conferring the donor immune system into the recipient. Here, the graft is capable of generating an immune response against the host, and this is termed “graft versus host” disease (GVHD). Immunosuppressive and antimicrobial treatment is required to block adverse consequences of GVHD, and a need exists for safer and more effective inhibitors of the adverse effects by the graft.

Because of some of the difficulties and inadequacies of conventional therapy for treating transplantation complications and associated side-effects, new therapeutic modalities are needed.

**SUMMARY**

Embodiments of the present invention provide for methods for treating a subject having or in need of a transplant. In accordance with these embodiments, a subject may be treated with a composition for reducing the risk of a transplant rejection or a side-effect of a transplant rejection in a subject. In accordance with this method the subject can be administered a composition including a compound that is capable of significantly reducing serine protease activity. The composition may be administered before transplantation, during transplantation, after transplantation or combination thereof. In addition, the composition may further include one or more anti-transplant rejection agent, anti-inflammatory agent, immunosuppressive agent, immunomodulatory agent, anti-microbial agent, or a combination thereof.

In certain embodiments of the invention, a composition capable of significantly reducing serine protease activity can include alpha-1-antitrypsin, an analog thereof or a combination thereof. A transplant of the present invention may include an organ transplant and/or a non-organ transplant. For example lung, kidney, heart, liver, cornea, skin, stem cells, soft tissue (e.g. facial component transplant), intestinal transplants, bone marrow, pancreatic islet, pancreas transplant or combination thereof are contemplated.

Embodiments of the present invention provide for methods for ameliorating symptoms or signs experienced by a subject having or in need of a transplant. In accordance with these embodiments, symptoms or signs may include conditions associated with graft versus host disease (GVHD), or graft rejection. In one example, methods disclosed herein may be used to treat a subject undergoing bone marrow transplantation. In another embodiment, symptoms or signs may include but is not limited to one or more of the following, kidney failure, lung failure, heart failure, malaise, fever, dry cough, anorexia, weight loss, myalgia, and chest pains, ventilatory compromise, sweating, nausea, vomiting, fever, abdominal pain, bloody diarrhea, mucosal ulcerations, reduced renal function (increased creatinine, decreased urine output), reduced pulmonary function (increased shortness of breadth, fever, cough, sputum, hypoxemia), reduced cardiac function (shortness of breath, chest pain, fatigue, pulmonary or peripheral edema, valvulopathy), reduced islet function (increased glucose, diabetes mellitus), graft versus host disease (gastrointestinal (GI) ulceration, pulmonary failure, skin ulceration, coagulopathy, CNS dysfunction (mental status changes, coma) CMV (cytomegalovirus infection, viral, fungal parasitic infection)).

Embodiments of the present invention provide methods for promoting prolonged graft survival and function in a subject including administering to a subject in need thereof a therapeutically effective amount of a composition including a substance exhibiting α1-antitrypsin or α1-antitrypsin analog or inhibitor of serine protease activity or a functional derivative thereof.

Embodiments of the present invention provide for methods for treating a subject in need of an immunotolerance therapy. In accordance with these embodiments, the subject may be treated with a composition for reducing the risk of a dysfunctional immune responses or a side-effect of a dysfunctional immune response in a subject. In another embodiment, methods herein provide for inducing immune tolerance specific for a graft and/or reduce the need for immunosuppressive therapy. In accordance with this embodiment, the immune system of the transplant recipient may have reduced or lost the specific ability to attack the graft while maintaining its ability to mount any other type of immune attack. In accordance with this method the subject can be administered a composition including a compound that is capable of significantly reducing serine protease activity or other activity associated with α1-antitrypsin or α1-antitrypsin analog. In certain embodiments, a composition capable of significantly reducing serine protease activity can include alpha-1-antitrypsin, an analog thereof or a combination thereof. In accordance with these embodiments, one example for immunotolerance therapy can include inhibiting cytokine production.

Embodiments of the present invention provide for methods for reducing TNFα (tumor necrosis factor alpha) levels in a subject including administering a composition including alpha-1-antitrypsin, an analog thereof or a combination thereof to a subject in need of such a treatment.

Embodiments of the present invention provide for methods for treating a subject in need of an immunotolerance therapy. In accordance with these embodiments methods are provided for reducing NO production and/or reducing apoptosis and/or inhibiting cytomegalovirus (infection and reactivation) including administering a composition including a compound that is capable of significantly reducing serine protease activity and/or other alpha-1-antitrypsin activity. In
certain embodiments of the invention, a composition capable of significantly reducing serine protease activity and/or mimicking other alpha-1-antitrypsin activity can include alpha-1-antitrypsin, an analog thereof, or a combination thereof.

In certain embodiments of the present invention, the anti-inflammatory compound or immunomodulatory drug can include but is not limited to one or more of interferon, interferon derivatives including betasemsin, beta-interferon, prostate derivatives including iloprost, ciprost; glucocorticoids including cortisol, prednisolone, methyl-prednisolone, dexamethasone; immunosuppressives including cyclosporine A, FK-506, methoxsalen, thalidomide, sulfaalazine, azathioprine, methotrexate; lipooxygenase inhibitors comprising zileutone, MK-886, WY-50295, SC-45662, SC-41661A, BI-L-357; leukotriene antagonists; peptide derivatives including ACTH and analogs thereof, soluble TNF-receptors; TNF-antibodies; soluble receptors of interleukin, other cytokines, T-cell proteins; antibodies against receptors of interleukins, other cytokines, T-cell proteins; and calcipotriols; Celcept®, mycophenolate mofetil, and analogues thereof taken alone or in combination.

In certain embodiments, synthetic and/or naturally occurring peptides may be used in compositions and methods of the present invention for example, providing serine protease inhibitor activity. Homologues, natural peptides, with sequence homologies to AAT including peptides directly derived from cleavage of AAT may be used or other peptides such as, peptides that inhibit serine proteases or have AAT-like activity. Other peptide derivatives, e.g., aldehyde or ketone derivatives of such peptides are also contemplated herein. Without limiting to AAT and peptide derivatives of AAT, compounds like oxadiazole, thiazazole and triazole peptides and substances comprising certain phenylenedialkanote esters, CE-2072, UT-77, and triazole peptides may be used. Examples of analogues are TLCK (tosyl-L-lysine chloromethyl ketone) or TPCK (tosyl-L-phenylalanine chloromethyl ketone).

In certain embodiments, an agent that reduces the occurrence of graft rejection, promotes prolonged graft function or promotes prolonged allograft survival can also be an inhibitor of serine protease activity, an inhibitor of elastase, or an inhibitor of proteinase-3. An inhibitor of serine protease activity can include, but is not limited to, small organic molecules including naturally-occurring, synthetic, and biosynthetic molecules, small inorganic molecules including naturally-occurring and synthetic molecules, natural products including those produced by plants and fungi, peptides, variants of α1-antitrypsin, chemically modified peptides, and proteins.

In some embodiments, AAT peptides contemplated for use in the compositions and methods of the present invention are also intended to include any and all of those specific AAT peptides other than the 10 amino acid AAT peptides of SEQ ID NO. 1 depicted supra. Any combination of consecutive amino acids depicting a portion of AAT or AAT-like activity may be used, such as amino acids 2-12, amino acids 3-13, 4-14, etc. of SEQ ID NO. 1, as well as any and all AAT peptide fragments corresponding to select amino acids of SEQ ID NO. 1. Applicants are herein entitled to compositions based upon any and all AAT peptide variants based upon the amino acid sequence depicted in SEQ ID NO. 1.

In one aspect of the invention, the pharmaceutical compositions of the present invention are administered orally, systemically, via an implant, intravenously, topically, intrathecally, intracranially, subcutaneously, intravitally, intraventricularly, intranasally such as inhalation, mixed with grafts by flushing of organ or suspension of cells, or any combination thereof.

Other embodiments concern methods for preventing or reducing the risk of developing an organ or cellular transplant rejection in a subject having had or undergoing a cellular transplant, the method comprising administering to the subject a composition comprising alanine-1 antiproteinase (AAT), α-1 antiproteinase-like compound or combination thereof. Any composition herein can be administered to the subject before transplantation, during transplantation, and after transplantation or combination thereof. A composition contemplated herein can further include one or more anti-transplant rejection agent, anti-inflammatory agent, immunosuppressive agent, immunomodulatory agent, anti-microbial agent, or a combination thereof. A composition herein can include, but is not limited to a carboxy-terminal peptide corresponding to AAT, an analog thereof, any derivative of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or a combination thereof.

Cellular transplant
can be a cornea, bone marrow, liver, stem cell, pancreatic islet, pancreas, kidney, lung, intestinal transplant, or a combination thereof. In certain embodiments, a cellular transplant can be a pancreatic islet cell transplant.

[0030] Immunosuppressive agent can be chosen from inhibitors of apoptosis, serine protease inhibitors, reducers of lymphocyte numbers, reducers of cytokine production, reducers of cytokine activities, monoclonal antibodies, reducers of cytokine receptors, reducers of nitric oxide production and a combination thereof.

[0031] Other agents contemplated herein can include reducers of cytokine production, reducers of cytokine activities, reducers of cytokine receptors is an inhibitor of one or more of TNFα (tumor necrosis factor alpha), IL-1 (interleukin-1), IL-12 (interleukin-12), IL-18 (interleukin-18), IL-17 (interleukin-17), IL-23 (interleukin-23), IL-32 (interleukin-32), IFNγ (interferon gamma) or a combination thereof.

[0032] In other methods contemplated herein, treating organ or cellular transplant rejection in a subject is contemplated by identifying a subject having or in need of a cellular or organ transplant; performing cellular or organ transplantation on the subject; and administering a therapeutically effective amount of a composition comprising AAT, AAT-like compound, AAT analog, AAT derivative, serine protease inhibitor, one or more carboxy-terminal peptides derived from AAT, any derivative of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof to the subject. In accordance with these methods, treating the subject with the composition reduces the risk of transplantation rejection by at least 10% compared to a subject not treated with the composition.

[0033] Other embodiments herein include treating diabetes in a subject by identifying a subject having or at risk of developing diabetes; performing pancreatic islet cell transplantation on the subject; and administering a therapeutically effective amount of a composition comprising AAT, AAT-like compound, AAT analog, AAT derivative, serine protease inhibitor, one or more carboxy-terminal peptides derived from AAT, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof to the subject. Administering the composition may include administering the composition to the organ or cells to be transplanted before transplant, administering the composition to the subject before transplant, administering the composition to the subject during transplantation, administering the composition to the subject after transplantation or a combination thereof. Certain subjects contemplated herein have or are at risk of developing Type 1 diabetes. These subjects may have been diagnosed with early phase type 1 diabetes. Other subjects may have or are at risk of developing Type 2 diabetes. It is contemplated that using compositions disclosed herein may reduce the symptoms associated with diabetes by 10%, 20%, or 30% or more.

[0034] Yet other embodiments herein include methods reducing a side-effect of cellular transplant rejection in a subject, the method comprising administering to the subject a composition comprising alpha-1 antitrypsin (AAT), one or more carboxy-terminal peptides derived from AAT, or alpha-1 antitrypsin-like compound. In accordance with these embodiments, a side effect of cellular or organ transplant can be production of pro-inflammatory cytokines, infiltration of immunocompetent cells, infiltration of inflammatory cells, infiltration of cytotoxic T-cells, infiltration of mature dendritic cells, infiltration of monocytes, production of nitric oxide, production of prostaglandins, production of reactive oxygen species, production of super oxide radicals, infiltration of natural killer cells, infiltration of natural killer T-cells and a combination thereof.

[0035] Other exemplary methods include preventing or reducing the risk of developing pancreatic islet cell transplant rejection in a subject having had or undergoing a pancreatic islet cell transplant, the method comprising administering to the subject a composition comprising alpha-1 antitrypsin (AAT), one or more carboxy-terminal peptides derived from AAT, engager of the SEC receptor, alpha-1 antitrypsin-like compound, serine protease inhibitor or combination thereof. A subject may have juvenile or late onset type 1 diabetes or type 2 diabetes. Reducing the risk of developing pancreatic islet cell transplant rejection in a subject can include reducing the risk by at least 10% in the subject compared to a second subject not treated with the composition.

[0036] In certain embodiments, the subject is a human. In some embodiments, the subject is a domesticated animal or livestock.

[0037] A pharmaceutical composition contemplated herein may include, AAT, AAT-like compound, serine protease inhibitor, AAT analog, AAT derivative, one or more carboxy-terminal peptides derived from AAT, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof and at least one of an anti-transplant rejection agent, an anti-inflammatory agent, an immunosuppressive agent, an immunomodulatory agent, and an anti-microbial agent. In accordance with these embodiments, the pharmaceutical composition can be AAT, serine protease inhibitor, AAT-like compound, AAT analog, AAT derivative, carboxy-terminal peptide corresponding to AAT, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof and one or more anti-transplant rejection agents.

[0038] Other exemplary methods can include methods for inducing immunological tolerance in a subject undergoing a cellular or organ transplant including, but not limited to, administering to the subject a composition comprising alpha-1 antitrypsin (AAT), alpha-1 antitrypsin-like compound or combination thereof wherein the subject is undergoing islet cell transplant. One cellular transplant can be pancreatic islet cell transplant. One transplant can be a temporary cadaver transplant of skin in a burn patient where compositions herein inhibit rejection of the temporary cadaver treatments.

[0039] Other exemplary methods can include, at least one of increasing numbers of or increasing effectiveness and/or of sustaining T-regulatory cells in a subject by administering an AAT or AAT derivative composition to the subject.

[0040] Yet other exemplary methods can include increasing immune tolerance in a subject in need thereof by administering an AAT or AAT derivative composition to the subject in need thereof. In addition, these methods may further include inhibiting dendritic cell maturation. For example, compositions contemplated herein may be administered to a subject having, undergoing or previously having had a transplant.

[0041] Other exemplary methods herein include reducing antigen presentation by dendritic cells by administering an AAT or AAT derivative composition to the cells. In accordance with these embodiments, the cells are cells of a human subject and administration is to the subject in need of reducing antigen presentation. Other examples herein include
inhibiting maturation of dendritic cells by administering an AAT, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or AAT derivative composition to a subject having, undergoing or previously having had a transplant.

[0042] As such, those skilled in the art will appreciate that the conception, upon which this disclosure is based, can readily be used as a basis for designing other methods for carrying out the several features and advantages of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] The following drawings form part of the present specification and are included to further demonstrate certain embodiments of the present invention. The embodiments may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0044] FIGS. 1A-1D illustrates an exemplary method of treating islet allografts with AAT. Islets from DBA/2 mice (H-2d) were transplanted under the renal capsule of streptozotocin-induced hyperglycemic C57BL/6 mice (H-2b). (A) Glucose levels from days 6-18. (B) Treatment protocols. Control and full AAT treatment are described in panel A. Early AAT treatment consists of treatment on days 1, 2 and 3 (2 mg, n=3). Late AAT treatment consists of treatment from day 2 and on every 2 days (2 mg, n=3). (C) Effect of mouse anti-human-AAT antibodies. Dashed line indicates post transplantation glucose levels of a mouse under full AAT treatment protocol (see A, B) that was immunized by multiple administrations of human AAT prior to transplantation (1 representative, n=3). Solid line indicates glucose levels of a non-immunized mouse treated under full AAT treatment protocol (1 representative, n=10). Arrow indicates detection of treatment-induced, anti-human-AAT antibodies in the non-immunized representative mouse. (D) Comparison of day 15 post-transplantation glucose levels in mice that were under full treatment protocol with ALB (n=3) or AAT (non-immunized n=10, immunized n=3). Of the AAT-treated group, antibodies were detected on day 15 in 3/3 immunized mice and in 6/10 non-immunized mice.

[0045] FIGS. 2A-2D illustrates an exemplary method of the effect of AAT on thioglycolate-elicited peritoneal cellular infiltrates. (A) Total cellular population of harvested cells of (B) saline or (C) AAT-treated (5 mg) thioglycollate-injected mice. (B) Percent cell population from saline-injected mice at 48 hours. (C) Oxidation of AAT. (D) Identification of elicited macrophages and neutrophils.

[0046] FIGS. 3A-3C illustrates an exemplary method of the effect of AAT on MHC-incompatible, N(N1-3T3-fibroblast-elicited peritoneal cellular infiltrates. (A) Cell numbers. The number of cells in each subpopulation was calculated from the percentages obtained by FACS analysis, and total number of cells in the infiltrate. (B) Representative FACS analysis. (C) Effect of AAT on intensity and function of infiltrate elicited by islet allograft. Left, Hematolymph and Eosi (HEE) staining of day 7 islet allografts. Right, Immunohistochemistry (IHC) with anti-insulin antibodies of day 15 islet grafts. R, renal parenchymal, G, graft, C, renal capsule.

[0047] FIGS. 4A-4H illustrates an exemplary method of the effect of AAT on islet response. (A-D) Means±SEM of A. nitric levels, B, Cell viability and C. MP-1α levels. Dashed line represents islets incubated at one-50th the concentration of IFNγ/IL-1β. D. TNFα levels. (E) Insulin induction assay, (F) Streptozotocin toxicity. Each image depicts a representative islet from one pancreas. (G) Cellular content of islets. (H) MHC class II expression.

[0048] FIGS. 5A-5D illustrates the effect of AAT on TNFα. (A) Islets from C57BL/6 mice were cultured (100 islets/well in triplicate) in the presence of AAT (0.5 mg/ml) or TACE inhibitor (10 mM) 1 hour before stimulation by IFNγ (5 ng/ml) plus IL-1β (10 ng/ml). Left, means±SEM change in TNFα in supernatants after 72 hours of incubation. Right, mean SEM fold change in membrane TNFα on islet cells after 5 hours of incubation, according to FACS analysis. (B) Representative FACS analysis of membrane TNFα on stimulated islet cells in the absence (open area) or presence (shaded area) of AAT. (C) Streptozotocin-induced hyperglycemia.

[0049] FIGS. 6A-6D illustrates the effect of AAT on islet allograft transplantation. 6A illustrates the time course study after transplantation. 6B illustrates an immune infiltrate found outside the graft area. 6C illustrates an increase in the presence of CD4+ and a comparative decrease in monocytes and neutrophils. 6D illustrates levels of glucose reflecting a level of tolerance with respect to days following allografting of the same donor (left) and a 3rd donor re-graft (right), indicating induction of specific immune tolerance.

[0050] FIGS. 7A-7E illustrates the production of AAT by islet cell and reflection of islet graft survival. 7A illustrates a time course expression of mouse AAT mRNAs after cytokine production (IL-1β and IFNγ) (left) and at 8 hours (right). 7B illustrates an example of islet injury during pancreatectomy, the histology of normal islets (top left), the histology of islets of an inflamed pancreas (top right) and expression of mouse AAT in islets obtained from the pancreas in an acute pancreatitis model (bottom). 7C illustrates an example of samples of islet allografts taken post grafting and the percent change in AAT mRNA levels were assessed. 7D illustrates an example of islet protection from cytokine injury with endogenous AAT by introducing oncostatin M (an interleukin 6 (IL-6) family member) that induces AAT expression in islets, oncostatin M and AAT levels (top left); nitric oxide and viability levels assessed (top right) and nitric oxide production representing islet viability after 4 day exposure to oncostatin M and AAT production decreasing cytokine effects on the islets (bottom).

[0051] FIGS. 8A-8D illustrates the effect of AAT on human islets and the production of nitric oxide (8A), TNF-α production (8B) IL-6 (8C) and IL-8 (8D).

[0052] FIGS. 9A-9D illustrate exemplary extended AAT monotherapy where exposure induces strain-specific immune tolerance towards islet allografts in mice. Islet-allograft transplantation was performed and blood glucose was followed in mice that received albumin (ALB, n=6) or hAAT monotherapy (n=24) for various periods of times. (A) Islet graft survival curve. (B) Summary of uninterrupted normoglycemic intervals achieved during and after hAAT monotherapy (“First graft”) and during a second grafting procedure that was carried out in explanted animals in the absence of therapy ("Second graft") (n=7). Double-underlined headings indicate number of hAAT monotherapy and therapy-free days. The outcome of the second grafting procedure is indicated per individual mouse. (C) Representative Blood glucose follow-up. Albumin (ALB)-treated animals are represented by dashed line. Day of hAAT treatment withdrawal is indicated. Treatment-free glucose levels were determined during the ensuing days. Graft removal by nephrectomy, resulting in hyperglycemia, is indicated. A second grafting without further hAAT treatment was performed with same
strain islet allograft (left) or third strain islet allograft (right). Transplantation outcome of the second grafting is monitored for 50 days. (D) Histology: Representative day 72 explanted graft from hAAT-treated mice 20 days after withdrawal of hAAT treatment. H&E stain, image of entire islet graft site. Islet mass appears flanked by a dense mononuclear cell population (thick arrows).

**[0053]** FIG. 10 represent exemplary effects of hAAT monotherapy on gene expression profile in islet allografts. RT-PCR of explanted islet allografts from albumin (ALB)-treated control and hAAT-treated mice. Left 4 columns, initial days after islet transplantation into control mice. Right column, day 72 after islet transplantation into hAAT-treated mice (see FIG. 9). Data are representative of n = 6 (ALB) and n = 3 (hAAT; time points between days 30 and 72 after transplantation).

**[0054]** FIG. 11 represent exemplary cell-specific effects of hAAT. Inducible IFNγ levels (left) and cell proliferation (right) assessed in Con A-primed PBMC that were stimulated with increasing concentrations of IL-2 in the presence of 0.5 mg/ml hAAT or albumin (CT). Data are mean±SEM of three individual donors.

**[0055]** FIGS. 12A-12B Identification of hAAT-induced IL-10-expressing Treg cells in non-rejecting islet allografts. (A) RT-PCR of explanted islet allografts in albumin (ALB)-treated graft recipients during acute allorejection (Left 4 columns, days 1-7) and hAAT-treated graft recipient 20 days after withdrawal of hAAT treatment (Right column, day 72, see FIG. 12). Data are representative of n = 6 (ALB) and n = 3 (hAAT; representative time point between days 30 and 72 after transplantation). (B) Intragraft gene expression profile throughout hAAT therapy. RT-PCR of explanted islet allografts in hAAT-treated graft recipients during hAAT treatment. K, tissue from pole opposite to the grafting site. G, intragraft gene profile.

**[0056]** FIGS. 13A-13C represent exemplary time-dependent hAAT-induced distribution of Treg cells between DLN and allograft. Foxp3-GFP knock-in mice (H-2b) were grafted with wild-type BALB/c tissue (H-2d). Mice received a 10-day hAAT treatment or albumin protocol (see FIG. 9). (A) Intracellular DIL, FACs analysis of CD44-sorted foxp3-GFP-positive DIL cells. Inset, RT-PCR for foxp3 mRNA transcripts in DIL. Illustrates are representative time-points. (B) Matrigel skin graft. Treg cells in matrigel grafted on day 10 identified by fluorescent microscopy of unstained material (left) plus DAPI-counter stained material (right). (C) Islet graft. Day 14 Treg cells identified in the “cut” site (see FIG. 9D). Anti-GFP antibody immunostaining and DAPI counterstaining. Representative image of three hAAT-treated grafts. Grafts from albumin-treated mice contained no “cut” (not shown).

**[0057]** FIG. 14 represents an exemplary method illustrating of early local and systemic effects of hAAT. Wild-type islet-matrigel grafts containing increasing concentrations of hAAT (indicated, amount per matrigel) were explanted 48 hours after transplantation into hAAT-1g recipients. Top, identification of CD144-positive cells (RT-PCR) and identification of host-cells inside the graft (genomic). Bottom, RT-PCR depiction of insulin and VEGF intragraft transcripts.

**[0058]** FIG. 15 represents an exemplary effect of AAT on dendritic cell migration and maturation. CD86, MHC class II and IL-10 expression in renal DLN. 72 hours after allogeneic skin grafting under the renal capsule. DLN were harvested and examined by RT-PCR. DLN from non-grafted mice (first bar on left) is compared to 72-hour DLN gene expression from untreated (CT) and hAAT-treated (AAI) mice. Mean±SEM from three experiments. * p<0.05, ** p<0.01 between CT and AAT.

**[0059]** FIGS. 16A-16B represent exemplary experiments cell-specific effects of hAAT. (A) Mouse splenocytes. Inducible IFNγ levels (top), cell proliferation (bottom) and clump formation (right) in Con A-stimulated splenocytes in the presence indicated concentrations of hAAT or albumin (ALB). CT, cells with no added Con A. Photomicrographs depict an example of Con A-driven cell clumping. The data represent mean±SEM of three independent experiments. (B) Mouse peritoneal macrophages. Inducible nitric oxide production in peritoneal macrophages that were stimulated with IFNγ (5 ng/ml) in the presence of increasing concentrations of hAAT. The data are mean±SD. * p<0.05, ** p<0.01.

**[0060]** FIG. 17 represents exemplary effects of early local and systemic effects of hAAT. hAAT-induced changes in serum cytokines. Top-Left, Unprovoked serum cytokines after a 10-day schedule (see FIG. 9) of hAAT-treatment (n = 3) compared to albumin treated mice (n = 3). Relative cytokine levels were determined in duplicate by the Proteome Profiler (see Methods). Results are presented as mean±SEM fold-change (all p<0.05) in hAAT-treated mice over that observed in albumin-treated mice. Out of 36 cytokines tested, those without statistically significant changes are not shown. Top-Right, LPS-elicited cytokines. Following a 10-day schedule of hAAT (n = 3) or albumin treatment (n = 3), mice were injected with LPS (1 mg/kg) and after 2 hours serum was collected. Differences are shown as mean±SEM fold-change in hAAT-treated mice compared to albumin-treated mice. Bottom, sterile-inflammation-induced serum cytokines. Sera from hAAT-treated and saline-treated mice that were injected intramuscularly with either turpentine or saline 24 hours earlier. IL-10 and IL-6 levels were measured by specific ELISA. Mean±SEM from 3 experiments.

**[0061]** FIGS. 18A-18B represent exemplary experiment effect of AAT on dendritic cell migration and maturation. (A) Graft-derived cell migration into DLN. GFP-transgenic skin grafts were transplanted into wild-type recipient mice treated with a 10-day treatment schedule of hAAT or albumin (see FIG. 9). PCR amplification of genomic DNA extracted from the graft tissue is shown in the left lane and from the inguinal DLN in the remaining lanes. Representative data from one of three independent experiments is shown. (B) In vitro dendritic cell maturation. Bone-marrow-derived GM-CSF-differentiated dendritic cell were cultured with no stimulant (CT) or LPS (100 ng/ml) in the absence or presence of hAAT (0.5 mg/ml) for 44 hours. FACs analysis of CD11c-positive cells for surface levels of MHCII (top) and CD86 (bottom). Proportion of the double-positive population is depicted as percent from total cells. Representative panels from three experiments performed in 6-plicate.

**[0062]** FIG. 19 represents exemplary effects of AAT on stimulated human islets.

**[0063]** FIG. 20 represents exemplary effects of AAT on stimulated human islets. Levels of IL-6, IL-8 and TNFα (percent from stimulated islets) and nitric oxide in supernatant are represented.

**[0064]** FIG. 21 represents exemplary experiments where right after isolation, islet cells were supplemented with AAT (or left untreated, CT) for 24 hrs. The cells were then washed...
and incubated for 72 hours with IL-1β and IFNγ, without AAT. LDH was measured in supernatants.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Definitions

[0065] Terms that are not otherwise defined herein are used in accordance with their plain and ordinary meaning.

[0066] As used herein, “a” or “an” may mean one or more than one of an item.

[0067] As used herein “analog of alpha-1-antitrypsin” may mean a compound having alpha-1-antitrypsin-like activity. In one embodiment, an analog of alpha-1-antitrypsin is a functional derivative of alpha-1-antitrypsin. In a particular embodiment, an analog of alpha-1-antitrypsin is a compound capable of significantly reducing serine protease activity. For example, an inhibitor of serine protease activity has the capability of inhibiting the proteolytic activity of trypsin, elastase, kallikrein, thrombin, cathepsin G, chymotrypsin, plasminogen activators, plasmin and/or other serine proteases.

[0068] As used herein “immunomodulatory drugs or agents”, it is meant, e.g., agents which act on the immune system, directly or indirectly, e.g., by stimulating or suppressing a cellular activity of a cell in the immune system, e.g., T-cells, B-cells, macrophages, or antigen presenting cells (APC, dendritic cells), or by acting upon components outside the immune system which, in turn, stimulate, suppress, or modulate the immune system, e.g., cytokines, e.g., hormones, receptor agonists or antagonists, and neurotransmitters; immunomodulators can be, e.g., immunosuppressants or immunostimulants.

[0069] It is to be understood that the terminology and phraseology employed herein are for the purpose of description and should not be regarded as limiting.

DETAILED DESCRIPTION OF THE INVENTION

[0070] In the following sections, various exemplary compositions and methods are described in order to detail various embodiments of the invention. It will be obvious to one skilled in the art that practicing the various embodiments does not require the employment of all or even some of the specific details outlined herein, but rather that concentrations, times and other specific details may be modified through routine experimentation. In some cases, well known methods or components have not been included in the description.

[0071] Embodiments of the present invention provide for methods for treating a subject having or in need of a transplant. In accordance with these embodiments, a subject may be treated with a composition capable of significantly reducing serine protease activity. In addition, one embodiment of the present invention provides for methods including treating a subject with a composition comprising a compound having α-1-antitrypsin activity. In one embodiment, the composition can include α-1-antitrypsin, analog thereof or a serine protease inhibitor to, for example, promote transplant survival or reduce a side effect of the transplant. Further, the administration of the composition can be before transplantation, during transplantation, after transplantation or combination thereof. In addition, the composition may further include one or more additional therapies such as immunosuppressive therapies. A transplant of the present invention may include transplantation of an organ such as lung, kidney, heart, liver, skin, pancreas, or bowel organ or non-organ such bone marrow, pancreatic islet, cornea, and/or soft tissue.

[0072] Human pancreatic islet transplantation has a low 5-year graft survival rate. The current immunosuppression protocol in this procedure is void of anti-inflammatory corticosteroids. Alpha-1-antitrypsin (AAT) reduces cytokine-mediated islet damage and interferes with inflammatory processes. Certain embodiments disclosed herein concern AAT monotherapy for allografts with anti-inflammatory conditions that impair dendritic cell maturation and favor development of antigen-specific T regulatory cell. Given its established safety in patients, AAT may be considered for use during human islet transplantation.

[0073] Islet transplantation can provide type-1 diabetes patients with tight glycemic control that can eliminate the need for exogenous insulin injections. In this procedure, isolated islets are introduced into the hepatic portal circulation of a diabetic patient. The immunosuppressive protocol used for islet transplantation excludes diabetogenic corticosteroids and therefore is void of anti-inflammatory activity. To date, islet loss in most transplant patients steadily progresses and results in a low 5-year graft survival rate.

[0074] Islets are particularly prone to injury during inflammatory conditions. Immediately after transplantation, viable islet mass rapidly decreases, regardless of algogenic discrepancy. As damage intensifies, necrotic islet beta cells secrete injurious cytokines and chemokines while presenting algogenic antigens to the host. Thus, grafted islets actively participate in the inflammatory flare and become activators, and targets, of resident macrophages.

[0075] Extent of inflammation and injury can determine the degree of antigen presentation and closely affects the expansion of allospecific effector cells. In addition, the favorable state of immune tolerance can be elaborated by a shift in balance between effector T cells and protective regulatory T (Treg) cells, a process which requires the uninterrupted activity of IL-2. By reducing the intensity of inflammation while allowing IL-2 activity one may provide optimal conditions for prolonged allograft survival.

[0076] In addition to its ability to inhibit serine proteases, alpha-1-antitrypsin (AAT) possesses anti-inflammatory properties. AAT has been disclosed to prevent the demise of islet beta cells from normal mice, enabling insulin secretion in the presence of IL-1β and IFNγ and reducing cytokine and chemokine secretion, previously described. Administered to animals, AAT reduced the susceptibility of islets to inflammation and prolonged islet allograft survival. In addition, AAT allows for uninterrupted IL-2 activity.

[0077] As disclosed herein, effects of extended AAT monotherapy on islet allograft rejection were examined. In order to allow extended therapy with hAAT, mice heterozygous for the human AAT transgene (hAAT-Tg) were used as graft recipients. In these mice, the human AAT sequence is preceded by a surfactant promoter, thus limiting hAAT expression to lung epithelial cells and circulating hAAT levels to less than 10 ng/ml. Thus, the impact of monotherapy on the process of allograft rejection was examined in the setting of a normal immune system. After rendered diabetic, the mice were grafted with islets from another mouse strain and were treated with hAAT for extended periods of time. Unexpectedly, therapy withdrawal revealed the fervent induction of strain-specific treatment-induced immune tolerance.

[0078] Embodiments herein provide for administration of compositions including, but not limited to, AAT, serine pro-
tease inhibitors, derivatives or fragments of the carboxy-terminus of AAT that bind to the SEC receptor, or AAT derivative or composition with AAT-like activity to a subject having or previously having had graft surgery for example, cellular implantation, cellular supplementation, organ implantation and/or tissue implantation. In certain embodiments, a subject in need of increased immune tolerance is contemplated to reduced rejection of the implanted or grafted cells, tissue or organ. In other embodiments, administration of AAT or AAT derivative or composition with AAT-like activity to a subject can be prior to implantation to reduce rejection and/or reduce immune response to the transplantation. Subject contemplated herein in need of immune tolerance include, but are not limited to, subjects having undergone a transplant or subjects scheduled for a transplant.

[0079] Embodiments herein provide for administration of compositions including, but not limited to, AAT or AAT derivative or composition with AAT-like activity to a subject having acute rejection from having undergone a transplant such as a kidney, liver or other transplant. In certain embodiments, a subject may be administered one or more infusions of an AAT composition to reduce rejection of the transplant or prolong acceptance of the transplanted organ or tissue in the subject. In certain examples, the subject may have undergone a transplant recently, a month ago or a year or more prior to administration of the AAT composition.

[0080] In certain embodiments, compositions of AAT or AAT derivative, or carboxy terminal fragment of AAT capable of binding to the SEC receptor or compositions with AAT-like activity may be administered to a subject in need thereof to induce immune tolerance in the subject. As used herein carboxy terminus of AAT can include the later half of the AAT molecule toward the carboxy end of the molecule (e.g. from the predominant naturally occurring form of AAT that would include from about AA 209 to about AA 418 of AAT).

[0081] Yet other embodiments herein concern administering AAT compositions or AAT derivative or compositions with AAT-like activity to a burn patient. For example, these compositions can be administered to a burn patient undergoing interim therapy of cadaver skin applied to the burn regions of the patient. In accordance with these embodiments, the patient can be administered iv AAT compositions at periodic times (e.g. daily, weekly or monthly) to prolong the tolerance period for the cadaver skin permitting re-growth of the patient’s own tissue.

[0082] Embodiments herein provide for administration of compositions including, but not limited to, AAT or AAT derivative or AAT-like activity to a subject in need of immune tolerance. Subject contemplated herein in need of immune tolerance include, but are not limited to, subjects in a biologically compatible

[0083] In other exemplary embodiments, a subject contemplated herein may have early phase type 1 diabetes. This disease often affects young children and can be called “juvenile type 1 diabetes.” There is also a similar type 1 diabetes that affects older individuals and is called “late onset” type 1 diabetes. Embodiments herein contemplate that AAT suppresses the immune response for example, by promoting the generation and proliferation of T regulatory cells, as well as sustaining the activity of T regulatory cells. Since both juvenile, as well as late onset type 1 diabetes, have an autoimmune response directed against the insulin producing beta cells in the pancreatic islets, AAT can be used as a treatment for both juvenile as well as late onset type 1 diabetes via the T-regulatory cells. In addition, the anti-inflammatory properties of AAT, contributes to protecting the beta cells from the cytotoxic effects of pro-inflammatory cytokines and inflammatory mediators.

[0084] In other embodiments, compositions contemplated herein can be used to treat a subject having type 2 diabetes. For example, a subject may be treated with periodic administration of the composition such as monthly or weekly administrations of compositions disclosed herein.

[0085] In some embodiments, AAT can be used to treat autoimmune diseases since these diseases reveal a low number and/or function of Tregs. Since AAT does not affect IL-2 production or activity, Tregs can be stimulated during AAT therapy to increase development of Tregs. Many immunosuppressive agents suppress IL-2 production and/or function, development of T regs is impaired. For example, in an autoimmune disease such as rheumatoid arthritis, the use of methotrexate may impair the development of Tregs. Lowering the dose of an immunosuppressive agent may be performed during AAT treatment. Other autoimmune diseases that are treated with immunosuppressive regimens may also be treated with AAT while lowering the dose of immunosuppressive agents include, but are not limited to, lupus erythematosus, Crohn’s disease, ulcerative colitis, psoriasis, biliary cirrhosis, and thrombocytopenia.

[0086] Serine protease inhibitors, have been found in a variety of organisms. At least five separate, well-characterized proteins are now identified, which share the ability to inhibit the activity of various proteases. Several of the inhibitors have been grouped together, such as the α, antitrypsin protease inhibitor. Serine proteases include but are not limited to leukocyte elastase, thrombin, cathepsin G, chymotrypsin, plasminogen activators, and plasmin.

[0087] Embodiments of the present invention provide for methods for promoting transplantation, graft survival, reducing graft rejection and/or reducing or preventing side-effects associated with graft rejection. In accordance with these embodiments, side-effects may include conditions associated with graft versus host disease (GVHD), or graft rejection. In one example, methods disclosed herein may be used to treat a subject undergoing bone marrow transplantation. In another embodiment, symptoms or signs may include but is not limited to one or more of the following, malaise, fever, dry cough, myalgia, and chest pain, ventilatory compromise, sweating, nausea, vomiting, fever, abdominal pain, bloody diarrhea, mucosal ulcerations, reduced renal function (increased creatinine, decreased urine output), reduced pulmonary function (increased shortness of breath, fever, cough, sputum, pyrexia), reduced cardiac function (shortness of breath, chest pain, fatigue, pulmonary or peripheral edema, valvulopathy), reduced islet function (increased glucose, diabetes mellitus), graft versus host disease (gastrointestinal (GI) ulceration, pulmonary failure, skin ulceration).

[0088] Embodiments of the present invention provide for methods for treating a subject in need of an immunotolerance therapy. In accordance with these embodiments, a subject may be treated with a composition for inducing immunotolerance. This achieved while reducing the risk of a dysfunctional immune responses or a side-effect of a dysfunctional immune response in a subject as typically encountered during standard immune suppression. For example, a dysfunctional immune response may be an effect of graft rejection, pneumonia, sepsis, fungal infection, cancer. In accordance with
this method the subject can be administered a composition including a compound that is capable of significantly reducing serine protease activity or other activity associated with α1-antitrypsin or α1-antitrypsin analog. In certain embodiments, a composition capable of significantly reducing serine protease activity can include α1-antitrypsin, an analog thereof or a combination thereof. In accordance with these embodiments, one example for immunotolerance therapy can include inhibiting cytokine production.

Any of the embodiments detailed herein may further include one or more a therapeutically effective amount of anti-microbial drugs anti-inflammatory agent, immuno-modulatory agent, or immunosuppressive agent or combination thereof.

Non-limiting examples of anti-rejection agents/drugs may include for example cyclosporine, azathioprine, corticosteroids, FK506 (tacrolimus), R561443, mycophenolate mofetil, rapamycin (sirolimus), mizoribine, 15-deoxyspergualin, and/or leflunomide or any combination thereof.

In addition, other combination compositions of methods disclosed in the present invention include certain antibody-based therapies. Non-limiting examples include, polyclonal anti-lymphocyte antibodies, monoclonal antibodies directed at the T-cell antigen receptor complex (OKT3, TIOH9), monoclonal antibodies directed at additional cell surface antigens, including interleukin-2 receptor alpha. Antibody-based therapies may be used as induction therapy and/or anti-rejection drugs in combination with the compositions and methods of the present invention.

Embodyments of the present invention provide for methods treating a subject in need of an immunotolerance therapy. In accordance with these embodiments, a subject may be treated with a composition capable of significantly reducing serine protease activity. In one embodiment, the composition can include α1-antitrypsin, analog thereof or a serine protease inhibitor to for example, to reduce or inhibit the production of cytokines. In accordance with these embodiments, combination therapies are contemplated, such as combining α1-antitrypsin composition with an anti-inflammatory agent.

In one particular embodiment, the present inventions provide for methods for reducing levels and activities of cytokines such as TNFα (tumor necrosis factor alpha). For example, the composition can include alpha-1-antitrypsin or analog thereof or combination thereof alone or in combination with other therapies.

In one embodiment, the reduction, prevention or inhibition of rejection of transplantation or side effects thereof associated with one or more of each of the above-recited conditions may be about 10-20%, 30-40%, 50-60%, or more reduction or inhibition due to administration of the disclosed compositions.

In one embodiment of the present invention a composition may include compounds that engage molecules for the SEC receptor to treat a subject undergoing a transplantation and/or in need of immunotolerance therapy. In each of the recited methods, an α1-antitrypsin (e.g. mammalian derived) or inhibitor of serine protease activity substance contemplated for use within the methods of the present invention can include a series of peptides including carboxylterminal amino acid peptides corresponding to AAT. These pentapeptides can be represented by a general formula (I): I-A-B-C-D-E-F-G-H-TT (note: in the Sequence Listing F=X), wherein I is Cys or absent; A is Ala, Gly, Val or absent; B is Ala, Gly, Val, Ser or absent; C is Ser, Thr or absent; D is Ser, Thr, Ans, Glu, Arg, Ile, Leu or absent; E is Ser, Thr, Asp or absent; F is Thr, Ser, Asn, Gln, Lys, Trp or absent; G is Tyr or absent; H is Thr, Gly, Met, Met(O), Cys, Thr or Gly; and I is Cys, an amide group, substituted amide group, an ester group or absent, wherein the peptides includes 4 or more consecutive amino acids and physiologically acceptable salts thereof. Among this series of peptides, several are equally acceptable including FVFLM (SEQ ID NO: 1), FVFLM (SEQ ID NO: 2), FVFLM (SEQ ID NO: 5), FLMMI (SEQ ID NO: 6), FLFLVM (SEQ ID NO: 7), FLVVF (SEQ ID NO: 8), FLFL (SEQ ID NO: 9), FLFL (SEQ ID NO: 10), FLMMI (SEQ ID NO: 11), FLMMI (SEQ ID NO: 12), FLMMI (SEQ ID NO: 13), FLFL (SEQ ID NO: 14), FLFAV (SEQ ID NO: 15), FVVFL (SEQ ID NO: 16), FFAFLM (SEQ ID NO: 17), AAFLM (SEQ ID NO: 18), and any combination thereof.

In several embodiments herein, AAT peptides contemplated for use in the compositions and methods of the present invention are also intended to include any and all of those specific AAT peptides of SEQ ID NO. 1 depicted supra. Any combination of consecutive amino acids simulating AAT or AAT-like activity may be used, such as amino acids 209-219, amino acids 314-324, 394-404, etc. In addition, combinations of consecutive amino acid sequences such as 5-mers or 10-mers of the carboxyl terminus can also be used. For example, any combinations of 5-mers or 10-mers from SEQ ID NO. 61 AAs 209-418 can be used in compositions contemplated herein. Another example is SEQ ID NO. 19 through 60 below may be combined in any composition contemplated herein.

As contemplated herein, the last amino acid is the carboxyl terminus. In certain embodiments, the carboxyl domain of AAT going backwards from the carboxyl terminus is defined as those amino acids most conserved among the difference species and do not participate in the protease binding domain of AAT. In addition, other embodiments, AAT protease binding domain can be mutated in order to reduce or eliminate the protease function of the molecule and not inhibit elastase activity; these molecules can be used in any composition contemplated herein. In certain embodiments, a mutated AAT can be used to protect islet cells before, after, and/or during transplantation either supplied directly to the islets and/or administered to a subject in need of islet cell infusion. In other embodiments, a mutated molecule (e.g. having reduced or essentially no protease activity) retains its an anti-inflammatory effects and can be used as an anti-inflammatory molecule in a subject having an autoimmune condition or undergoing an organ or cellular transplant. It is also contemplated herein that the carboxyl domain is the non-protease binding domain. One skilled in the art would understand a non-protease binding domain of AAT.

In each of the above-recited methods, α1-antitrypsin or analogs thereof are contemplated for use in a composition herein. These analogs may include peptides. The peptides may include but are not limited to amino acid peptides containing MPPSVSWGL (SEQ ID NO:19), LLA-GLCCLVP (SEQ ID NO:20), VSLAEDPGQDM (SEQ ID NO:21), AAKQTDTSHI (SEQ ID NO:22), DQDIHTFTNK (SEQ ID NO:23), TPNLEAFAFS (SEQ ID NO:24), LVRQAIHQS8N (SEQ ID NO:25), STNIFFSPVS (SEQ ID NO:26), LATAFAMLSL (SEQ ID NO:27), GTKADTHDL (SEQ ID NO:28), LLEGIPNMG (SEQ ID NO:29), IPIA-QHILGF (SEQ ID NO:30), QLQILRIKLQNP (SEQ ID NO:31),
DSQLQLTTGNN(SEQ ID NO:32), GLFLSLGKLK(SEQ ID NO:33), VDKFLDVLKK(SEQ ID NO:34), LYSHLAFTVYN(SEQ ID NO:35), HGDLTLLAKQQ(SEQ ID NO:36), INDYVLKGTQ(SEQ ID NO:37), GIKVIDLVQK(SEQ ID NO:38), DRTDIVFALVQV(SEQ ID NO:39), YIFFGKWKER (SEQ ID NO:40), PFLVBKTLLL(SEQ ID NO:41), DFIG-VDQVTVTV(SEQ ID NO:42), KVPMMKRRGKMQ(SEQ ID NO:43), FNIQHCXKLSK(SEQ ID NO:44), SWVVLMKKYLG (SEQ ID NO:45), NATAPIFFLDQ(SEQ ID NO:46), LGKXLQHNLQ(SEQ ID NO:47), LTIDITTFFXLF(SEQ ID NO:48), LNLDRSASL(SEQ ID NO:49), HPKLIKSTGT (SEQ ID NO:50), YDLKSVLQLQ(SEQ ID NO:51), GIT- KTVSNQGAP(SEQ ID NO:52), DLGQVTLLAP(SEQ ID NO:53), LKLKAKAVHAKA(SEQ ID NO:54), VTLIDLKTGL (SEQ ID NO:55), AAGAMFLLAI(SEQ ID NO:56), PMSIP- PLVKE(SEQ ID NO:57), NKPFTPFLMIL(SEQ ID NO:58), QNTKSPFLMG(SEQ ID NO:59), KVVNPQTK(SEQ ID NO:60), or any combination thereof.

Pharmaceutical Compositions

[0100] Embodiments herein provide for administration of compositions to subjects in a biologically compatible form suitable for pharmaceutical administration in vivo. By "biologically compatible form suitable for administration in vivo" is meant a form of the active agent (i.e. pharmaceutical chemical, protein, peptide, gene, antibody etc. of the embodiments) to be administered in which any toxic effects are outweighed by the therapeutic effects of the active agent. Administration of a therapeutically active amount of the therapeutic compositions is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a compound may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimal therapeutic response.

[0101] In one embodiment, the compound (i.e. pharmaceutical chemical, protein, peptide etc. of the embodiments) may be administered in a convenient manner such as subcutaneous, intravenous, by oral administration, inhalation, transdermal application, intravaginal application, topical application, intranasal or rectal administration. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the degradation by enzymes, acids and other natural conditions that may inactivate the compound. In a preferred embodiment, the compound may be orally administered. In another preferred embodiment, the compound may be administered intravenously. In one particular embodiment, the compound may be administered intranasally, such as inhalation.

[0102] A compound may be administered to a subject in an appropriate carrier or diluent, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. The term "pharmaceutically acceptable carrier" as used herein is intended to include diluents such as saline and aqueous buffer solutions. It may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. The active agent may also be administered parenterally or in injectable forms. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

[0103] Pharmaceutical compositions suitable for injectable use may be administered by means known in the art. For example, sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion may be used. In all cases, the composition cannot be sterile and can be fluid to the extent that easy syringability exists. It might be unstable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms such as bacteria and fungi. The pharmaceutically acceptable carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyols (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can 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. Prevention of microorganisms can be achieved by heating, exposing the agent to detergent, irradiation or adding various antibacterial or antifungal agents.

[0104] Sterile injectable solutions can be prepared by incorporating active compound (e.g. a compound that reduces serum protease activity) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[0105] Aqueous compositions can include an effective amount of a therapeutic compound, peptide, epitopic core region, stimulator, inhibitor, and the like, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Compounds and biological materials disclosed herein can be purified by means known in the art.

[0106] Solutions of the active compounds as free-base or pharmaceutically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can 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. 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.

[0107] 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 the type of injectable solutions described above. It is contemplated that slow release capsules, timed-release microparticles, and the like can also be employed. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration.

[0108] The active therapeutic agents may be formulated within a mixture to comprise about 0.001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 1 to 10 gram per dose. Single dose or multiple doses can also be administered on an appropriate schedule for a predetermined condition.

[0109] In another embodiment, nasal solutions or sprays, aerosols or inhalants may be used to deliver the compound of
interest. Additional formulations that are suitable for other modes of administration include suppositories and pessaries. A rectal pessary or suppository may also be used. In general, for suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%.

[0110] Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate and the like. In certain defined embodiments, oral pharmaceutical compositions will comprise an inert diluent or 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. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 75% of the weight of the unit, or preferably between 25-60%. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

[0111] A pharmaceutical composition may be prepared with carriers that protect active ingredients against rapid elimination from the body, such as time-release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyalkylenes, polyglycolic acid, polylactic acid, polyethylene glycol, and others are known.

[0112] Pharmaceutical compositions are administered in an amount, and with a frequency, that is effective to inhibit or alleviate side effects of a transplant and/or to reduce or prevent rejection. The precise dosage and duration of treatment may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Dosages may also vary with the severity of the condition. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. In general, an oral dose ranges from about 200 mg to about 1000 mg, which may be administered for example 1 to 3 times per day.

[0113] It will be apparent that, for any particular subject, specific dosage regimens may be adjusted over time according to the individual need. The preferred doses for administration can be anywhere in a range between about 0.01 mg and about 100 mg per ml of biologic fluid of treated patient. In one particular embodiment, the range can be between 1 and 100 mg/kg which can be administered daily, every other day, biweekly, weekly, monthly etc. In another particular embodiment, the range can be between 10 and 75 mg/kg introduced weekly to a subject. The therapeutically effective amount of α-l-antitrypsin, peptides, or drugs that have similar activities as α-l-antitrypsin or peptides can be also measured in molar concentrations and can range between about 1 nM to about 2 mM.

[0114] The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent.

[0115] Liposomes can be used as a therapeutic delivery system and can be prepared in accordance with known laboratory techniques. In addition, dried lipids or lyophilized liposomes prepared as previously described may be reconstituted in a solution of active agent (e.g. nucleic acid, peptide, protein or chemical agent), and the solution diluted to an appropriate concentration with a suitable solvent known to those skilled in the art. The amount of active agent encapsulated can be determined in accordance with standard methods.

[0116] In a preferred embodiment, a nucleic acid (e.g. α-l-antitrypsin or analog thereof) and the lipid dioleoylphosphatidylcholine may be employed. For example, nucleic acid-resistant oligonucleotides may be mixed with lipids in the presence of excess t-butanol to generate liposomal-oligonucleotides for administration.

[0117] The pharmaceutical compositions containing the α-l-antitrypsin, analog thereof, or inhibitor of serine protease activity or a functional derivative thereof may be administered to individuals, particularly humans, for example by subcutaneously, intramuscularly, intranasally, orally, topically, transdermally, parenterally, gastrointestinally, transbrachially and transvulvarly. Topical administration is accomplished via a topically applied cream, gel, rinse, etc. containing therapeutically effective amounts of inhibitors of serine proteases. Transdermal administration is accomplished by application of a cream, rinse, gel, etc. capable of allowing the inhibitors of serine proteases to penetrate the skin and enter the blood stream. In addition, osmotic pumps may be used for administration. The necessary dosage will vary with the particular condition being treated, method of administration and rate of clearance of the molecule from the body.

[0118] In each of the aforementioned compositions and methods, a compound having serine protease inhibitor activity and/or having α-l-antitrypsin activity or analog thereof may be used in a single therapeutic dose, acute manner or a chronic manner to treat episodes or prolonged bouts, respectively, in promoting graft survival, treating graft rejection and/or associated graft rejection-induced side-effects.

[0119] In certain embodiments of the methods of the present invention, the subject may be a mammal such as a human or a veterinary and/or a domesticated animal.

Therapeutic Methods

[0120] In one embodiment of the present invention, methods provide for treating a subject in need of or undergoing a transplant. For example, treatments for reducing graft rejection, promoting graft survival, and promoting prolonged graft function by administering to a subject in need thereof a therapeutically effective amount of a composition. The composition can include a compound capable of inhibiting at least one serine protease for example, α-l-antitrypsin, or analog thereof.

Preserving the Graft during Transplant before Engraftment

[0121] According to the methods of the present invention, transplantation complications can be reduced or inhibited to obtain important therapeutic benefits. Therefore, administration of a therapeutic composition contemplated by embodiments
ments of the invention, i.e., α1-antitrypsin, derivative or analog thereof, can be beneficial for the treatment of transplantation complications or conditions.

[0122] Another beneficial effect of use of the compositions and methods of the present invention include reducing negative effects on an organ or non-organ during explant, isolation, transport and/or prior to implantation. For example, the composition can reduce apoptosis, reduce production of cytokines, reduce production of NO, or combination thereof in an organ for transplant. In one particular embodiment, a composition can include a compound that includes α1-antitrypsin, an analog thereof, a native protease inhibitor, serine protease inhibitor-like activity, analog thereof or a combination thereof. The transplant organ or non-organ can include but is not limited to, lung, kidney, heart, liver, soft tissue, skin, pancreas, intestine, soft tissue corns, bone marrow, stem cell, pancreatic islet, and combination thereof.

[0123] In a further embodiment, the methods and compositions of the invention are useful in the therapeutic treatment of graft rejection associated side effects. In a yet further embodiment, graft rejection associated side effects can be prevented by the timely administration of the agent of the invention as a prophylactic, prior to onset of one or more symptoms, or one or more signs, or prior to onset of one or more severe symptoms or one or more signs of a graft rejection associated disease. Thus, a patient at risk for a particular graft rejection or graft rejection-associated disease or clinical indication can be treated with serine protease inhibitors, for example, (Benzyloxy carbonyl)-L-valyl-N-[1-(3-(5-(3-trifluoromethyl)benzyl)-2,4-oxadiazolyl) carbonyl]-2-(S)-methylpropyl-L-prolinamide; and (benzyloxy carbonyl)-L-valyl-N-[1-(3-(5-(2-methoxy benzyl)-1,2,4-oxadiazolyl) carbonyl]-2-(S)-methylpropyl]-L-prolinamide.

[0127] α1-antitrypsin is a glycoprotein of MW 51,000 with 417 amino acids and 3 oligosaccharide side chains. Human α1-antitrypsin is a single polypeptide chain with no internal disulfide bonds and only a single cysteine residue normally intermolecularly disulfide-linked to either cysteine or glutathione. The reactive site of α1-antitrypsin contains a methionine residue, which is labile to oxidation upon exposure to tobacco smoke or other oxidizing pollutants. Such oxidation reduces the elastase-inhibiting activity of α1-antitrypsin; therefore substitution of another amino acid at that position, i.e. alanine, valine, glycine, phenylalanine, arginine or lysine, produces a form of α1-antitrypsin which is more stable. α1-antitrypsin can be represented by the following formula: SEQ ID NO: 61: mpssvsvgall lagleclgyv selapeqdfm aauqtdshq dqfliptfklk tpanaeafs 60 lryqlaqhnsn stfispsv itatamfisl gktdhfliegfl nfnitltepeaqhi 120 qeqliriqsp dqslqgtg fllsleqgik vdklfewyf kyheasefntn fgdteedalqg 180 inlyveqgk gqkidflke drrdvlf yivfkkgl yevekudee dflh vdpvttv 240 kypmnkrlggm ffnqekckls swvlmmkygk nataifdpd eqgkhleqk lthaidflk 300 enedrsasl hplksqst gylkplvqsg ylglvse cpk lklkavkka 360 vlqtdekgk aagamflkpn qmsipveqf kqfplmvlf ngqactplng kvntqpfk 418

[0128] One important amino acid sequence near the carboxyterminal end of α1-antitrypsin is shown in bold and underlined and is pertinent to this invention (details of the sequence can be found for example in U.S. Pat. No. 5,470,970, as incorporated by reference).

[0129] Extrahapatic sites of AAT production include neutrophils, monocytes and macrophages, and the expression of AAT is inducible in response to LPS, TNFα, IL-1 and IL-6 in various cell types. Deficiency in AAT is associated with immune dysfunctional conditions such as rheumatoid arthritis and systemic lupus erythematosus.

[0130] Other serine protease inhibitor molecules, which may be used in any of the disclosed compositions may include compounds disclosed in the following: WO 98/20034 disclosing serine protease inhibitors from fleas; WO/98/23565 disclosing aminoguanidine and alkoxyguanidine compounds useful for inhibiting serine proteases; WO/98/50342 disclosing bis-aminomethylcarybol compounds useful for treating cysteine and serine protease disorders; WO98/50420 disclosing cyclopeptide and other amino acid derivatives useful for thrombin-related diseases; WO/97/21690 disclosing D-amino acid containing derivatives; WO/97/10231 disclosing ketomethylene group-containing inhibitors of serine and cysteine proteases; WO 97/05679 disclosing phosphorous containing inhibitors of cysteine and cysteine proteases; WO 98/21186 benzothiazole and related heterocyclic inhibitors of serine proteases; WO 98/22619 disclosing a combination of inhibitors binding to P site of serine proteases with chelating site of divalent cations; WO 98/22008 disclosing a composition which inhibits conversion of pro-enzyme CPP32 subfamily including caspase 3 (CPP32/Yama/Apopain); WO 97/47006 disclosing pyrrolo-
pyrazine-diones; and WO 97/33996 disclosing human placental bikunin (recombinant) as serine protease inhibitor.

[0131] Other compounds having serine protease inhibitory activity are equally suitable and effective for use in the methods of the present invention, including but not limited to: tetrazole derivatives as disclosed in WO 97/24339; guanidinobenzonic acid derivatives as disclosed in WO 97/37969 and in a number of U.S. Pat. Nos. 4,283,418; 4,843,094; 4,310,533; 4,283,418; 4,224,342; 4,021,472; 5,376,655; 5,247,084; and 5,077,428; phenylsulfonamide derivatives represented by general formula in WO 97/45402; novel sulfide, sulfoxide and sulfone derivatives represented by general formula in WO 97/49679; novel amidino derivatives represented by general formula in WO 99/41231; other amidinophenol derivatives as disclosed in U.S. Pat. Nos. 5,432,178; 5,622,984; 5,614,555; 5,514,713; 5,110,602; 5,004,612; and 4,889,723 among many others.

Graft Rejection and Graft Survival-Side-Effects and Conditions

[0132] One of the beneficial effects of use of the compositions and methods of the present invention include, for example, and not by way of limitation, reduced infiltration of graft with cells or serum factors (including but not limited to, complement, anti graft antibody that generate inflammation and graft rejection), reduced cytokines, reduced nitric oxide, reduced apoptosis, and reduced specific immune response against the graft or any combination thereof.

Management of Graft Rejection

[0133] By preventing or reducing the side effects or conditions associated with graft survival or graft rejection using this novel approach, several advantages are obtained compared to alternative approaches, for example, and not by way of limitation:

[0134] 1. Reduced infiltration of graft with cells or serum factors (for example, and not by way of limitation, complement, anti graft antibody that generate inflammation and graft rejection); reduced production of cytokines or nitric oxide (NO) that can induce inflammation or apoptosis; inhibits apoptosis; inhibits immune activation, inhibits CMV or any combination thereof.

[0135] 2. Synthetic inhibitors of serine proteases (AAT-like mimics or analogs) can and have been developed by means known in the art. Such a pharmaceutical agent may be formulated as for example, a cream to treat graft rejection and/or promote graft survival.

[0136] 3. Commercially available agents already approved for different use in humans will work as a treatment for graft rejection and/or promote graft survival. These agents are currently used for indications other than graft rejection and/or to promote graft survival, and include injectable AAT, plasma preparations, aprotinin and others (American J. of Resp Critical Care Med 1998, VII 158: 49-59, incorporated herein by reference in its entirety). In one embodiment, serine protease inhibitors may be delivered by inhalation. An inhaled agent (natural AAT or a synthetic AAT-like mimic/or other inhibitor of serine protease) may be especially useful due to elevated local concentrations, ease of drug delivery, and lack of side effects (since administration is not systemic). This mode of focused drug delivery may augment serine protease inhibitor activity within the lung tissues and associated lymphatics, which are two of the principal sites where diseases and/or clinical conditions associated with graft rejection and/or promotion of graft survival develop.

[0137] 4. By promoting graft survival and/or treating graft rejection, the direct cause of the side effect is disrupted in affected individuals. This invention specifically contemplates inhibiting host cell serine proteases or induce the SEC receptor or combination thereof as a method of treating graft rejection and/or promoting graft survival in a mammal in need thereof in conjunction with administration of one or more anti-rejection and/or anti-microbial.

[0138] 5. There is an extensive clinical experience using injectable AAT to treat patients with genetic AAT deficiency. No long-term negative effects have been detected to date (American J. of Resp Critical Care Med 1998, VII 158: 49-59; Weneker et al. Chest 2001 119:737-744). Moreover, a small molecule inhibitor of host serine protease has been administered to patients with Kawasaki’s Disease (Ulinstatin, Ono Pharmaceuticals).

Isolated Proteins For Use In The Compositions And Methods Of The Invention

[0139] One aspect of the invention pertains to proteins, and portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise antibodies directed against a polypeptide of the invention. In one embodiment, the native polypeptide can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, polypeptides of the invention are produced by recombinant DNA techniques. Alternative to recombinant expression, a polypeptide of the invention can be synthesized chemically using standard peptide synthesis techniques.

[0140] Recombinant unmodified and mutant variants of alpha.sub.1-antitrypsin produced by genetic engineering methods are also known (see U.S. Pat. No. 4,711,848). The nucleotide sequence of human alpha.sub.1-antitrypsin and other human alpha.sub.1-antitrypsin variants has been disclosed in international published application No. WO 86/00,337, the entire contents of which are incorporated herein by reference. This nucleotide sequence may be used as starting material to generate all of the AAT amino acid variants and amino acid fragments depicted herein, using recombinant DNA techniques and methods known to those of skill in the art.

[0141] An isolated and/or purified or partially purified protein or biologically active portion thereof may be used in any embodiment of the invention. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein. When the protein or biologically active portion thereof is recombinantly produced, it can also be substantially free of culture medium. When the protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals. Accordingly, such preparations of the protein have less than about 30%, 20%, 10%, and 5% (by dry weight) of chemical precursors or compounds other than the polypeptide of interest.

[0142] Biologically active portions of a polypeptide of the invention include polypeptides including amino acid sequences sufficiently identical to or derived from the amino acid sequence of the protein (e.g., the amino acid sequence shown in any of SEQ ID Nos: 1 to 60, which exhibit at least one activity of the corresponding full-length protein). A biologically active portion of a protein of the invention can be a polypeptide; which is, for example, 5, 10, 25, 50, 100 or more
amino acids in length. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of the native form of a polypeptide of the invention.

[0144] Preferred polypeptides have the amino acid sequence of SEQ ID Nos: 1 to 60. Other useful proteins are substantially identical (e.g., at least about 45%, preferably 55%, 65%, 75%, 85%, 95%, or 99%) to any of SEQ ID NOS: 1 to 60, and retain the functional activity of the protein of the corresponding naturally-occurring protein yet differ in amino acid sequence due to natural allelic variation or mutagenesis.

[0145] The compounds of the present invention can be used as therapeutic agents in the treatment of a physiological (especially pathological) condition caused in whole or part, by excessive serine protease activity. In addition, a physiological (especially pathological) condition can be inhibited in whole or part. Peptides contemplated herein may be administered as free peptides or pharmaceutically acceptable salts thereof. The polypeptides should be administered to individuals as a pharmaceutical composition, which, in most cases, will include the polypeptide and/or pharmaceutically acceptable carrier.

[0146] When utilizing BLAST, Gapped BLAST, and PSI-BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

[0147] The present invention also pertains to variants of the polypeptides of the invention. Such variants have an altered amino acid sequence which can function as either agonists (mimetics) or as antagonists. Variants can be generated by mutagenesis, e.g., discrete point mutation or truncation. An agonist can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the protein. An antagonist of a protein can inhibit one or more of the activities of the naturally occurring form of the protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the protein of interest. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the protein.

[0148] Variants of a protein of the invention which function as either agonists (mimetics) or as antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the protein of the invention for agonist or antagonist activity.

Fusion Polypeptides

[0149] In other embodiments, compounds having serine protease inhibitor activity such as α1-antitrypsin and/or analog thereof, may be part of a fusion polypeptide. In one example, a fusion polypeptide may include α1-antitrypsin (e.g., mammalian α1-antitrypsin) or an analog thereof and a different amino acid sequence that may be heterologous to the α1-antitrypsin or analog substance.

[0150] In one embodiment, fusion polypeptides of the invention are produced by recombinant DNA techniques. Alternative to recombinant expression, a fusion polypeptide of the invention can be synthesized chemically using standard peptide synthesis techniques. The present invention also provides compositions that comprise a fusion polypeptide of the invention and a pharmaceutically acceptable carrier, excipient or diluent.

[0151] In particular, in one embodiment the fusion protein comprises a heterologous sequence that is a sequence derived from a member of the immunoglobulin protein family, for example, comprise an immunoglobulin constant region, e.g., a human immunoglobulin constant region such as a human IgG1 constant region. The fusion protein can, for example, include a portion of α1-antitrypsin, analog thereof or inhibitor of serine protease activity polypeptide fused with the amino-terminus or the carboxyl-terminus of an immunoglobulin constant region, as disclosed, e.g., in U.S. Pat. No. 5,714,147, and U.S. Pat. No. 5,116,964. In accordance with these embodiments, the FeR region of the immunoglobulin may be either wild-type or mutated. In certain embodiments, it is desirable to utilize an immunoglobulin fusion protein that does not interact with an Fe receptor and does not initiate ADCC reactions. In such instances, the immunoglobulin heterologous sequence of the fusion protein can be mutated to inhibit such reactions. See, e.g., U.S. Pat. No. 5,985,279 and WO 98/06248.

[0152] In yet another embodiment, α1-antitrypsin, analog thereof, or inhibitor of serine protease activity polypeptide fusion protein comprises a GST fusion protein in which is fused to the C-terminus of GST sequences. Fusion expression vectors and purification and detection means are known in the art.

[0153] Expression vectors can routinely be designed for expression of a fusion polypeptide of the invention in prokaryotic (e.g., E. coli) or eukaryotic cells (e.g., insect cells (using baculovirus expression vectors), yeast cells or mammalian cells)) by means known in the art.

[0154] Expression of proteins in prokaryotes may be carried out by means known in the art. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification.

[0155] In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector as described in the art. In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferably in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid) such as pancreas-specific promoters (Edlund et al. (1985) Science 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). A host cell can be any prokaryotic (e.g., E. coli) or eukaryotic cell (e.g., insect cells, yeast or mammalian cells). Vector DNA can be introduced
into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques.

Combination Therapies

[0156] In each of the aforementioned methods of the present invention, the use of a compound capable of inhibiting serine protease or α1-antitrypsin or analog thereof alone or in combination with standard immunosuppressive agents enables transplantation of grafts into immunosuppressed or immunocompromised recipients. This combination therapy will expand the eligible patient population able to receive this form of treatment.

[0157] In each of the aforementioned aspects and embodiments of the invention, combination therapies other than those already enumerated above are also specifically contemplated herein. In particular, the compositions of the present invention may be administered with one or more macrolide or non-macrolide antibiotics, anti-bacterial agents, anti-fungals, anti-viral agents, and anti-parasitic agents. Examples of macrolide antibiotics that may be used in combination with the composition of the present invention include but are not limited to clarithromycin, azithromycin, and roxithromycin. Examples of preferred anti-bacterial agents include, but are not limited to, penicillin, quinolones, aminoglycosides, vancomycin, monobactams, cephalosporins, carbacephem, cephamycins, carbapenems, and monobactams and their various salts, acids, bases, and other derivatives.

[0158] Examples of anti-fungal agents include, but are not limited to, caspofungin, terbinafine hydrochloride, nystatin, and selenium sulfide.

[0159] Anti-viral agents include, but are not limited to, gancyclovir, acyclovir, valacyclovir, amantadine hydrochloride, rimantadine and ecodoxine.

[0160] Examples of macrolide antibiotics that may be used in combination with the composition of the present invention include but are not limited to clarithromycin, azithromycin, and roxithromycin. Examples of preferred anti-bacterial agents include, but are not limited to, penicillin, quinolones, aminoglycosides, vancomycin, monobactams, cephalosporins, carbacephem, cephamycins, carbapenems, and monobactams and their various salts, acids, bases, and other derivatives.

[0161] Anti-fungal agents include, but are not limited to, caspofungin, terbinafine hydrochloride, nystatin, and selenium sulfide.

[0162] Anti-viral agents include, but are not limited to, gancyclovir, acyclovir, valacyclovir, amantadine hydrochloride, rimantadine, and ecodoxine.

[0163] In another aspect, in the method of the present invention, one may, for example, supplement the composition by administration of a therapeutically effective amount of one or more an anti-inflammatory or immunomodulatory drugs or agents. By ‘anti-inflammatory drugs’, it is meant, e.g., agents which treat inflammatory responses, i.e., a tissue reaction to injury, e.g., agents which treat the immune, vascular, or lymphatic systems.

[0164] Anti-inflammatory or immunomodulatory drugs or agents suitable for use in this invention include, but are not limited to, interferon derivatives, (e.g., betaseron); prostanoid derivatives, (e.g., compounds disclosed in PCT/DE93/0013, iloprost, cortisol, dexamethasone; immuno-suppressives, (e.g., cyclosporine A, FK-506 (mofetil); lipooxygenase inhibitors, (e.g., zileutone, MK-886, WY-50295); leukotriene antagonists, (e.g., compounds disclosed in DE 40091171 German patent application P 42 42 390.2); and analogs; peptide derivatives, (e.g., ACTH and analogs); soluble TNF-receptors; TNF-antibodies; soluble receptors of interleukins, other cytokines, T-cell-proteins; antibodies against receptors of interleukins, other cytokines, and T-cell-proteins.

Kits

[0165] In still further embodiments, the present invention concerns kits for use with the methods described above. Small molecules, proteins or peptides may be employed for use in any of the disclosed methods. In addition, other agents such as anti-bacterial agents, immunosuppressive agents, anti-inflammatory agents may be provided in the kit. The kits will thus can include, in suitable container means, a protein or a peptide or analog agent, and optionally one or more additional agents.

[0166] The kits may further include a suitably aliquoted composition of the encoded protein or polypeptide antigen, whether labeled or unlabeled, as may be used to prepare a standard curve for a detection assay.

[0167] The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the antibody or antigen may be placed, and preferably, suitably aliquoted. Where a second or third binding ligand or additional component is provided, the kit will also generally contain a second, third or other additional container into which this ligand or component may be placed. The kits of the present invention will also typically include a means for containing the antibody, antigen, 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.

EXAMPLES

[0168] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated that those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors 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

Alpha-1-Antitrypsin Prolongs Islet Graft Survival in Mice

[0169] FIG. 1A-1D. Islets from DBA/2 mice (H-2d) were transplanted under the renal capsule of streptozotocin-induced hyperglycemic C57BL/6 mice (H-2b). (A) Glucose levels from days 6–18. Control consists of mice that were untreated (n=3) or treated from day –1 every 3 days with human albumin (ALB, 6 mg, n=3). Prolonged islet graft survival is observed in mice treated from day-1 every 3 days with human AAT (2 mg, n=10). *P<0.05, **P<0.01, ***P<0.001
between glucose levels on same day. (B) Treatment protocols. Control and full AAT treatment are described in panel A. Early AAT treatment consists of treatment on days -1, 1 and 3 (2 mg, n=3). Late AAT treatment consists of treatment from day 2 and on every 2 days (2 mg, n=3). Rejection indicates the day that glucose levels exceed 300 mg/dL. (C) Effect of mouse anti-human-AAT antibodies. Dashed line indicates post transplantation glucose levels of a mouse under full AAT treatment protocol (see A, B) that was immunized by multiple administrations of human AAT prior to transplantation (1 representative, n=3). Solid line indicates glucose levels of a non-immunized mouse treated under full AAT treatment protocol (1 representative, n=10). Arrow indicates detection of treatment-induced, anti-human-AAT antibodies in the non-immunized representative mouse. (D) Comparison of day 15 post-transplantation glucose levels in mice that were under full treatment protocol with ALB (n=3) or AAT (non-immunized n=10, immunized n=3). Of the AAT-treated group, antibodies were detected on day 15 in 3/5 immunized mice and in 6/10 non-immunized mice. ** P=0.005 between mice that produced antibodies (n=6) and mice that did not produce antibodies (n=4).

[0170] Treatment with human albumin (6 mg) resulted in graft rejection comparable to that of untreated recipient mice. In contrast, recipient mice that received AAT (2 mg) exhibited prolonged graft function. As depicted in FIG. 10, neither of the partial treatment protocols, i.e., days -1, 1 and 3 (‘early treatment’) or days 2 and beyond (‘late treatment’) prolonged allograft survival.

[0171] AAT-treated mice developed anti-human-AAT antibodies (FIGS. 1C and D). Individual mice exhibited anti-human-AAT antibodies at various time points (data not shown). To ascertain that the antibodies reduce the protective effect of AAT, a group of mice was pre-exposed (“immunized”) to human AAT two months before being rendered hyperglycemic and transplanted with allogeneic islets. These graft recipients were treated with the full AAT protocol, despite exhibiting high titers of specific antibodies before engraftment, and displayed rapid graft rejection (FIG. 1C). Day 15 was chosen to depict an association between antibody formation and loss of AAT protective activity; at this time point AAT-treated mice were divided into positive and negative producers of anti-human-AAT antibodies. As shown in FIG. 1D, on day 15 all antibody-positive mice were hyperglycemic and all antibody-negative mice were normoglycemic.

Example 2

[0172] FIG. 2A-2D illustrates an exemplary method of the effect of AAT on thioglycolate-elicted peritoneal cellular infiltrates. Mice were administered intraperitoneal 0.1 ml saline, ALB, AAT or oxidized-AAT followed by 1 ml of saline or thioglycolate (ThG, 3% w/v, n=3 per group). Peritoneal lavage was performed on separate groups after 24 and 48 hours. (A) Total cell population of lavaged cells (open bars) saline or (closed bars) AAT-treated (5 mg) thioglycolate-injected mice. ** P<0.05. (B) Percent cell population from saline-treated mice at 48 hours. ** P<0.05. (C) Oxidation of AAT. AAT was subjected to oxidative radicals (see Methods). Loss of serine protease activity of oxidized AAT was assessed in an elastase assay. Activity of elastase in the absence of native AAT was set at 100% and the percentage of activity in the presence of native and oxidized AAT was calculated (n=3). *** P<0.001. In FIG. 2D, elicited macrophages and neutrophils are identified. Peritoneal infiltrates from 48 hour lavages of ALB (6 mg) and AAT-treated (6 mg), thioglycolate-injected mice were stained for FACS analysis by specific antibodies. Macrophages and neutrophils were identified on the basis of F4/80 and GR1 versus side scatter flow cytometry profiles. Top, FACS analysis representative graphs (n=3). Quantified FACS results (n=3) are depicted in the bottom.

AAT Inhibits Cellular Infiltration

[0173] To address the possibility that AAT affects effector cell infiltration, two models of cell emigration were examined: thioglycolate (ThG)-elicited peritoneal infiltration, and cellular infiltration due to intraperitoneal injection of MHC-incompatible fibroblasts.

[0174] As shown in FIG. 2A, there was a progressive increase in total cell count at 24 and 48 hours in mice injected with ThG, whereas no significant increase was observed in mice injected with AAT and ThG. At 48 hours, total cell count in peritoneal lavage of AAT-treated mice was 50% of that of control (FIG. 2B). Total cell count in mice that received albumin control was similar to that of saline-treated mice. There was a dose-dependent effect of AAT in that one-sixth the dose was found to reduce cell count to a lesser extent in a significant manner. Oxidized AAT, which had lost its in vitro anti-elastase activity (FIG. 2C), did not affect cellular infiltrate at 1 mg (FIG. 2B).

[0175] The decrease in total cell count is primarily attributed to a decrease in the number of neutrophils (FIG. 2D), identified by their GR-1high/intermediate side-scatter (SSC) profile. No major difference was observed with the infiltration of macrophages, identified by their F4/80int, GR-1int, intermediate SSC profile, which is distinct from the F4/80 very high, GR-1low, high SSC profile of resident macrophages (data not shown).

Example 3

[0176] FIG. 3A-3C illustrates an exemplary method of the effect of AAT on MHC-incompatible, NIH-3T3-fibroblast elicited peritoneal cellular infiltrates. Mice (C57BL/6; H-2b) were injected i.p. 0.1 ml saline or AAT (1 mg) followed by 1 ml NIH-3T3 cells (1*107 cells in saline; H-2d). Peritoneal lavage was performed daily on days 1-5 and cell subpopulations were identified by FACS analysis. (n=3 per treatment). (A) Cell numbers. The number of cells in each subpopulation was calculated from the percentages obtained by FACS analysis, and total number of cells in the infiltrate * P<0.05, ** P<0.01 between cell numbers on the same day. (B) Representative FACS analysis. (C) Effect of AAT on intensity and function of infiltrate elicited by islet allograft. Left, Hematoxilin and Eosin (H&E) staining of day 7 islet allografts. A section of AAT-treated islet graft (white frame) is compared to a similar section of ALB-treated diabetic recipient mouse (full treatment protocol, see FIG. 1A). Arrow points at border between islet and surrounding infiltrate. Right, Immunohistochemistry (IHC) with anti-insulin antibodies of day 15 islet grafts. A section of autologous islet graft (white frame) is compared to similar sections of allografts of AAT- and ALB-treated recipient mice. R, renal parenchyma; G, graft; C, renal capsule.

[0177] As illustrated in FIG. 3A, introduction of allogeneic cells evoked a cellular infiltrate that consisted of early appearing neutrophils and activated macrophages, and late appear-
ing CD3+ and NK cells (FIG. 3B). AAT-treated mice exhibited a reduction in neutrophils, CD3+ and NK cells, dark color is insulin staining.

**Example 4**

**[0179]** FIG. 4A-4H illustrates an exemplary method of the effect of AAT on islet responses. (A-D) Islets from C57BL/6 mice were cultured at 100 islets/well in duplicate. AAT was incubated at the indicated concentrations for 1 hour before the addition of IFNγ (5 ng/ml) plus IL-1β (10 ng/ml). 72 hours later, supernatants were collected and islet viability was assessed. Islet cell responses in the absence of AAT were set at 100%. Data are combined from 3 individual experiments, in duplicate. **P<0.01**, ***P<0.001** between AAT-treated and untreated islets. Mean±SEM of a. nitrite levels, b. Cell viability and c. MIP-1α levels. Dashed line represents islets incubated at one-third the concentration of IFNγ/IL-1β. d. TNFα levels. (E) Insulin induction assay. Islets were incubated in triplicate (20 islets/well) in the presence of AAT (0.5 mg/ml) or AB 1B (0.5 mg/ml) 1 hour before addition of IFNγ (5 mg/ml) plus IL-1β (10 ng/ml). 24 hours later, islets were transferred to a 3 mM or 20 mM glucose solution for 30 minutes and insulin levels were measured. Vertical axis depicts the ratio between insulin levels at both glucose concentrations. * P<0.05 between AAT-treated and AB-treated islets. (F) Streptozotocin toxicity. C57BL/6 mice were injected i.p. with AAT (5 mg) or saline, one day before, on same day and one day after injection of streptozotocin (225 mg/kg) or saline (n=3 per group). 48 hours later, pancreata were removed and insulin-containing cells were identified by immunohistochemistry. Each image depicts a representative islet from one pancreas. Graph, mean±SEM percent change of insulin-containing cells as determined manually from images of 2 islets per pancreas (n=6 per treatment group). * P<0.05. (G) Cellular content of islets. Freshly isolated islets (100 islets in triplicate) and residual non-islet pancreatic debris were dissociated into single cell suspensions and stained for FACS analysis with anti-CD45-APC or isotype control antibody. Shaded area, islets. Open area, debris. (H) MHC class II expression. Islets from C57BL/6 mice were cultured (100 islets/well in duplicate) in the presence of AAT (0.5 mg/ml) 1 hour before the addition of IFNγ (5 ng/ml) plus IL-1β (10 ng/ml). 24 hours later, islets were dissociated into single cell suspensions and double-stained for FACS analysis with anti-CD45-APC and anti-MHCII-PE, or isotype control antibodies. Left, Mean±SEM percent change from control (CT) unstimulated islets. * P<0.05 between AAT-treated and untreated islets. Right, Representative FACS analysis; Shaded area, AAT-treated islets. Open area, stimulated islets. Events are gated for CD45+.

AAT Modifies Islet Response to Proinflammatory Mediators

**[0180]** Various islet responses to IL-1β/IFNγ were examined in vitro. Islets exposed to IL-1β/IFNγ for 72 hours produce nitric oxide (NO) in a concentration-dependent manner and exhibit NO-dependent loss of viability. As shown in FIGS. 4A and B, in the presence of AAT, less NO was produced and greater islet viability was obtained. The production of MIP-1α was decreased in the presence of AAT, particularly when stimulated by low concentrations of IL-1β/IFNγ (FIG. 4C). Notably, TNFα level in supernatants was markedly diminished by AAT (FIG. 4D). Insulin induction was inhibited by IL-1β/IFNγ, but was intact in the presence of IL-1β/IFNγ plus AAT (FIG. 4E). To test the effect of AAT on islets in vivo, STZ toxicity was evaluated. AAT (2 mg) was administered one day before, on the same day and a day after STZ injection. Immunohistochemistry of pancreata with anti-insulin antibodies at 48 hours after STZ injection reveals more insulin-producing cells in islets of AAT- than ALB-treated mice (26.3±2.6 and 12.8±2.3 insulin-producing cells per islet, respectively, FIG. 4F). White cell content of freshly isolated islets was evaluated by FACS analysis. Islets contain CD45+ cells (FIG. 4G) that are also positive for the monocyte/granulocytic markers GR1 and F4/80 (data not shown). This cell population responded to AAT with decreased surface MHC class II (FIG. 4H).

**Example 5**

**[0181]** FIG. 5A-5D illustrates the effect of AAT on TNF-α. (A) Islets from C57BL/6 mice were cultured (100 islets/well in triplicate) in the presence of AAT (0.5 mg/ml) or TACE inhibitor (10 mM) 1 hour before stimulation by IFNγ (5 ng/ml) plus IL-1β (10 ng/ml). Left, mean±SEM change in TNFα in supernatants after 72 hours of incubation. Right, mean SEM fold change in membrane TNFα on islet cells after 5 hours of incubation, according to FACS analysis. ***P<0.001*** compared control (CT) levels in the absence of AAT. (B) Representative FACS analysis of membrane TNFα on stimulated islet cells in the absence (open area) or presence (shaded area) of AAT. Events are gated for CD45+. (C) Streptozotocin-induced hyperglycemia. C57BL/6 mice were injected i.p. with saline (n=3), AAT (5 mg, n=3) or TNFα (1 mg/kg, n=3) or administered p.o. with TACE inhibitor (TACEi, 60 mg/kg, n=6) one day before injection of STZ (225 mg/kg, i.p.). Subsequently, AAT and TNFα were injected daily; TACE inhibitor was administered twice a day. At 48 hours, mean±SEM glucose levels are compared to those of normal littermates (n=3). * P<0.05, **P<0.01 compared to saline-treated, STZ-injected mice.

AAT Inhibits Release of Membrane TNFα

**[0182]** Proteolytic cleavage of membrane TNFα releases soluble TNFα from activated cells by the action of TNFα-converting-enzyme (TACE). The inventors examined the levels of membrane TNFα on stimulated islets in the presence of AAT. The effect of AAT was compared to that of a TACE inhibitor. Both AAT and TACE inhibitor decreased TNFα levels in supernatants of islets exposed to IL-1β/IFNγ (FIG. 5A, left). Under these conditions, membrane TNFα accumulated on the cell surface of CD45+ islet cells (FIG. 5A, right).
To assess the possibility that islet protection occurs via inhibition of release of membrane TNFα in vivo, TACE inhibitor, p75 TNF receptor (TNF BP) or AAT were introduced to mice prior to STZ injection. Although all mice developed hyperglycemia after day 4, the progression of β-cell toxicity was significantly affected by treatments. As shown in FIG. 5C, the effect of STZ at 48 hours was decreased in the presence of AAT (a decrease of 23.2%±2.3 in fasting glucose levels compared to STZ/saline injected mice). The effect of TACE inhibitor and p75 TNF receptor was not as profound. Similarly, TACE inhibitor prolonged islet graft survival to a lesser extent than AAT (preliminary data not shown).

Splenocytes that were harvested 48 hours after ThG injection produced TNFα in culture (FIG. 5D). AAT administered prior to thioglycolate decreased TNFα release from cultured splenocytes. A similar trend was found with IFNγ (data not shown), signifying that the response to ThG had effects that extend beyond the peritoneal compartment and that pretreatment with AAT reduced these effects.

Example 6

FIG. 6A-6D illustrates the effect of AAT on islet allograft transplantation. 6A illustrates the time course study after transplantation of islet cells. This example indicates that treated mice maintain normoglycemia over a 60 day period (n=4), after the AAT therapy was withdrawn. After withdrawal of the therapy, the normoglycemia lasted another 20 days. 6A illustrates the glucose follow-up. Positive insulin staining in a day-85 treated islet graft was also demonstrated (data not shown). 6B illustrates an immune infiltrate found outside the graft area. 6C illustrates an increase in the presence of CD4+ and a comparative decrease in monocytes and neutrophils. It was also shown that massive vascularization was evident inside the graft (data not shown). It has been observed that long-lasting accepted islet grafts can be spared an immune allorejection even after therapy removal, whether the therapy had evoked an immune tolerance specific for the strain of donor islets was evaluated. For this, grafts were explanted by nephrectomy and the new-hyperglycemic original recipients were re-transplanted with either the same strain of islets as before (n=2), or a 3rd strain which they had never encountered before (n=2). In accordance with established strain specific immune tolerance, mice accepted grafts from original donors, but had acutely rejected 3rd-strain grafts (6D); the same donor (left) and a 3rd donor re-graft (right).

Example 7

FIG. 7A-7E illustrates the production of AAT by islet cell and reflection of islet graft survival. 7A illustrates a time course expression of mouse AAT mRNA after cytokine production (IL-1β and IFNγ) (left) and at 8 hours (right). To demonstrate the relevance of endogenous alpha-1-antitrypsin in physiological conditions, the issue of islet injury during pancreatitis was addressed. In mouse model of acute pancreatitis, isolated islets of pancreata that are inflamed express inducible alpha-1-antitrypsin. 7B illustrates an example of islet injury during pancreatitis; the histology of normal islets (top left), the histology of islets of an inflamed pancreas (top right) and expression of mouse AAT in islets obtained from the pancreas in an acute pancreatitis model (bottom). Alpha-1-antitrypsin levels during pancreatitis (caerulein model for acute pancreatitis). Top, histology of an islet in a normal pancreas (left) and an islet in an inflamed pancreas (right), representative of n=3. Bottom, expression of mouse alpha-1-antitrypsin in islets obtained from pancreata in acute pancreatitis model. Treatment of mice with exogenous alpha-1-antitrypsin resulted in down-regulation of endogenous alpha-1-antitrypsin expression, as well as decrease in serum TNFα levels (not shown).

To demonstrate the relevance of endogenous alpha-1-antitrypsin in islet transplantation, islet allografts from untreated transplanted mice on days 1 through 7 after transplantation (n=3) were examined. These were examined for alpha-1-antitrypsin expression and reveal a pattern which may fit inflammation phase (days 1-3) followed by loss of islet mass (days 4-7). 7C illustrates an example of samples of islet allografts taken post grafting and percent change in AAT mRNA levels were also assessed. Total RNA was extracted and mRNA for alpha-1-antitrypsin evaluated by RT-PCR.

Islet protection from cytokine injury was examined using endogenous alpha-1-antitrypsin by introducing oncostatin M, a member of IL-6 family that induces alpha-1-antitrypsin expression in islets without causing islet death. After 4 days that human islets were incubated with oncostatin M, for the purpose of accumulation of sufficient alpha-1-antitrypsin for the protection of islet allografts from cytokines. 7D illustrates an example of islet protection from cytokine injury with endogenous AAT by introducing oncostatin M (an interleukin 6 (IL-6) family member) which inhibits AAT expression in islets, oncostatin M and AAT levels (top left); nitric oxide and viability levels assessed (top right). Bottom, human islets exposed to oncostatin M for 4 days produce enough alpha-1-antitrypsin to diminish the effects of IL-1β/IFNγ added for an additional 48 hours.

Example 8

In one exemplary study, alpha-1-antitrypsin on human islets was examined. FIG. 8A-8D illustrates the effect of AAT on human islets. The production of nitric oxide (SA), TNF-α production (SB) IL-6 (SC) and IL-8 (SD) was examined. 100 human islets per well were seeded in triplicates and added alpha-1-antitrypsin (AAT) 2 hours before stimuli. Supernatants were assayed 72 hours later. 3A, nitric oxide; 3B, TNFα; 3C, IL-6; 3D, IL-8. Results are mean±SEM and are representative of separate islet isolations from three human donors.

Methods

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Animal Cell Culture, R. J. Freshney, ed., 1986).

Mice. C57BL/6 and DBA/2 females were purchased from Jackson Laboratories.

Induction of hyperglycemia by streptozotocin, islet isolation and islet transplantation. In one exemplary method, 5-6-weeks-old C57BL/6 mice were treated intraperitoneally (i.p.) with 225 mg/kg Streptozotocin (STZ) (Sigma). Mice
with established hyperglycemia were used at least 5 days after STZ administration. Islets were isolated from DBA/2 mice on day of transplantation, or 24 hours before in vitro assays, by enzymatic digestion of pancreata, by means known in the art, with minor modifications. Briefly, mice were anesthetized with i.p. ketamine (50 mg/kg, Vedco Inc.) and xylazine (10 mg/kg, Vedco Inc.). Each pancreas was inflated with 3.5 ml cold collagenase (1 mg/ml, type XI, Sigma), excised and immersed for 40 minutes at 37°C in water bath. Pancreata were gently vortexed and filtered through 500-micron metal sieve. The pellet was washed twice in cold HBSS containing 0.5% BSA (Sigma) and reconstituted in RPMI-1640 (Cellgro, Mediatech) supplemented with 10% FCS (Cellgro), 50 IU/ml Penicillin (Cellgro) and 50 μg/ml streptomycin (Cellgro). Islets were collected on a 100-micron nylon cell strainer (BD Falcon), released into a petri dish by rinsing with HBSS (Cellgro, Mediatech) and 0.5% BSA (Sigma) and hand picked under a stereomicroscope. For transplantation, 450 islets were thoroughly washed from residual FCS in HBSS and 0.5% BSA and mounted on 0.2 ml tip for immediate transplantation. For in vitro assays islets were left to incubate for 24 hours at 37°C. Islet transplantation was performed into the left renal subcapsular space. Recipient mice were anesthetized, as described above. An abdominal wall incision was made over the left kidney. Islets were released into the subcapsular space through a puncture and the opening was sealed by means known in the art. Blood glucose follow-up was performed 3 times a week from end-tail blood drop using glucosticks (Roche). (Nañji, S. A. & Shapiro, A. M. Islet transplantation in patients with diabetes mellitus: choice of immunosuppression. BioDrugs 18, 315-28 (2004).)

[0193] Development of anti-human-AAT antibodies in mice. In another exemplary method, in order to evoke specific antibody production against human AAT, mice were injected i.p. with 10 mg human AAT per 20-gram mouse for four times in intervals of 1 week. Mice were used in experiments 2 months after last administration. Antibody production was evaluated before transplantation experiments were carried out.

[0194] In one example, assaying for anti-human-AAT antibody levels was performed as described in the art. Briefly, mouse sera were kept at -70°C until assayed for anti-human-AAT levels. Plates were coated with human AAT or albumin (2 μg/ml) in PBS at 4°C overnight, then washed and blocked for 1 hour at 25°C as described. Negative control serum was used in addition to test serum. Bound anti-AAT antibody using standard TMB substrate solution was measured (Sigma).

[0195] Cells. NIH-3T3 cell line (e.g. ATCC) were cultured. On day of peritoneal inoculation, 1x10^6 cells were freshly collected by trypsinization and washed with cold PBS. Pellet was resuspended in 1 ml cold PBS for immediate injection.

[0196] Infiltration experiments. Peritoneal infiltration was elicited by i.p. injection of 1 ml autoclaved thioglycollate (5% w/v, Sigma) or allogeneic cells (NIH-3T3), together with 0.1 ml saline, human albumin, human AAT or oxidized AAT. Peritoneal lavage was performed at 24 and 48 hours (thioglycollate) or on days 1-5 (allogeneic cells). For lavage, mice were anesthetized by isoflurane inhalation and injected immediately with 5.5 ml cold PBS containing 5% FCS and 5 U/ml heparin into the peritoneal cavity. After massaging the abdomen, peritoneal fluid was recovered. Red blood cells were lysed (RBC lysing buffer, BD PharMingen) and cell counts were performed with a hemocytometer. Cells were then isolated. Cells (about 1x10^9/polypropylene vial) were incubated with FcγRII/II receptor block antibodies (Table 1) for 10 min. Cells were then divided into two groups and incubated with mAbs for leukocytes and either CD3/NK cells or neutrophil/macrophages (Table 1) for 30 min. Cells were washed and fixed. The number of cells expressing a particular marker was calculated by multiplying percentages obtained from flow-cytometry by the concentration of cells in lavage fluid.

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tbody>
<tr>
<td><strong>Rat Anti-Mouse mAb Used for Flow Cytometry</strong></td>
</tr>
<tr>
<td><strong>Purpose</strong></td>
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<tr>
<td>Blocking</td>
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<tr>
<td>Leukocytes</td>
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<tr>
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<td>MHC class II</td>
</tr>
<tr>
<td>Isotype control</td>
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</table>

[0197] An insulin assay and immunohistochemistry was performed by means known in the art (Nañji, S. A. & Shapiro, A. M. Islet transplantation in patients with diabetes mellitus: choice of immunosuppression. BioDrugs 18, 315-28 (2004)).

[0198] AAT oxidation by myeloperoxidase (MPO) system. In one example, AAT (4 mg/ml) was incubated at 37°C for 45 minutes with MPO (1 U/ml, Sigma), H2O2 (80 μM, Sigma) and NaCl (2.5 mM) in PBS, pH 7.4, by means known in the art. Reaction was terminated by boiling for 1 hour followed by filter-centrifugation of the system products. In this example, boiling was needed for the inactivation of MPO but this did not inactivate AAT (data not shown). Loss of activity of oxidized AAT was confirmed by elastase activity assay.

[0199] Elastase activity assay. In another exemplary method, inhibition of a the serine protease elastase was evaluated 30 minutes after co-incubation of AAT or oxidized AAT with porcine elastase (Sigma) in triplicate, by known methods. The ability of elastase to liberate 4-nitroaniline (A340) from SucAla-Pro-PNA was determined by kinetic measurement of light absorbance at 410 nm. Activity in the absence of inhibitors was set as 100% at the linear range of the assay.

[0200] Cytokine assays. An electrochemiluminescence (ECL) assay as known in the art was used for the measure-
ment of mouse TNFα and MIP-1α. Briefly, cytokine-specific goat anti-mouse affinity purified antibodies were labeled with ruthenium (e.g. BioVeris) according to manufacturer’s instructions. Biotinylated polyclonal anti-mouse antibodies (e.g. R&D Systems) were used. The amount of TNFα and MIP-1α chemiluminescence was determined using an Origen Analyzer (BioVeris).

[0201] Membrane TNFα. Membrane TNFα on islet cells was detected by modification of a method for the evaluation of membrane TNFα on human PBMC. Briefly, single-cell suspension of islets was incubated with anti-mTNFα-PE mAb (Table 1). Cells were washed with FACS buffer and resuspended in 0.5 ml 2% EM-grade formaldehyde.

[0202] Nitric oxide assay. Nitrite levels in supernatants were determined using Griess reagent (Promega), as previously described (Chan, E. D. & Riches, D. W. Am J Physiol Cell Physiol 280, C441-50 (2001)).

[0203] Apoptosis Assay. The protective effect of AAT on islets may address one of the major obstacles in islet transplantation today, namely the inadequacy of islet mass and post-isolation islet viability. Freshly isolated human islets activate stress signaling pathways and exhibit high rate of apoptosis due to the process of isolation, necessitating the use of more than one islet donor per diabetic patient (Nanj, 2004); Abdelli, S. et al. Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure. Diabetes 53, 2815-25 (2004)).

[0204] In this example, apoptosis that follows islet isolation is diminished when islets are cultured with AAT (data not shown) and demonstrate that islets that are cultured with AAT for 24 hours prior to transplantation are able to normalize serum glucose levels of diabetic mice when transplanted autologously at an otherwise sub-functional mass (data not shown).

[0205] AAT dosage. Normal human plasma contains 0.8-2.4 mg/ml AAT, with a half life of 5-6 days. In gene transfer studies in C57BL/6 mice, plasma levels of 0.8-1.0 mg/ml were achieved and provided protection from type I diabetes in NOD mice (Song, S. et al Gene Therapy 11, 181-6 (2004)). AAT administered intraperitoneally at 0.3-1.0 mg per mouse protected from TNFα-induced lethal response, and 0.8 mg AAT protected from D-galactosamine/IPS induced hepatic injury. Libert, C., et al., J Immunol 157, 5126-9 (1996).

[0206] Since AAT levels rise 3- to 4-fold during the acute phase response 1, 2 mg per mouse results in plasma levels that do not exceed physiological levels.

[0207] Statistical analysis. Comparisons between groups were analyzed by two-sided t-test or ANOVA for experiments with more than two subgroups. Results are presented as mean±SEM.

[0208] Prolongation of Islet Graft Survival by AAT.

[0209] In the present study, administration of clinical grade AAT to mice transplanted with allogeneic islets prolonged graft survival. In addition, AAT reduced migration of neutrophils and the subsequent infiltration of lymphocytes and NK cells in models of peritonitis. AAT also decreased secretion of TNFα and MIP-1α from islets and inhibited surface MHC class II expression on CD4+ islet cells in vitro. AAT was protective in a model of streptozotocin (STZ)-induced β-cell toxicity. Thus, it appears that AAT monotherapy targets several aspects of an activated inflammatory immune system, culminating in prolongation of islet allograft survival.

[0210] Effect of AAT on Cell Infiltration.

[0211] AAT diminished neutrophil migration into the peritoneum of mice injected with either thioglycollate or MHC-incompatible fibroblast cells. Other studies demonstrate that AAT inhibits neutrophil infiltration into kidneys during ischemia/reperfusion injury and into lungs following intratracheal administration of silica. In the present study AAT decreased islet production of MIP-1α and TNFα, resulting in islets deficient in chemotactic capabilities and therefore less immunogenic. The detrimental effect of neutrophils recruited to islets has been clearly demonstrated.

[0212] The involvement of macrophages in islet destruction is critical; their presence precedes insulin in NOD mice and in prediabetic BB rat, and their depletion is protective during islet transplantation in rats. Islets are potent recruiters of macrophages; of the 51 gene products identified in freshly isolated human islets by RNA array expression, expression of MCP-1 was found to be high. In mice, blockade of MCP-1 prolongs islet allograft survival when combined with a short subtherapeutic course of rapamycin. Islet allograft rejection is associated with a steady increase in intragraft expression of MCP-2, MCP-5, CCL5, CXCL-10 and CXCL-9, and the chemokine receptors CCR2, CCR5, CCR1, and CXCR5. Accordingly, CCR2−/−, CCR5−/−, and CXCR3−/− mice exhibit prolongation of islet allograft survival. In transplant settings, cytokines that are produced locally, as TNFα and IL-1β, cause damage to proximal cells independent of antigen recognition, and complement activation is critical for graft survival independent of allspecific immunity. The relevance of macrophages during early events in islet graft rejection is strengthened by the identification of CD45, F4/80 and Gr1 positive cells that express MHC class II in freshly isolated islets. In the presence of AAT, MHC class II levels were decreased below those of IL-1β/IFNγ-stimulated and unstimulated islets, supporting the idea that the process of islet isolation is sufficient to provoke activation of inflammatory pathways in islet cells. In light of the involvement of neutrophils and macrophages in graft rejection, interference with their functions by AAT provides an unusually non-inflammatory environment for the survival and recovery of engrafted islets.

[0213] As shown in the present study and elsewhere intraperitoneal injection of allogeneic NIH-3T3 cells evokes infiltration of macrophage and neutrophil on days 1-2 and of CD3+ and NK cells on days 4-5. The intensity of the latter infiltration was decreased by administration of AAT prior to allogeneic cell-line injection, but not by administration of AAT on day 3 (data not shown). In transplant settings, early non-specific factors contribute to subsequent specific immune response. It is therefore possible that the decrease in CD3+ and NK cell infiltration in the present study is secondary to the functional failure of the early innate response. However, regardless of AAT treatment, histological examination of islet grafts demonstrated that the infiltrate evoked by allogeneic islets consists of neutrophils and lymphocytes. Nevertheless, day 7 infiltration was diminished in AAT-treated recipients, and, according to day 15 insulin immunohistochemistry, the infiltrate caused less islet destruction.

AAT Inhibits Release of TNFα.

[0214] Supernatants of IL-1β/IFNγ-stimulated islets contained strikingly less TNFα when incubated with AAT (induction of 100.0±22.0 mean±SEM at 0 mg/ml AAT; 10.2%±11.2 at 0.5 mg/ml and 0.8%±0.1 at 1.0 mg/ml). In stimulated human PBMC, AAT was shown to diminish TNFα release without affecting TNFα-mRNA levels. In mice,
accordingly, serum TNFα levels are decreased in LPS-injected AAT-treated mice. Importantly, treatment of mice with AAT blocks TNFα-mediated LPS-induced, but not TNFα-induced lethality in mice. In the present study, cultured mouse splenocytes isolated from thioglycolate-injected mice secreted less TNFα, 48 hours after injection of AAT.

[0215] In the presence of AAT, membrane TNFα accumulated in IL-1β/IFNγ-stimulated CD45+ islet cells. TNFα is released from the cell surface of macrophages by the action of TNFα converting enzyme (TACE), a metalloproteinase that cleaves membrane TNFα into the soluble form of TNFα. Inhibitors of TACE reduce TNFα release and increase the levels of membrane TNFα, as demonstrated by FACS analysis. Although the regulation of TACE activity is unclear, there is evidence to suggest that extracellular proteases are involved: TACE does not require its cytoplasmic domain for its activation, its activity does not depend on the amount of TACE on the cell surface, co-expression of TACE and transmembrane TNFα is not sufficient for processing of TNFα and the enzyme is expressed constitutively in various cells. Serpins, such as serpin PN-152, are suggested to possess extracellular regulatory effects on various surface proteins.

[0216] TACE is likely to be relevant for graft rejection since TACE inhibitor decreased injury parameters in a rat model of post-transplant lung injury. In addition to a decrease in TNFα levels, the study shows lower expression of MCP-1 and ICAM-1, and a reduction in neutrophil infiltration. Similar findings were obtained with both AAT and a broad spectrum metalloproteinase inhibitor in a model of silica induced neutrophil influx into lungs. However, TACE inhibitor only partially reproduced the protective effect of AAT on islet graft survival (preliminary data). Similarly, AAT protection from STZ-induced hyperglycemia was only partially reproduced by TACE inhibition and by recombinant p75-TNF-receptor. Despite the fact that locally secreted TNFα is detrimental to islet graft function, there is, to our knowledge, no report that describes protection of islet grafts by neutralization of TNFα activity. This distinction between AAT and TACE inhibition supports the possibility that AAT affects multiple aspects of the immune system, including not only TNFα release but also events that are downstream to TNFα activities.

[0217] In one embodiment, it is contemplated that a composition of the present invention may include AAT, an analog thereof, a serine protease, TACE inhibitor (TACEi) or any combination thereof. These compositions may be administered to a subject having or in need of a transplant and or in need of immunotolerance therapy.

Transplanted Islets are Stimulated by the Process of Isolation.

[0218] The process of islet isolation initiates in the islets an inflammatory cascade of cytokines and chemokines. Thus, isolated islets contain an intrinsic proinflammatory potential that may affect local host immune responses. The mechanism of cytokine-induced islet toxicity is believed to involve expression of inducible nitric oxide synthase and subsequent production of nitric oxide (NO) by non-β-cells. In the present study, AAT decreased NO production in IL-1β/IFNγ-treated islets. Accordingly, islet viability was increased in a low NO environment, as attained by either incubation with a low concentration of stimulators (data not shown) or by introduction of AAT. Insulin induction, which is typically incomplete in the presence of cytokines, was intact in the presence of AAT and cytokines. In vivo, AAT protected islets in mice injected with STZ, as concluded by lower serum glucose levels. The portion of viable β-cells was visually assessed by insulin immunohistochemistry and was proportional to the decrease in serum glucose levels. The protection of AAT was limited to the initial days that follow STZ administration, suggesting that AAT interferes with NO production and immune activation and not with intracellular DNA alkylation. Freshly isolated non-stimulated CD45+ islet cells expressed MHC class II, which is involved in immune responses against islets. The levels of MHC class II were elevated in the presence of IL-1β/IFNγ and decreased in the presence of AAT. Interestingly, MHCII expression was unaffected by the presence of TACE (TNFα converting enzyme) inhibitor (data not shown), confirming that AAT activities extend beyond those of TACE inhibition.

[0219] According to the present study, the activities of AAT are directed against multiple components of the innate immune system, culminating in a protective effect on islet graft destruction. Islets in particular exhibited a high degree of protection from inflammatory processes in the presence of AAT. Pretreatment with AAT prior to islet transplantation may reduce both islet loss and the immunological response against the graft.

[0220] The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed herein, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or sub-group of members of the Markush group and that other members of the described groups are included but may not be listed.

[0221] All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

Example 9

Prolonged Administration of hAAT to Diabetic Islet Allograft Recipient Mice Results in Strain-Specific Immune Tolerance

[0222] In one exemplary method, to examine the outcome of islet allograft transplantation during extended monotherapy with hAAT, mice heterozygous for tissue-specific hAAT (hAAT-Tg) that exhibit levels of circulating hAAT that are below detection were used as graft recipients. hAAT-Tg mice (H-2b) were rendered diabetic, transplanted with allogeneic islets (H-2d) and treated with serial doses of hAAT (n=24) or albumin (n=6). As shown in FIG. 9A, control albumin-treated mice rejected allografts by day 12. In contrast, all hAAT-treated mice that were treated for the various durations
indicated exhibited extended normoglycemia. The shortest 14-day course of hAAT therapy resulted in delayed loss of function in 50% of the transplants and in graft acceptance in the remainder. A 21-day course resulted in a single delayed graft failure event out of the six transplanted islet grafts. All twelve mice that received hAAT treatment for 30 days or more achieved graft acceptance (treatment duration 30 days n=8, 41 days n=1, 52 days n=2, 60 days n=1).

[0223] Removal of grafts by nephrectomy restored hyperglycemia. In another exemplary method, as illustrated in FIG. 9C left and right, by day 12 after transplantation the albumin-treated mouse (broken line) had mounted an acute allograft rejection response and developed overt hyperglycemia, whereas the hAAT-treated mice maintained normoglycemia for the duration of therapy. After withdrawal of hAAT, continued graft-derived insulin production was observed (FIG. 9B second column and FIG. 9C days 52-72), raising the possibility that allospecific immune tolerance was achieved. To examine this, grafts were removed and a second grafting procedure was undertaken in the subcapsular space of the remaining kidney without further treatment, using the same strain of islets that had been originally transplanted (n=4, H-2d, FIG. 9B and illustrated in an example in Fig. 9C left). In each case, following re-engraftment, recipient mice remained normoglycemic for over 50 days. To ascertain that antigen-specific immune tolerance had been induced, islets from a third strain (H-2k) were used as the source of the second graft in three hyperglycemic mice without further hAAT treatment (FIG. 9B and FIG. 9C right). As shown, all three mice exhibited acute rejection of third-strain allografts.

[0224] According to histology, a “cuff” of mononuclear cells surrounded the entire islet mass in all explanted grafts (FIG. 9D). The mononuclear cells were located at the intersection between the renal parenchyma and capsule, flanking an intact islet graft mass. By staining for several cell-specific markers we found the near absence of activated macrophages (CD11b, not shown), and a predominance of CD4 or CD8-positive cells, interspersed with CD25-positive cells (not shown).

Example 10
Inflammatory and Anti-Inflammatory Gene Expression in Islet Allografts

[0225] In another example, in light of the sensitivity of islet beta cells to inflammatory mediators, expression of inflammation-related genes in explanted islet grafts was examined. FIG. 10 represents a comparison between steady-state mRNA patterns present early after transplantation in albumin-treated mice (days 1, 3, 5 and 7) and in long-lasting hAAT-treated islet grafts (representative day 72). As illustrated, transcripts of genes coding for islet-injurious ligands were low in grafts from hAAT-treated mice. These include the beta cell toxic IL-1α, in addition to CD 14, a marker for invading macrophages, IL-2, carried by invading T cells and ICAM-1, which represents a pivotal adhesion molecule typically essential for cell migration. In addition, mRNAs transcripts that encode for the pro-neutrophilic CXC chemokines, KC and macrophage inflammatory protein (MIP)-2, were undetectable in long-lasting islet allografts. Islet allograft explants from hAAT-treated mice also exhibited elevated expression of IL 1 receptor antagonist (IL-1Ra) and isoforms of IL-18 binding protein (IL-18BP), both reported to protect islet allografts. In contrast, explants of albumin-treated mice exhibited either low or undetectable expression of IL-1Ra and IL-18BP (FIG. 10, days 1, 3, 5 and 7). The intensity of the cellular infiltration can be appreciated by the progressive increase in GAPDH-mRNA levels in grafts from albumin-treated mice. The identification of insulin transcripts confirms the presence of beta cells in the explants.

Example 11
Some Cell-Specific Effects of AAT

[0226] In another example, expression of anti-inflammatory molecules observed here belong to grafts that were explanted several weeks after withdrawal of hAAT treatment (see FIG. 9). It is therefore likely that the intragraft anti-inflammatory gene expression profile reflects the acquired cellular components that have progressively accumulated in the antigen-rich site. In order to examine the effects of hAAT on major cell subpopulations, in vitro assays were performed for lymphocytic and non-lymphocytic responses. As illustrated in FIG. 11, IL-2-stimulated human peripheral blood mononuclear cells (PBMC) were able to produce IFNγ and proliferate, as expected, in the presence of hAAT. Similarly, mouse splenocytes responded to Con A with secretion of IFNγ, as well as increased cell proliferation and cell clumping, each response unaffected by hAAT (FIG. 16A). In contrast to lymphocytic responses, peritoneal macrophages responded to hAAT by secreting significantly less IFNγ-induced nitric oxide in a concentration-dependent manner (FIG. 16B).

Example 12
Treg-Related Gene Expression in hAAT-Treated Islet Allografts

[0227] In the unique set of genes expressed within grafts of hAAT-treated recipient mice, we also observed the expression of genes indicative of Treg cells (FIG. 12). As shown, grafts from hAAT-treated mice (FIG. 12A representative day 72) exhibit a significantly elevated expression of foxp3, TGFβ and CTLA-4, representing the expected phenotype of Treg cells. In contrast, the expression of these genes was either below detection or terminated early in grafts from albumin-treated mice (days 1, 3, 5 and 7). As depicted in FIG. 4B, the presence of foxp3-positive cells was observed as early as day-14 of hAAT therapy in sections that contained the graft site (FIG. 4B, G). Notably, in renal tissue from kidney portions that did not contain the grafted islets (FIGS. 12B, K), foxp3-positive cells were also observed. CTLA-4 expression was only present inside the graft (G). Of particular importance, IL-10 transcript levels were closely associated with foxp3-expression, suggesting that the identified Treg cells are also producers of IL-10.

Example 13
Time-Dependent Distribution of Treg Cells between Draining Lymph Nodes (DLN) and Allograft

[0228] In another example, in order to examine the effect of hAAT on Treg cell development during transplantation, foxp3-GFP knock-in mice were used as graft recipients (C57BL/6 background, H-2b). A vigorous allo-recognition response was evoked by implanting wild-type skin grafts (H-2d) under the surface of both left and right thighs. Animals were treated with hAAT (n=13) or albumin (n=13) using the
same dosing schedule employed in the islet transplantation protocol (see FIG. 1). Inguinal DLN were removed on various days after grafting and CD4+ sorted cells were examined by FACS and by RT-PCR for Foxp3-positive cells. As shown in FIG. 13A, between transplantation and 3 days after engraftment of islets the number of Foxp3-positive cells in the DLN unvaryingly decreases in both the albumin-control and hAAT-treated graft recipient mice. However, between days 4 and 9 DLN from hAAT-treated mice had more Foxp3-positive cells. In the days that followed, the gap in the size of the Treg population was restored. Gene expression analysis corroborated FACS findings (FIG. 13A inset). By using Foxp3-GFP knock-in mice, we were able to observe Treg cells infiltrating into allografts by day 10 after transplantation. This model offers a particular advantage as invading fluorescent cells can be directly identified in freshly-obtained, unstained specimens using fluorescent microscopy. As shown in FIG. 13B, invading Foxp3-positive cells localized to grafts in hAAT-treated animals (bottom). In this technique, autofluorescent fur can be observed. Total intensity of infiltrating cells can be appreciated by DAPI counter-staining. Similarly, as shown in FIG. 13C, islet allografts that had been transplanted into Foxp3-GFP knock-in recipient mice also contained Foxp3-positive cells in the “cuff” site. The proportion of Foxp3-positive cells approximated that found with CD4/CD25 co-staining (not shown). From these findings, it appears that hAAT treatment promotes early accumulation of Treg cells in the draining lymph nodes and a progressive migration into the allograft-recipient site.

Example 14
Early Local and Systemic Effects of hAAT

In another exemplary method, events that might precede the changes observed in the DLN were studies. Islets embedded into matrigel offer a model for examination of islet-driven cellular invasion during the first 48 hours of provocation. Allogeneic islets were introduced into hAAT-containing matrigel plugs (12.5-100 μg hAAT per graft) and implanted subcutaneously into mice. Grafts were retrieved 48 hours after transplantation and intragraft steady-state mRNA levels were assessed. As shown in FIG. 14, a dose-dependent decrease in CD14 mRNA levels had occurred, reflecting hAAT-dependent inhibition of macrophage invasion. Distinctively, the recipient mice carry the hAAT genomic insert and invading host cells can thus be identified. The amount of invading cells was decreased in the presence of hAAT, as corroborated by histological examination of the explanted matrigel at 48 hours (not shown). Copies of insulin mRNA transcripts correlated with the amount of added hAAT, representing improved hAAT-mediated beta cell viability. hAAT treatment also resulted in a dose-dependent increase in VEGF mRNA levels. VEGF mRNA in the matrigel-islet graft is likely to be of islet-cell origin since VEGF mRNA copies coincided with near absence of host genomic DNA (FIG. 14).

In another method, in addition to local events that reflect an inflammation-damped antigen presentation environment, more comprehensive changes that may support the generation of Tregs were examined. Mice were subject to 10 days of hAAT treatment in order to reproduce the circulating cytokine environment of a treated islet graft recipient. Control mice received albumin. Serum levels of cytokines were then measured. As shown in FIG. 17, serum levels of Th17-related cytokines, IL-17 and IL-23, were 3-fold lower compared to levels in control mice. Serum IL-6 and MCP-1 were also decreased. On the other hand, serum IL-10 levels increased 2-fold and the levels of IL-309, a chemottractant for Treg cells, increased 2-fold. To examine the circulating cytokines evoked during a vigorous inflammatory response, hAAT or albumin-treated mice (10 days) were challenged with LPS, and serum cytokines were assessed after 2 hours (FIG. 17). Once more, IL-309 and MIP-2 levels exhibited the favorable changes observed in hAAT-treated non-challenged mice. Most strikingly, circulating IL-1RA levels increased 3-fold. The effect of hAAT treatment on serum IL-6 and IL-10 levels were also studied during a sterile inflammatory response (FIG. 17). In this procedure the inflammatory response results in increased levels of IL-1β-dependent IL-6. However, mice treated with hAAT exhibited a 50% decrease in serum IL-6 protein levels and a 27% increase in serum IL-10 protein levels.

Effect of AAT on Dendritic Cell Migration, Maturation and Function

To investigate the implications of a dampened antigen presentation process during transplantation, we studied dendritic cell activation in vitro and in vivo. Using transgenic GFP-positive donor skin grafts and subsequent PCR amplification of DNA isolated from DLN, we evaluate the migration of graft-derived cells towards DLN in vivo (FIG. 18A). Mice received hAAT one day before grafting, as a result, allograft transplantation. Graft-derived DNA was present in DLN after transplantation in both control and hAAT-treated mice.

Example 15

Effects of hAAT on the transcript levels of CD86, CD3 and IL-10 in renal DLN of mice receiving skin grafts into the renal subcapsular space were examined (FIG. 15). For background gene expression, DLN from non-transplanted mice were examined. Seventy-two hours after transplantation, CD86 mRNA transcript levels were reduced by hAAT treatment 2-fold. At the same time point, DLN contained 2-fold less total CD3 mRNA transcripts. Notably, a 2.5-fold rise in IL-10 gene expression was observed in DLN from hAAT-treated grafted mice.

In another example, to examine the direct effect of hAAT on dendritic cell activation and maturation, dendritic cells were cultured in vitro with LPS in the absence and presence of hAAT (FIG. 18B). According to FACS analysis, LPS stimulation in the presence of hAAT resulted in a marked decrease in the levels of inducible surface MHC class II and CD86.

To address the possibility that hAAT treatment had specifically induced IL-10 production in Treg cells in vivo, hAAT or albumin were administered 3 days before LPS challenge to foxp3-GFP knock-in mice. 16 hours later spleens were harvested and splenocytes were isolated to examine IL-10 release in a cytometric secretion assay. LPS administration alone resulted in foxp3-positive cells that released IL-10 (6.1±0.1%, compared to 0.2±0.1% without LPS). The number of IL-10-secreting Treg cells increased in hAAT-treated mice to 10.6±1.2% (mean±SEM, p=0.0167).
These data support the possibility that hAAAT mono therapy modifies the antigen presentation process towards the generation of a migrating, yet immature and tolerogenic dendritic cell phenotype, culminating in the expansion of functioning, IL-10-producing Tregs.

**Methods**

**[0236]** Mice. hAAAT-Tg mice, background strain C57BL/6, were engineered as described previously and studied as detailed in Supplementary Methods. Circulating levels of hAAAT in heterozygote hAAAT-Tg mice were determined by a specific ELISA for human AAAT, as described previously. Serum levels were below the limit of detection (10 ng/ml). Wild-type Balb/c, CBA/Ca and DBA/2 mice were purchased from Jackson Laboratories.

**[0237]** Islet allograft transplantation. Renal subcapsular islet transplantation was performed as described previously. Briefly, hAAAT-Tg heterozygote mice weighing 25-30 g were rendered diabetic by a single i.p. streptozotocin injection (225 mg/kg, Sigma). Donor islets were isolated and collected on 100-micron cell strainer (BD Falcon, Franklin Lakes, N.J.), as described previously. 450 hand-picked isolated islets from DBA/2, Balb/c, C57BL/6 or CBA/Ca donor mice were grafted under the renal subcapsular space. hAAAT treatment was initiated one day before transplantation and every third day (2 mg per mouse, Amlast, Baxter, Westlake Village, Calif.). Control hAAAT-Tg mice received the same amount of human serum albumin (Abbott, North Chicago, Ill.). In the experiments in which monotherapy exceeded 14 days the amount of hAAAT was increased by 0.5 mg every third day until a 6 mg maintenance dose was reached. Islet allograft rejection was defined as the day blood glucose exceeded 300 mg/dl after a period of at least 3 days normoglycemia.

**[0238]** Skin allografts. 1 mm² freshly-prepared skin derived from shaved avascular portion of the abdominal midline was used as donor tissue. A graft was inserted into the subcutaneous space of each thigh in foxp3-GFP knock-in mice through a 1 mm-long incision. Incision site was sealed with a 3-0 suture.

**[0239]** Immune responses in vitro. Peripheral blood mononuclear cells (PBMC) were isolated from healthy individuals, as described previously. Studies of human blood were approved by the Colorado Multiple Institutional Review Board. Splenocytes and resident peritoneal macrophages were obtained from C57BL/6 mice, as described previously. Response assays, see Supplementary Methods.

**[0240]** Statistical analysis. Comparisons between groups were performed by two-tailed t-test.

**[0241]** hAAAT transgenic Mice. Experiments were performed with heterozygote hAAAT-Tg mice, obtained by mating of hAAAT-Tg mice with wild-type C57BL/6 mice (Jackson Laboratories, Bar Harbor, Me.). Litters were screened for the presence of the human AAAT gene by standard tail DNA extraction (XNAT2 Extraction Kit, Sigma, St. Louis, Mo.) followed by two-step nested PCR amplification using the following primers: outer sequence (450 bp): forward 5'-ACTGTCGCTACTTCTTACACC-3' (SEQ. ID NO. 62) and reverse 5'-CATGCTAGGCCGACCACT-3' (SEQ. ID NO. 64); inner sequence (249 bp): forward 5'-ACTGTCGCTACTTCTTACACC-3' (SEQ. ID NO. 62) and reverse 5'-CATGCTAAACACCCTTTACAC-3' (SEQ. ID NO. 65).

**[0242]** Assessment of explanted renal allografts. Subcapsular renal grafts were removed by nephrectomy between first and second grafting procedures under anesthesia by ligation of renal vessels and severing of the kidney together with the islet graft. Incision site was sealed with a 3-0 suture and mice were allowed to recover. Upon graft explantation, tissue was maintained on ice and islet graft sites were identified macroscopically on the surface of the kidneys. For RT-PCR, the region containing the graft was removed with a number 11 blade and immediately transferred to liquid nitrogen. An equivalent size of tissue was removed from the opposite renal pole to control for background gene expression of non-graft tissue. For histology, samples were fixed in 10% formalin.

**[0243]** Histology. Kidneys or matrigel explants were fixed in buffered formalin and 24 hours later cut into two equal portions through the center of the graft for embedding in paraffin. Blocks were sliced serially for multiple staining with either H&E, DAPI or with the following antibodies: Insulin (as previously described), VWF, CD4, CD8, CD11b and GFP (eBiosciences, San Diego, Calif.). Immunostaining methods previously described.

**[0244]** RT-PCR. Total RNA was extracted (Qiagen) and reverse transcription followed (Invitrogen, Carlsbad, Calif.). Primers: mouse IL-1β forward 5'-CTCCATGAGCTTTGTACAAAGG-3' (SEQ. ID NO. 66) and reverse 5'-TGGTGTAGTACAGTGGGAGG-3' (SEQ. ID NO. 67), CD4 forward 5'-CATTGCACTCTGTGGTCTTGAG-3' (SEQ. ID NO. 68) and reverse 5'-GAGTGTAGTTTCCTGCTCGGTG-3' (SEQ. ID NO. 69), IL-2 forward 5'-TCCAGACCTCAGCTACGGCAAG-3' (SEQ. ID NO. 70) and reverse 5'-GACAGAGGCTCATCTCCTCACAAGG-3' (SEQ. ID NO. 71), IL-10 forward 5'-TGTGAAAGTGATGCAAGGCAAGTGC-3' (SEQ. ID NO. 72) and reverse 5'-CATTGCACTCTGTGGTCTTGAG-3' (SEQ. ID NO. 73), CD3e forward 5'-GCTTGAAAGCACATGATAAGGC-3' (SEQ. ID NO. 74) and reverse 5'-GCCAAGTGATGACAGATGTC-3' (SEQ. ID NO. 75), CD86 forward 5'-TCCAGAAGTTTGGAACCCACCG-3' (SEQ. ID NO. 76) and reverse 5'-CAGGCTCAGCTGAATCTGGCCGATC-3' (SEQ. ID NO. 77), ICAM-1 forward 5'-AGGGCTTGGCATAGTGTCATC-3' (SEQ. ID NO. 78) and reverse 5'-CTTGAGAGGAGGAGAACACAGG-3' (SEQ. ID NO. 79), KC forward 5'-GCCTGCCTCTGCTGTGAGCC-3' (SEQ. ID NO. 80) and reverse 5'-ATTTTCACTTCCACCAAGGAGAC-3' (SEQ. ID NO. 81), MIP-2 forward 5'-TGCCGCTCAGCTGTGCT-3' (SEQ. ID NO. 82) and reverse 5'-AACCTTTTGGAGGGTTCCCTGA-3' (SEQ. ID NO. 83), GAPDH forward 5'-ATGGACACTCACTGGGCA-3' (SEQ. ID NO. 84) and reverse 5'-GAGATACACTCACTGGGCA-3' (SEQ. ID NO. 85), insulin forward 5'-CAGAAACACATGCAGCAAGGACG-3' (SEQ. ID NO. 86) and reverse 5'-TGCAAGAAAGCGCTCTGCTGGG-3' (SEQ. ID NO. 87), IL-1Ra forward 5'-GACCCAGAACCTTGCACACCC-3' (SEQ. ID NO. 88) and reverse 5'-GACGGATGGAAGTGAAG-3' (SEQ. ID NO. 89), IL-18BP forward 5'-CCCACCATCAAGAATTTACA-3' (SEQ. ID NO. 90) and reverse 5'-CTGGTCAGGACGTGTCATGAC-3' (SEQ. ID NO. 91), foxp3 forward 5'-CCCACCATCAAGATTTACA-3' (SEQ. ID NO. 92) and reverse 5'-GGCATATGAGGCCATCCA-3' (SEQ. ID NO. 93).
CAGT-3' (SEQ. ID NO. 93), TGfβ1 forward ‘5-GAA-CAAAAGGTACATGCCTGCTCAGTGAGTTA-3’ (SEQ. ID NO. 94) and reverse ‘5-CCCTCTGTTCCTCCTCAGTTAGGTA-3’ (SEQ. ID NO. 95), TGfβ2 forward ‘5-ATGCCCCCATCTG-CAAGGAGGACCTA-3’ (SEQ. ID NO. 96) and reverse ‘5-GCTTCTGCCAAGCTGCTGAGA-3’ (SEQ. ID NO. 97), CTLA-4 forward ‘5-GTAGGCTCTGACTCTTCCTTCT-3’ (SEQ. ID NO. 98) and reverse ‘5-AGGTACGTCCGGTGTCAAC-3’ (SEQ. ID NO. 99), VEGF forward ‘5-GGAGATCCCTGGAGGACGACACT-3’ (SEQ. ID NO. 100) and reverse ‘5-GGGCGATTAGGCAGCA-GATATAAGAA-3’ (SEQ. ID NO. 101).

[0245] FACS analysis. Analyses were conducted using a flow cytometer (FACS Calibur, Becton Dickinson, Mountain View, Calif.). Fluorescence data were analyzed by the Cell Quest program. At least 50,000 cells were analyzed per sample. Foxp3-GFP-positive cell analysis was performed on unstained CD4+–sorted lymphocytes (1 x 10⁶ per sample, see ‘DLN analysis’). Dendritic cells (1 x 10⁶ per sample) were double-stained with CD11c-APC and anti-CD86–PE, or anti-CD11c–APC and anti-MHCl–PE (all antibodies obtained from ebioscience). Antibodies were diluted and recommended concentrations according to the manufacturer’s instructions. Nonspecific binding of antibodies was assessed with cells labeled with matching isotype control antibodies. Nonspecific Fc staining was excluded by the addition of Fc–blocking antibodies (ebioscience).

[0246] IL-10 cytokine secretion assay. The assay was performed according to manufacturer’s instructions (Miltenyi Biotech, Bergisch Gladbach, Germany). Briefly, an anti-CD45-pan leukopenic chimeric antibody that also specifically binds to IL-10 was added to the freshly isolated spleen cells. During a short incubation, IL-10 that is released from activated cells is captured by the chimeric antibody and is bound to the surface of the secreting cell. Culture conditions preclude cell-to-cell association. The assay was performed on cells from foxp3-GFP knock-in mice. As such, the foxp3+ cell subpopulation was identified by GFP and surface bound IL-10 was identified by using anti-IL-10–PE in the same cell preparation (Miltenyi Biotech).

[0247] Lymphocytic response assays. PBMC were cultured in RPMI supplemented with 10% FCS, 50 U/ml penicillin and 50 µg/ml streptomycin (Cellgro, Herndon, Va.). Cells (5 x 10⁶ per well) were primed for 72 hours with concanavalin A (Con A, 1 µg/ml, Sigma). PBMC were then washed, resuspended (5 x 10⁶ per well) and activated with human IL-2 (Peprotech, Rocky Hill, N.J.) in the presence of hAAT or human serum albumin. Splenocytes (5 x 10⁶ well) were stimulated with Con A (1 µg/ml) for 24 hours in the presence of hAAT or albumin. Clumping was examined microscopically. Peritoneal macrophages Cells (5 x 10⁶ per well) were stimulated for 24 hours with murine IFNγ (Peprotech) in the presence of hAAT or human serum albumin. Bone-marrow derived dendritic cells were obtained by growing bone marrow cells from wild-type mouse femurs in Dulbecco’s Modified Eagle’s Medium (DMEM) (Biological Industries, Bet Hae- mek, Israel) supplemented with 10% FCS, 50 µM β-mercaptoethanol (Sigma), 100 U/ml penicillin and 100 µg/ml streptomycin (Biological Industries). Cells were cultured in the presence of 10 ng/ml granulocyte-macrophage colony-stimulating factor (Biological Industries) both in the initial seeding step and again after 5 days of culture. For stimulation with LPS, the dendritic cells from 10-day cultures were mechan-ically removed from the culture wells with a rubber spatula, washed, and then 3 x 10⁶ cells were incubated for 18 h with 100 ng/ml LPS (Sigma).

[0248] DLN analysis. Depending on the graft site, renal or inguinal DLN were harvested on indicated days. To examine gene expression, the lymphoid tissue was snap-frozen, total RNA extracted, quantified and normalized, and RT-PCR performed as described below. To examine the proportion of foxp3-positive Treg cells in DLN by FACS analysis, lymphoid tissue from foxp3-GFP knock-in mice was mechanically dissociated immediately after lymph node removal and CD4-positive cells sorted by magnetic-bead enrichment, according to manufacturer’s instructions (EasySep MuCD4, Stem Cell Technologies, Vancouver, Canada). FACS analysis was carried out to measure percent of GFP-positive cells in the population. In migration experiments, DLN were harvested from mice that were grafted with GFP-positive allogeneic skin and the specific presence of graft-derived cells was assessed by DNA isolation and PCR amplification of the transgenic sequence as described.

[0249] Matrigel-islet grafts. Growth factor reduced matrigel (BD Pharmingen, Erembodegen, Belgium) remains at a liquid state at 4°C and at a semi-solid state when introduced to mice subcutaneously. Fluid phase matrigel (0.3 ml) was mixed with 100 freshly isolated allogeneic islets from wild-type mice together with albumin or hAAT. Immediately after mixing, the matrigel-islet allografts were injected subcutaneously into the scuff region of the neck of anesthetized hAAT-Tg mice through a 21G needle. Exploitation of matrigel plugs was performed under anesthesia. One section was fixed in 10% formalin and processed for histology, the other section was immersed in 37°C in constant stirring with dispase (BD Pharmingen) for 2 hours for gentle digestion of the matrigel and release of inhabitant and invading cells. The cells recovered from the digested matrigel were washed three times with PBS and divided into two equal parts: one part was processed for RNA extraction (Qiagen, Inc., Valencia, Calif.) followed by RT-PCR (see below). The second part was processed for genomic DNA extraction (GenScript, Piscataway, N.J.) followed by PCR for hAAT (see ‘Mice’).

[0250] Matrigel skin grafts. A single 1 mm² freshly-prepared skin section (see ‘Skin allograft’ above) was mixed with matrigel and grafted subcutaneously into the scuff region of the neck of foxp3-GFP knock-in mice. Upon matrigel harvest, one section was placed immediately on a cover slip and analyzed by fluorescent microscopy for GFP-positive cells. DAPI mounting medium was added in order to evaluate total cell content. An equal size portion of the matrigel was processed for RT-PCR analysis (see ‘Matrigel-islet grafts’).

[0251] Cytokine measurements. Murine IFNγ was measured by ELISA (BD Pharmingen) and human IFNγ was measured by electrochemiluminescence (ECL) assay using the Origin Analyzer (BioVeris, Gaithersburg, Md.), as previously described. Murine IL-6 and IL-10 levels were determined by specific ELISA (ebioscience). Murine serum cytokine levels were also measured using cytokine Proteome Profiler™ blotting according to manufacturer’s instructions (R&D Systems, Minneapolis, Minn.). Nitric oxide levels were determined by Griess reaction (Promega, Madison, Wis.).
Normoglycemia after islet transplantation in diabetic recipient mice during and after hAAAT treatment, after graft removal and following second allografting.

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| °Donor strain: DBA/2, H-2b, recipient strain: hAAAT-Tg, H-2b |
| °Nephrectomized hyperglycemic mice grafted with islets in contralateral renal subcapsular space |

Example 16

Human islets, as well as mouse islets, are extremely sensitive to inflammatory mediators. In the presence of certain cytokines, islets will lose their rounded dense morphology and become injured; critical islet mass will be lost in the first 48 hours after transplantation, requiring more than one donor per diabetic human recipient of an islet graft. In one exemplary method, FIG. 19 represents the effects of AAT on stimulated human islets. Human islets were cultured in the presence of IL-1β plus IFNγ for 72 hours with indicated concentrations of AAT. As illustrated in FIG. 19, there are morphological indications in islets incubated with AAT compared to control islets from the same donor without AAT.

The multicellular islet contains cells capable of secreting important inflammatory agents. Upon transplantation, these contribute to loss of graft. AAT reduced the amounts of critical cytokines secreted by islets in response to inflammation, and lowered nitric oxide levels. Islets were cultured in the presence of IL-1β plus IFNγ for 72 hours with indicated concentrations of AAT (micrograms/mL) as shown in FIG. 20, levels of IL-6, IL-8 and TNFα (percent from stimulated islets) and nitric oxide were determined in supernatants. Data are mean levels 5 islet donors.

When added immediately after isolation, a procedure possible only in a center where human islet cells are isolated, islet cell death driven by exogenous cytokines (as would occur during transplantation) is abolished. The effect of AAT inhibition in the experiment is represented in FIG. 21. As shown, CT (control) are islets incubated without AAT and the LDH levels (indicator of cell death) are elevated when exposed to the combination of IL-1β plus IFNγ. On the right, islets were exposed to the combination of IL-1β plus IFNγ in the presence of AAT (0.5 mg/mL) and LDH levels were the same as in islets without IL-1β plus IFNγ.

Example 17

In another exemplary method, a subject scheduled for a transplant is treated with an AAT composition intravenously. In this example, a patient is identified in need of transplant surgery. The patient is administered an infusion via iv of an AAT composition (e.g. 60 mg/kg of Aralast AAT). Then the patient is sent to the fluoroscopy room where a catheter is implanted in a portal vein under fluoroscopy and islet cells are infused directly into the subject (e.g. where the cells then lodge in the liver). Later, for example 2 days after transplant of the islet cells, a patient is treated with another iv infusion of an AAT composition (e.g. 60 mg/kg of Aralast AAT). In addition, the patient may be treated in another several days, for example, about 5 days later with another iv infusion of an AAT composition (e.g. 60 mg/kg of Aralast AAT). In order to assess whether periodic administration of an AAT composition is needed, blood glucose levels of the patient can be measured to assess immune tolerance of the transplanted cells and administration of additional AAT composition infusions can be determined and delivered as needed. In addition, the patient can be monitored for anti-inflammatory levels using for example drawing one or more blood samples from a patient and assessing the level of anti-inflammatory compounds in the blood (e.g. cytokine levels in the blood). In this example, depending on patient need, AAT compositions can be administered, before, during and/or after islet cell transplantation.

All of the COMPOSITIONS and METHODS disclosed and claimed herein may be made and executed without undue experimentation in light of the present disclosure. While the COMPOSITIONS and METHODS have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variation may be applied to the COMPOSITIONS and METHODS and in the steps or in the sequence of steps of the METHODS described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.
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Gln Pro Asp Ser Gin Leu Glu Leu Thr Thr Gly Asn Gly Leu Phe Leu
130  135  140

Ser Glu Gly Leu Leu Val Asp Lys Phe Leu Glu Asp Val Lys Lys
145  150  155  160

Leu Tyr His Ser Glu Ala Phe Thr Val Asn Phe Gly Asp Thr Glu Glu
165  170  175

Asa Lys Lys Gin Ile Asn Asp Tyr Val Glu Lys Gly Thr Gin Gly Lys
180  185  190

Ile Val Asp Leu Val Lys Glu Leu Asp Arg Asp Thr Val Phe Ala Leu
195  200  205

Val Asp Tyr Ile Phe Phe Lys Gly Lys Trp Glu Arg Pro Phe Glu Val
210  215  220

Lys Asp Thr Glu Glu Asp Phe His Val Asp Gin Val Thr Thr Val
225  230  235  240

Lys Val Pro Met Met Lys Arg Leu Gly Met Phe Asn Ile Gin His Cys
245  250  255

Lys Lys Leu Ser Ser Thr Val Leu Met Lys Tyr Leu Gly Asn Ala
260  265  270

Thr Ala Ile Phe Phe Leu Pro Asp Glu Gly Lys Leu Gin His Leu Glu
Aan Glu Leu Thr His Asp Ile Ile Thr Lys Phe Leu Glu Aan Glu Asp 275 280 285
Arg Arg Ser Ala Ser Leu His Leu His Thr Ser Ile Thr Gly Thr 290 295 300
Tyr Asp Leu Lys Ser Val Leu Gly Gin Leu Gly Ile Thr Lys Val Phe 310 315 320
Ser Aan Gly Ala Asp Leu Ser Gly Val Thr Glu Glu Ala Pro Leu Lye 330 335
Leu Ser Lys Ala Val His Lys Ala Val Leu Thr Ile Asp Glu Lys Gly 350 355
Thr Glu Ala Ala Gly Ala Met Phe Leu Glu Ala Ile Pro Met Ser Ile 370 375 380
Pro Pro Glu Val Lys Phe Aan Lys Pro Phe Val Phe Leu Met Ile Glu 390 395 400
Gln Aan Thr Lys Ser Pro Leu Phe Met Glu Lys Val Val Aan Pro Thr 410 415 420
Gln Lys

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 63
Ala Cys Thr Cys Cys Thr Cys Cys Gly Thr Ala Cys Cys Cys Thr Cys
1 5 10 15
Ala Ala Cys Cys
20

<210> SEQ ID NO 63
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 64
Gly Cys Ala Thr Thr Gly Cys Cys Cys Ala Gly Gly Thr Ala Thr Thr
1 5 10 15
Thr Cys Ala Thr
20

<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 64
Ala Cys Thr Gly Thr Cys Ala Ala Cys Thr Thr Cys Gly Gly Gly Gly
1 5 10 15
Ala Cys Ala Cys
20

<210> SEQ ID NO 65
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse
Cys Ala Thr Gly Cys Cys Thr Ala Ala Ala Cys Gly Cys Thr Thr Cys
1  5    10  15

Ala Thr Cys Ala
20

<210> SEQ ID NO 66
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 66

Cys Thr Cys Cys Ala Thr Gly Ala Gly Cys Thr Thr Cys Gly Gly Thr Ala
1  5    10  15

Cys Ala Ala Gly Gly
20

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 67

Thr Gly Cys Thr Gly Ala Thr Gly Thr Ala Cys Cys Ala Gly Thr Thr
1  5    10  15

Gly Gly Gly Gly
20

<210> SEQ ID NO 68
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 68

Cys Ala Thr Thr Gly Cys Ala Thr Cys Cys Thr Cys Cys Thr Gly Cys
1  5    10  15

Gly Thr Thr Thr Cys Thr Gly Ala
20

<210> SEQ ID NO 69
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 69

Gly Ala Gly Thr Gly Ala Gly Thr Thr Thr Thr Thr Cys Cys Cys Thr
1  5    10  15

Thr Cys Cys Gly Thr Gly Thr Gly
20

<210> SEQ ID NO 70
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 70

Thr Thr Cys Ala Ala Gly Cys Thr Cys Cys Ala Cys Thr Thr Cys Ala
1  5    10  15

Ala Gly Cys Thr Cys Thr Ala Cys Ala Gly Cys Gly Gly Ala Ala Gly
<210> SEQ ID NO 71
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 71

Gly Ala Cys Ala Gly Ala Ala Gly Cys Thr Ala Thr Cys Cys Ala
1  5    10  15
Thr Cys Thr Cys Thr Cys Ala Gly Ala Ala Gly Thr Cys Cys
20  25  30

<210> SEQ ID NO 72
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 72

Thr Gly Thr Gly Ala Ala Ala Thr Ala Ala Gly Ala Gly Cys Ala
1  5  10  15
Ala Gly Gly Cys Ala Gly Thr Gly
20

<210> SEQ ID NO 73
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 73

Cys Ala Thr Thr Cys Ala Thr Gly Cys Cys Thr Thr Gly Thr Ala
1  5  10  15
Gly Ala Cys Ala Cys Cys
20

<210> SEQ ID NO 74
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 74

Gly Cys Cys Thr Cys Ala Gly Ala Ala Gly Cys Ala Thr Gly Ala Thr
1  5  10  15
Ala Ala Gly Cys
20

<210> SEQ ID NO 75
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 75

Cys Cys Cys Ala Gly Ala Gly Thr Gly Ala Thr Ala Cys Ala Gly Ala
1  5  10  15
Thr Gly Thr Cys
20

<210> SEQ ID NO 76
<211> LENGTH: 25
<212> TYPE: PRT

<400> SEQUENCE: 76
Thr Cys Cys Ala Gly Ala Ala Cys Thr Thr Ala Cys Gly Gly Ala Ala
1 5 10 15
Gly Cys Ala Cys Cys Cys Ala Gly
20 25

Cys Ala Gly Gly Thr Thr Cys Ala Cys Thr Gly Ala Ala Gly Thr Thr
1 5 10 15
Gly Gly Cys Gly Ala Ala Thr Ala Cys
20 25

Ala Gly Gly Gly Cys Thr Gly Gly Cys Ala Thr Thr Gly Thr Thr Cys
1 5 10 15
Thr Cys Thr Ala
20

Cys Thr Thr Cys Ala Gly Ala Gly Cys Ala Gly Gly Ala Ala Ala Ala
1 5 10 15
Cys Ala Gly Gly
20

Cys Gly Cys Thr Cys Cys Thr Thr Cys Thr Cys Thr Gly Thr Cys
1 5 10 15
Cys Ala

Ala Thr Thr Thr Thr Cys Thr Gly Ala Ala Cys Cys Ala Ala Gly Gly
1 5 10 15
Gly Ala Gly Cys Thr
   20

Thr Gly Cys Cys Gly Gly Cys Thr Cys Thr Cys Thr Cys Ala Gly Thr Gly
   1  5   10   15
Cys Thr Gly

<210> SEQ ID NO 93
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 93

Ala Ala Ala Cys Thr Thr Thr Thr Gly Ala Cys Cys Gly Cys Cys
   1   5   10   15
Cys Thr Thr Gly Ala
20

<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 94

Ala Thr Thr Gly Ala Cys Cys Ala Cys Thr Ala Cys Cys Thr Gly Gly
   1  5   10   15
Gly Cys Ala Ala
20

<210> SEQ ID NO 95
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 95

Gly Ala Gly Ala Thr Ala Cys Cys Thr Thr Cys Ala Ala Cys Ala
   1  5   10   15
Cys Thr Thr Thr Gly Ala Cys Cys Thr
20 25

<210> SEQ ID NO 96
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 96

Cys Ala Gly Ala Ala Cys Ala Thr Cys Ala Gly Cys Ala Ala
   1  5   10   15
Gly Cys Ala Gly Gly
20

<210> SEQ ID NO 97
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 97
Thr Thr Gly Ala Ala Ala Ala Ala Gly Cys Thr Gly Gly Gly
1 5 10 15
Thr Gly Gly Gly
20

Gly Ala Cys Cys Cys Thr Gly Cys Ala Ala Gly Ala Thr Gly Cys Ala
1 5 10 15
Ala Gly Cys Cys
20

Gly Ala Gly Cys Gly Gly Ala Thr Gly Ala Ala Gly Gly Thr Ala Ala
1 5 10 15
Ala Gly Cys Gly
20

Cys Cys Cys Ala Cys Cys Cys Thr Ala Cys Gly Ala Ala Gly Thr Ala
1 5 10 15
Cys Cys Ala Ala
20

Cys Thr Gly Gly Thr Ala Ala Gly Ala Gly Ala Cys Thr Ala Thr Gly Gly
1 5 10 15
Thr Gly Thr Gly
20

Cys Cys Cys Ala Cys Cys Thr Ala Cys Ala Gly Gly Cys Cys Cys Thr
Thr Cys Thr Cys
20

<210> SEQ ID NO 93
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 93
Gly Gly Cys Ala Thr Gly Gly Cys Ala Thr Cys Ala Cys Ala
1   5   10   15
Gly Thr

<210> SEQ ID NO 94
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 94
Gly Ala Ala Cys Ala Ala Ala Ala Gly Gly Thr Ala Cys Ala Thr
1   5   10   15
Gly Gly Cys Cys Cys Thr Gly Ala
20   25

<210> SEQ ID NO 95
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 95
Cys Cys Thr Thr Cys Thr Gly Thr Thr Cys Cys Thr Cys Thr Thr
1   5   10   15
Cys Ala Gly Thr Gly Ala Gly Gly Thr Ala
20   25

<210> SEQ ID NO 96
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 96
Ala Thr Gly Cys Cys Cys Ala Thr Cys Gly Thr Gly Cys Ala Cys Ala
1   5   10   15
Gly Gly Gly Ala Cys Cys Thr Cys Ala
20   25

<210> SEQ ID NO 97
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 97
Cys Gly Thr Thr Cys Thr Gly Cys Cys Ala Cys Ala Cys Thr Gly Gly
1   5   10   15
Gly Cys Thr Gly Thr Gly Ala
20

<210> SEQ ID NO 98
<211> LENGTH: 21
What is claimed:

1. A method for preventing or reducing the risk of developing an organ or cellular transplant rejection in a subject having had or undergoing a cellular transplant, the method comprising administering to the subject a composition comprising alpha-1 antitrypsin (AAT), alpha-1 antitrypsin-like compound or combination thereof.

2. The method of claim 1, wherein the composition is administered to the subject before transplantation, during transplantation, after transplantation or combination thereof.

3. The method of claim 1, wherein the composition further comprises one or more anti-transplant rejection agent, anti-inflammatory agent, immunosuppressive agent, immunomodulatory agent, anti-microbial agent, or a combination thereof.

4. The method of claim 1, wherein the composition comprises a carboxy-terminal peptide corresponding to AAT, an analog thereof, any derivative of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or a combination thereof.

5. The method of claim 1, wherein the cellular transplant is selected from the group consisting of cornea, bone marrow, liver, stem cell, pancreatic islet, pancreas, kidney, lung, intestine, and a combination thereof.

6. The method of claim 5, wherein the cellular transplant is pancreatic islet cell transplant.

7. The method of claim 3, wherein the immunosuppressive agent is chosen from inhibitors of apoptosis, serine protease inhibitors, reducers of lymphocyte numbers, reducers of cytokine production, reducers of cytokine activities, monoclonal antibodies, reducers of cytokine receptors, reducers of nitric oxide production and a combination thereof.

8. The method of claim 7, wherein reducers of cytokine production, reducers of cytokine activities, reducers of cytokine...
ine receptors is an inhibitor of one or more of TNFα (tumor necrosis factor alpha), IL-1 (interleukin-1), IL-12 (interleukin-12), IL-18 (interleukin-18), IL-17 (interleukin-17), IL-23 (interleukin-23), IL-32 (interleukin-32), IFNγ (interferon gamma) or a combination thereof.

9. A method for treating organ or cellular transplant rejection in a subject in need of such a treatment comprising:
identifying a subject having or in need of a cellular or organ transplant;
performing cellular or organ transplantation on the subject; and
administering a therapeutically effective amount of a composition comprising AAT, AAT-like compound, AAT analog, AAT derivative, serine protease inhibitor, one or more carboxy-terminal peptides derived from AAT, any derivative of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof to the subject.

10. The method of claim 9, wherein administration comprises administering the composition to the organ or cells to be transplanted before transplant, administering the composition to the subject before transplant, administering the composition to the subject during transplantation, administering the composition to the subject after transplantation or a combination thereof.

11. The method of claim 9, wherein treating the subject with the composition reduces the risk of transplantation rejection by at least 10% compared to a subject not treated with the composition.

12. The method of claim 9, wherein the cellular transplant is selected from the group consisting of cornea, bone marrow, liver, stem cell, pancreatic islet, pancreas, kidney, lung, intestine, skin and a combination thereof.

13. The method of claim 9, wherein the composition further comprises one or more anti-transplant rejection agent, anti-inflammatory agent, immunosuppressive agent, immunomodulatory agent, anti-microbial agent, or a combination thereof.

14. A method for treating diabetes in a subject in need of such a treatment comprising:
identifying a subject having or at risk of developing diabetes;
performing pancreatic islet cell transplantation on the subject; and
administering a therapeutically effective amount of a composition comprising AAT, AAT-like compound, AAT analog, AAT derivative, a serine protease inhibitor, one or more carboxy-terminal peptides derived from AAT, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof to the subject.

15. The method of claim 14, wherein administration comprises administering the composition to the organ or cells to be transplanted before transplant, administering the composition to the subject before transplant, administering the composition to the subject during transplantation, administering the composition to the subject after transplantation or a combination thereof.

16. The method of claim 14, wherein the subject has or is at risk of developing Type 1 diabetes.

17. The method of claim 14, wherein the subject is in early phase Type 1 diabetes.

18. The method of claim 14, wherein the subject has or is at risk of developing Type 2 diabetes.

19. The method of claim 14, wherein treating the subject with the composition decreases the risk of transplantation rejection by at least 10% compared to a subject not treated with the composition.

20. The method of claim 14, wherein the compound comprises one or more carboxy-terminal peptides derived from AAT, an analog thereof, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof.

21. A method for reducing a side-effect of cellular transplant rejection in a subject, the method comprising administering to the subject a composition comprising alpha-1 antitrypsin (AAT), one or more carboxy-terminal peptides derived from AAT, or alpha-1 antitrypsin-like compound.

22. The method of claim 21, wherein administration comprises administering the composition to the organ or cells to be transplanted before transplant, administering the composition to the subject before transplant, administering the composition to the subject during transplantation, administering the composition to the subject after transplantation or a combination thereof.

23. The method of claim 21, wherein the composition further comprises one or more anti-transplant rejection agent, anti-inflammatory agent, immunosuppressive agent, immunomodulatory agent, anti-microbial agent, or a combination thereof.

24. The method of claim 21, wherein the compound comprises a carboxy-terminal peptide corresponding to AAT, an analog thereof, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or a combination thereof.

25. The method of claim 21, wherein the cellular transplant is selected from the group consisting of cornea, bone marrow, liver, stem cell, pancreatic islet, pancreas, kidney, lung, intestine, and a combination thereof.

26. The method of claim 21, wherein the cellular transplant is pancreatic islet cell transplant.

27. The method of claim 21, wherein the side effects of cellular or organ transplant are selected from the group consisting essentially of production of pro-inflammatory cytokines, infiltration of immunocompetent cells, infiltration of inflammatory cells, infiltration of cytotoxic T-cells, infiltration of mature dendritic cells, infiltration of monocytes, production of nitric oxide, production of prostaglandins, production of reactive oxygen species, production of super oxide radicals, infiltration of natural killer cells, infiltration of natural killer T-cells and a combination thereof.

28. The method of claim 23, wherein the immunosuppressive agent is chosen from inhibitors of apoptosis, reducers of cytokine production, reducers of cytokine activities, reducers of nitric oxide production and a combination thereof.

29. The method of claim 21, wherein the composition inhibits the production of TNFα (tumor necrosis factor alpha), IL-1 (interleukin-1), IL-12 (interleukin-12), IL-18 (interleukin-18), IFNγ (interferon gamma), nitric oxide (NO) or a combination thereof.

30. A method for preventing or reducing the risk of developing pancreatic islet cell transplant rejection in a subject having had or undergoing a pancreatic islet cell transplant, the method comprising administering to the subject a composition comprising alpha-1 antitrypsin (AAT), one or more carboxy-terminal peptides derived from AAT, engager of the SEC receptor, alpha-1 antitrypsin-like compound, serine protease inhibitor or combination thereof.
31. The method of claim 30, wherein administration comprises administering the composition to the organ or cells to be transplanted before transplant, administering the composition to the subject before transplant, administering the composition to the subject during transplantation, administering the composition to the subject after transplantation or a combination thereof.

32. The method of claim 30, wherein the composition further comprises one or more anti-transplant rejection agent, anti-inflammatory agent, immunosuppressive agent, immunomodulatory agent, anti-microbial agent, or a combination thereof.

33. The method of claim 30, wherein the composition comprises a carboxy-terminal peptide corresponding to AAT, an analog thereof, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or a combination thereof.

34. The method of claim 30, wherein the subject has juvenile or late onset type 1 diabetes.

35. The method of claim 30, wherein the subject has type 2 diabetes.

36. The method of claim 30, wherein reducing the risk of developing pancreatic islet cell transplant rejection in a subject comprises reducing the risk by at least 10 percent in the subject compared to a second subject not treated with the composition.

37. The method of claim 30, wherein the subject is a human.

38. The method of claim 30, wherein the subject is a domesticated animal or livestock.

39. A pharmaceutical composition comprising, AAT, AAT-like compound, serine protease inhibitor, AAT analog, AAT derivative, one or more carboxy-terminal peptides derived from AAT, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof and at least one of an anti-transplant rejection agent, an anti-inflammatory agent, an immunosuppressive agent, an immunomodulatory agent, and an anti-microbial agent.

40. The pharmaceutical composition of claim 39, wherein the compositions is AAT, serine protease inhibitor, AAT-like compound, AAT analog, AAT derivative, carboxy-terminal peptide corresponding to AAT, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or combination thereof and one or more anti-transplant rejection agents.

41. A method for inducing immunological tolerance in a subject undergoing a cellular or organ transplant comprising: the method comprising administering to the subject a composition comprising alpha-1 antitrypsin (AAT), alpha-1 antitrypsin-like compound or combination thereof.

42. The method of claim 41, wherein the subject is undergoing islet cell transplant.

43. The method of claim 41, wherein the composition further comprises one or more anti-transplant rejection agent, anti-inflammatory agent, immunosuppressive agent, immunomodulatory agent, anti-microbial agent, or a combination thereof.

44. The method of claim 41, wherein the composition comprises one or more carboxy-terminal peptides corresponding to AAT, an analog thereof, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or a combination thereof.

45. The method of claim 41, wherein the cellular transplant is selected from the group consisting of cornea, bone marrow, stem cell, pancreatic islet, kidney, lung, intestine, skin and a combination thereof.

46. The method of claim 41, wherein the cellular transplant is pancreatic islet cell transplant.

47. The method of claim 46, wherein the immunosuppressive agent is chosen from inhibitors of apoptosis, reducers of cytokine production, reducers of cytokine activities, reducers of nitric oxide production and a combination thereof.

48. A method comprising, at least one of increasing numbers of or increasing effectiveness and/or of sustaining T-regulatory cells in a subject by administering an AAT or AAT derivative composition to the subject.

49. A method comprising, increasing immune tolerance in a subject in need thereof by administering an AAT or AAT derivative composition to the subject in need thereof.

50. The method of claim 49, further comprising inhibiting dendritic cell maturation.

51. The method of claim 49, wherein the subject is having, undergoing or previously having had a transplant.

52. A method comprising, reducing antigen presentation by dendritic cells by administering an AAT or AAT derivative composition to the cells.

53. The method of claim 52, wherein the cells are cells of a human subject and administration is to the subject in need of reducing antigen presentation.

54. A method comprising, inhibiting maturation of dendritic cells by administering an AAT, any derivative or fragment of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or AAT derivative composition to a subject having, undergoing or previously having had a transplant.

* * * * *